Chronic Viral Hepatitis
Second Edition
Edited by Kirti Shetty, Georgetown University, Washington DC
George Y. Wu, University of Connecticut, CT

Around the world hepatitis has infected more than 2 billion people (10 million in the US) and is, in its chronic form, the leading factor in end-stage liver disease and the rising incidence of hepatocellular carcinoma. In Chronic Viral Hepatitis: Diagnosis and Therapeutics, leading scientists, clinicians, and clinical investigators comprehensively review the epidemiology, molecular virology, diagnosis, treatment, and prevention of chronic hepatitis caused by the B and C viruses. The discussion of patient management includes contributions on developing novel therapeutics, supporting patients during therapy, alternative treatments, the use of drugs in chronic viral hepatitis, liver transplantation, and pregnancy in chronic viral hepatitis. Attention is also given to the treatment of patients with concomitant autoimmune disorders, the management of HIV co-infection, and the management of HBV/HCV co-infection. Each chapter provides an extensive review of available literature and is based on broad experience in clinical trials and the management of patients with chronic viral hepatitis.

Authoritative and eminently practical, Chronic Viral Hepatitis: Diagnosis and Therapeutics offers today’s gastroenterologists, primary care physicians, hepatologists, and infectious disease specialists a critical understanding of the state-of-the-art in chronic viral hepatitis and insightful projections of the many powerful new therapeutics now emerging.
DIAGNOSIS
AND THERAPEUTICS

Second Edition

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Humana Press
I wish to dedicate this book to my students and patients who have taught me so much and to my family who make this all worthwhile.  

K.S.

I would like to dedicate this book to my family for their patience, our students who inspire us, and especially Roy Lopata, Sigmund, and Jenny Walder who have been so supportive for our efforts in research, teaching, and treating liver disease.  

G.Y.W.
PREFACE

Since the publication of the first edition in 1991, a great deal of new information has become available regarding pathogenesis and treatment of chronic viral hepatitis. While the armamentarium for use against viral hepatitis has expanded, especially for the treatment of hepatitis B, in many ways the evaluation and therapeutic decision making has become more complex. Problematic issues not previously addressed include the appearance of resistance, side effects some of which are irreversible, and their management. Chronic viral hepatitis still affects hundreds of millions of people worldwide, and millions more new infections occur every year. This edition is intended to provide the readership with the most current information, concentrating on clinically useful practical information and new advances in diagnostic and therapeutic technology. Special attention is devoted to reactivation of hepatitis B with chemotherapy and immunosuppression, herbal and non-traditional therapies, HCV extrahepatic manifestations and their treatment, chronic viral hepatitis in the pediatric population, and immunology and immunotherapy of HCV.

As in the previous edition, the authors have strived to make this volume focused on clinically relevant information, presented in a practical and convenient format. It has been a pleasure to work with this group of expert authors in producing this volume. We hope the readers will find the information useful and helpful.

Kirti Shetty
George Y. Wu
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Molecular Virology of Hepatitis B and C: Clinical Implications

Marcy Coash, MD and George Y. Wu, MD, PhD

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Key Principles

- HBV and HCV are hepatotropic, noncytotoxic viruses.
- Both viruses mutate rapidly to produce quasispecies.
- HBV, but not HCV, can integrate into the host genome.
- Both viruses require viral and host proteins for replication.
- Few animal models for HBV and HCV are available.
- Specialized features of viral replication may offer targets for new antiviral agents.

1. INTRODUCTION

Despite fundamental differences in genome structure, hepatitis B (HBV) and C (HCV) viruses use many of the same strategies to achieve
high-level replication and persistence of infection in hepatocytes. The limited genome size of these viruses necessitates an efficient use of host cell machinery to carry out viral gene expression. This strong dependence on virus–host interactions restricts HBV and HCV cell tropism to the hepatocytes of higher primates and previously precluded their reliable and reproducible propagation in cell culture. Recent studies have begun to overcome this experimental limitation, so as to permit a more complete understanding of the molecular basis for viral replication, and the concomitant induction or evasion of the host antiviral immune response. Some of the important molecular biological properties of these viruses, as well as how they are reflected in the clinical manifestations of viral infection, are presented below.

2. HEPATITIS B VIRUS

HBV is a member of the Hepadnavirus family, a group of hepatotropic mammalian and avian DNA viruses, which replicate via reverse transcription of a full-length genomic RNA intermediate carried out by reverse transcriptase activity present in the viral DNA polymerase (1–3). The frequency of mutation during HBV genome replication is 10-fold higher than the normal rate for DNA viruses due to the fact that RTs do not correct errors of transcription. HBV populations may evolve more quickly than most DNA viruses as a result of environmental factors allowing the development of viral HBV mutants. Diversification of HBV strains has produced genotypes, subgenotypes, and HBsAg subtypes. They have emerged in specific populations and migrated with their hosts to other areas of the world. Based on sequence divergence in the entire genome of >8%, human HBV strains have been classified into eight genotypes (A–H), further divided into 24 subgenotypes that differ by at least 4% from each other. Additionally, nine different serologic subtypes exist as determined by HBsAg epitopes. Each of the various genotypes exhibits a characteristic serotype profile and geographical distribution, allowing an evaluation of HBV phylogenetic evolution and possible genotypic influence on the natural course of HBV infection (4). The sequencing of clinical isolates has even been used as an epidemiological marker to trace routes of transmission.

HBV infection is highly cell type- and species-specific: hepatocytes of the great apes are the only confirmed site of HBV replication. Reported infection of other cell types, e.g., lymphocytes, is controversial and generally thought to be of little clinical relevance. The lack of infection models to study the mechanisms of hepadnaviral entry and disease has been a significant problem in the past. Other hepadnaviruses, particularly duck and woodchuck hepatitis viruses, provided
convenient model systems for *HBV* infection and associated liver disease. With regard to cell culture models for *HBV*, recently primary hepatocytes from tree shrews, i.e., *Tupaia belangeri*, and a human hepatoma cell line (HepaRG) have been demonstrated to have susceptibility for *HBV* infection, the latter upon induction of differentiation in vitro (5). Besides the chimpanzees, which are extremely expensive and in short supply, a SCID mouse model based on primary human hepatocytes (PHH) and more recently an immunocompetent human hepatocyte-tolerant rat model have been described (6–8).

### 2.1. Structure of HBV

*HBV* is a 42-nm-coated DNA virus with a partially double-stranded 3.2-kb genome. The virion is composed of a nucleocapsid covered with the envelope proteins. The nucleocapsid, or core particle (also known as Dane particle), is a 27-nm icosahedron made up of the core protein HBcAg, an incomplete double-stranded viral DNA, and a DNA polymerase. The envelope is composed of lipids derived from the host in combination with the viral surface antigen (HBsAg), which is made up of three related proteins (S, M, L), variably glycosylated. Excess HBsAg can aggregate to form noninfectious quasispherical particles 20 nm in diameter as well as tubular structures 22 nm in diameter (9, 10).

### 2.2. Replication Cycle

*HBV* commonly achieves infection in virtually 100% of the hepatocyte population. The *HBV* replication cycle is complicated. Viral entry involves various components of the large and middle HBsAg proteins and annexins as well as other hepatocellular membrane proteins. Following entry into the host cell, the virus uncoats and the 3.2-kb partially double-stranded (pdsDNA) *HBV* genome is transported to the nucleus and converted by host RNA polymerase to a covalently closed circular (cccDNA) template, which is then organized into viral minichromosomes from which all viral mRNAs are transcribed, including the pregenomic RNA (pgRNA), which is essential for *HBV* replication (Fig. 1). Although the viral genome is a 3.2-kb partially double-stranded DNA molecule, it is generated by the reverse transcription of a longer-than-genome length 3.5-kb pregenomic RNA (11). The pgRNA is translated to produce the core protein and DNA polymerase and plays a role in nucleocapsid formation. The viral pgRNA transcript, encompassing the entire genome sequence, is subsequently encapsidated and reverse-transcribed into the pdsDNA form by a viral DNA polymerase, which functionally resembles that of a retrovirus.
**Fig. 1. HBV life cycle.** After cell entry, HBV nucleocapsids transport the partially double-stranded circular genomic HBV DNA to the nucleus, where the DNA is converted into covalently closed circular (ccc) DNA. The cccDNA functions as the template for the transcription of four viral RNAs, which are exported to the cytoplasm and used as mRNAs for the translation of the HBV proteins. The longest pregenomic RNA also functions as the template for replication, which occurs within nucleocapsids in the cytoplasm. Nucleocapsids are enveloped during their passage through the ER or Golgi complex and are secreted through the cell. Adapted from Rehermann et al. (45).

Although direct cytopathic liver injury is seen under conditions of very high viral load—as in fibrosing cholestatic hepatitis, a rare condition observed in some patients with recurrent posttransplantation hepatitis B—the virus itself is generally noncytolytic. Rather, HBV-associated injury is attributed to the lysis of infected hepatocytes by the host immune response (12).

### 2.3. Cellular Entry

Virion endocytosis, uncoating, and nuclear transport of the pdsDNA genome remain poorly understood, although the host cell contribution to these events is believed to be a major determinant of HBV-cell
tropism. Interaction of the viral envelope protein with a specific cell surface receptor is considered the initial step of HBV infection. Aside from host-derived phospholipids, the envelope of an HBV virion contains virus-derived (S), (M), and (L) surface proteins that are translated from distinct initiation codons, but share a common carboxyl domain. A generally accepted view is that the pre-S domain which resides on the N-terminal domain of the LHBs of the HBV envelope protein, specifically residues 21–47, is involved in mediating HBV attachment to a viral receptor on hepatocytes. The putative cellular receptor of HBV is still an enigma in the field of HBV research although studies with duck hepatitis B virus suggest the involvement of a carboxypeptidase receptor. More than 10 proteins including interleukin-6, human squamous cell carcinoma antigen 1, immunoglobulin A receptor, asialoglycoprotein receptor, transferrin receptor, apolipoprotein H, polymerized serum albumin, annexin V, fibronectin and more recently lipoprotein lipase have been demonstrated to interact with the HBV PreS or S proteins in vitro and may be potential receptor candidates although none of them has been experimentally proven to be the true receptor (13). Nuclear import of pdsDNA may be facilitated by the covalently attached HBV polymerase, or by the noncovalently associated capsid protein, which bears a nuclear localization signal. Host components involved in this nucleocytoplasmic transport have yet to be determined. Interestingly, the translocation event does not occur in transgenic mouse models of HBV infection, despite the ability of mouse hepatocytes to support subsequent steps in HBV replication.

2.4. cccDNA

The mechanism by which HBV pdsDNA is converted to covalently closed circular form is not known, but presumably involves host cell DNA-repair pathways. Conversion of the relaxed circular DNA genomes of the incoming virus to covalently closed circular (ccc) DNA within the nucleus of an infected hepatocyte is the first marker of a productive infection, but is difficult to detect (14). Once formed, the cccDNA does not undergo semiconservative replication in the nucleus, as the intracellular HBV DNA pool can be supplemented only by reverse transcription in the cytoplasm, as described below. In contrast to serum HBV DNA which has a half-life of just 1–3 d, cccDNA is a remarkably stable species in hepatocyte nuclei despite the fact that it is not integrated into the cellular genome. It is resistant to direct elimination by antiviral agents and serves as a reservoir for viral reactivation when antiviral therapy is interrupted. The half-life of infected hepatocytes is quite long, 10–100 d or more, depending on the extent of liver injury. Accordingly, antiviral therapy with reverse transcriptase inhibitors requires an extended course of treatment to achieve
complete clearance, especially in mild disease with a relatively low rate of hepatocyte turnover.

2.5. Transcription

In contrast to other DNA viruses, HBV appears not to utilize posttranscriptional alternative mRNA splicing to maximize its coding capacity. Instead, HBV makes exquisitely economical use of its genome at the transcriptional level by embedding regulatory elements—even overlapping open-reading frames (ORFs) (C, P, S, and X)—within concise protein coding sequences for seven viral proteins (Fig. 2). Despite its small size, the HBV cccDNA genome is sufficient to direct the synthesis of four classes of RNA transcripts by host RNA polymerase II. A pair of ~3.5-kb RNAs, differentially regulated by the core promoter, function as templates for HBV DNA synthesis and serve as mRNAs for precore (HBeAg) which contains the full amino acid sequence of the core protein, and is not incorporated into the virus particle, but is secreted.

Fig. 2. Structure of HBV DNA and genes. Four genes (S, C, X, and P) are encoded and these partially overlap. The pre-S gene is located upstream of the S gene and consists of the pre-S1 and pre-S2 genes. The precore region is upstream of the core gene.
in a soluble form by the infected cell, core (HBcAg), and polymerase (DNA-pol) proteins. The 2.4-kb and 2.1-kb pre-S1/pre-S2/S mRNAs encode the three envelope proteins. A 0.7-kb transcript directs the synthesis of the HBV X protein (HBxAg), which is a transactivator of virus replication that is not incorporated into the virion. The mature viral particle is composed of a single core protein, three envelope glycoproteins, and the viral DNA polymerase (2).

Transcription of each HBV RNA initiates from one or more unique promoters, which are regulated by two shared enhancer elements, EN I and II. Activity of liver-enriched transcription factors (TFs) at these sites is thought to contribute to tissue specificity of HBV infection, as the observed multifactorial cis-regulation of viral promoters takes place only in well-differentiated liver cells (15). Additionally, the HBV genome also contains two 11-base direct repeats (DR1 and DR2) located at the 5’-end of both strands, a polyadenylation signal, and a site for glucocorticoid response (GRE). All transcripts terminate at a common polyadenylation signal, which is located in the core ORF. This signal is read-through during transcription of the pregenomic RNA, generating a terminally redundant greater-than-genome length transcript. Hepatitis delta RNA selectively inhibits transcription of pregenomic HBV RNA in vitro. This effect likely contributes to the frequently observed precore antigen seroconversion in carriers superinfected with HDV.

### 2.6. Translation

Efficient use of coding sequences, the hallmark of HBV biology, is also apparent during translation. The surface antigens are generated by three different in-phase start codons. Translation of the precore/core ORF originates from one of two in-frame start codons; initiation from the upstream site generates the precore polypeptide containing a signal for secretion, which is posttranslationally modified to form the hepatitis B e-antigen. Translation of the polymerase gene initiates from an out-of-frame start codon located in the C-terminal coding region of the core ORF. The inefficiency of this unconventional and, as yet, poorly understood process yields a core:polymerase molar ratio of 250:1. Interaction between the HBV polymerase and the 5’-end of its own mRNA is sufficient to block further translation and to precipitate the events of genomic replication.

### 2.7. Encapsulation and Reverse Transcription

HBV RNA packaging and reverse transcription have been well characterized (16). Among the viral transcripts made from cccDNA, the
3.5-kb pregenomic RNA (pgRNA) encodes the core and polymerase proteins, and carries at its 5'-end a stem and loop structure known as epsilon that directs its encapsidation into the viral replication complex. The N-terminal domain of the polymerase protein binds to “epsilon” and is facilitated by host cell chaperones, such as p23 and heat-shock protein (Hsp)70; inhibitors of Hsp90 have been shown to block HBV RNA packaging and subsequent DNA synthesis. Formation of the polymerase–RNA complex triggers assembly of the viral nucleocapsid. The capsid is composed of 120 disulfide-linked core dimers, which collectively bind RNA via a C-terminal domain of the core polypeptide. The N-terminal domain is responsible for core protein dimer formation and capsid assembly (17).

Reverse transcription occurs within the nucleocapsid, which contains small pores to allow influx of deoxynucleotides and other small molecules. Initiation of reverse transcription is accomplished by a protein-priming mechanism, utilizing an amino-terminal tyrosine residue of the polymerase and the epsilon structure as a replication origin. The C-terminal catalytic domains of the HBV polymerase protein exhibit reverse transcriptase, DNA-dependent DNA polymerase, and RNase H activities. In the presence of requisite host cell chaperones, and possibly other cofactors, the polymerase carries out synthesis of viral pdsDNA and nearly complete degradation of the pregenomic RNA template. Phosphorylated metabolites of the antiviral compounds lamivudine, famciclovir, lobucavir, and adefovir dipivoxil inhibit this step of HBV replication by competing with deoxynucleotide triphosphates for incorporation into HBV DNA, thereby causing premature termination of nascent polynucleotide chains.

One notable feature of the HBV polymerization process is its discontinuous nature. pdsDNA synthesis requires dissociation of the polymerase, and nascent DNA from the RNA template, and annealing to a homologous direct repeat sequence on the 3'-end of the pregenome. Polymerization from primers that fail to undergo template switching (“in situ priming”) results in the formation of double-stranded linear (dsIDNA) genomes. Although illegitimate recombination events can convert dsIDNA into cccDNA, they may also cause integration of HBV sequences into host DNA.

2.8. Integration

The integration of the HBV DNA is apparently a common event that occurs early during HBV infection even after acute self-limiting hepatitis. It is not essential for the viral replication, but it allows persistence of the viral genome. Transcription from integrated DNA accounts for
hepatitis B surface antigen HBsAg secretion observed in the absence of viral replication. Even in the majority of patients who develop spontaneous or treatment-induced HBeAg seroconversion and normalization of aminotransferase (ALT) levels and histology, HBV DNA sequences can be detected by PCR-based diagnostic tests. HBV dsDNA is likely the predominant integrating species, although the gap structure in the pdsDNA may facilitate integration of this form as well. Commonly, the core and polymerase genes are disrupted by integration, whereas the X and pre-S2/envelope ORFs remain intact. It has been demonstrated that the regulatory proteins HBx and the pre-S2 activators that can be encoded by the integrant can exert a tumor promoter-like effect and lead to a selection of cells that produce a functional regulatory protein.

Integrated HBV DNA is found in the majority of HBsAg-positive hepatocellular carcinomas (HCCs). As with other viruses, these integrated viral genomes are characterized by rearrangements or partial deletions. HBV integration can induce deletions in the host chromosome at the integration site, as well as cause insertional mutagenesis, leading to activation of a proto-oncogene or inactivation of a tumor suppressor gene in cis. In woodchuck hepatitis virus, there is evidence of specific activation of the myc family of genes by this mechanism. In isolated cases of human HCC, HBV insertion has been observed near cyclin A, retinoic acid receptor β, mevalonate kinase gene, sarco/endoplasmic reticulum calcium ATPase1 gene, and p53 loci. Integration in these genes can cause alterations in cellular signal transduction cascades, proliferation control, and cell viability. However, the HBV integration site is apparently random, and integration of full-length HBV genomes does not commonly lead to transformation of cultured cells.

2.9. X Protein and Hepatocarcinogenesis

The oncogenic potential of HBV integration likely derives from the activation of host gene transcription in trans, rather than in cis (18). Truncated forms of the HBV pre-S/S envelope proteins may be formed as a result of integration. These exhibit potent transactivation in vitro and can stimulate the c-myc promoter, as seen in woodchuck hepatitis virus-related HCC. Integrated HBV DNA isolated from tumor nodules preferentially encompasses the X protein ORF. X is required for infection in vivo, but is dispensable for viral replication in transfection-based cell culture models. The X protein regulates transcription from viral and host RNA polymerase I, II, and III promoters in vitro (19). Although X has been shown to dimerize—a feature common to DNA-binding TFs—it likely acts as a coactivator. X associates with a myriad of host cell proteins, including transcriptional regulators such as NF-κB,
AP-1, AP-2, c-EBP, ATF/CREB, and the calcium-activated factor
NF-AT (20). It influences other TFs without binding them directly and
so is thought to act by more global mechanisms, such as interaction
with general transcription factors (TFIIB, RNA polymerase subunit 5).

Among the multitude of cis-acting genetic elements responsive to
X activity is HBV enhancer I: thus X may regulate its own syn-
thesis. Enh I is responsive to many extracellular signals including
12-O-tetradecanoylphorbol-13-acetate (TPA), okadaic acid, IL-6, and
genotoxic stimuli that are important in an acute inflammatory response
and cell proliferation in the liver. X-responsive host genes include many
involved in acute inflammatory and immune responses [e.g., major
histocompatibility complex (MHC), tumor necrosis factor (TNF)-α,1
interleukin (IL)-6, signal transduction, cell proliferation, and apop-
tosis]. HBxAg sensitizes cells to TNF-α and is suspected to influence
other apoptotic pathways. Overall, the transcriptional regulatory effects
of X may contribute to viral persistence, increased cell turnover, and
HBV-related hepatocarcinogenesis.

Distinct from its transactivation function, HBxAg may disrupt DNA
repair and normal cell-growth regulation by direct interaction with
host regulatory proteins. X associates with a cellular factor called UV-
damaged DNA-binding protein and downregulates nucleotide excision
repair pathways. Sequestration by X protein also inhibits p53 nuclear
translocation and tumor suppressor activities, disrupting transcription-
coupled repair mechanisms. Consequently, X may sensitize cells to
genotoxic stimuli and promote integration events and overall genetic
instability common in HCC. Interestingly, HBxAg has been reported to
exhibit endogenous adenosine monophosphate (AMP) kinase/ATPase
activities and to colocalize with proteasome subunits, which may be
mechanistically relevant to its observed effects on growth regulators.
Stimulation of the Ras-Raf-mitogen-activated protein kinase pathway
and activation of protein kinase C, c-Src, and c-Fyn have been reported
in cell culture models of X expression. The retinoblastoma (Rb) tumor
suppressor is inactivated by hyperphosphorylation in the presence of
HBxAg.

In vitro and transgenic studies indicate that X promotes cell trans-
formation and carcinogenesis, but only upon overexpression to levels
well beyond those typical of infected cells. Integration of X may lead
to such overexpression, as subcellular localization studies indicate that
expression of X from integrated templates leads to its accumulation
and redistribution. Notably, an X-responsive bZip-family TF was found
to be overexpressed in HCCs. However, cell transformation has been
observed with the expression of the X gene product N-terminal domain,
which lacks the transactivation function altogether. Indeed, most of the
proposed actions of X have not been demonstrated in the context of HCC-derived cells; it is possible that HBV-associated carcinogenesis is solely due to the induction of chronic hepatocellular necrosis and regeneration. Given that HBV-related HCC is closely associated with cirrhosis and that the peak incidence occurs 30–50 years after HBV infection, it is likely that the contribution of X expression alone is limited, relative to other factors, such as the extent of immune-mediated pathogenesis.

2.10. Virion Production

Newly synthesized pdsDNA may be imported into the nucleus, resulting in the replenishment of the cccDNA pool, or secreted from the cells in virions. Completion of pdsDNA synthesis in some way facilitates nucleocapsid translocation into the endoplasmic reticulum (ER) and entry into the secretory pathway. The mechanism of this coupled maturation process is unknown, but may involve a conformational shift in the nucleocapsid exterior. Other factors, such as a core protein phosphorylation state or regulation by HBeAg, may influence the relative rate of cccDNA accumulation and virion production. HBeAg has been shown to slow HBV DNA replication in vitro by reducing HBcAg dimerization, thereby reducing encapsidation of pregenomic RNA and may induce immunological tolerance. Secretion is also dependent on the concentration of L envelope protein as the pre-S1 domain mediates association of the envelope with core particles. Through budding, the nucleocapsid acquires a coating of the S, M, and L transmembrane proteins. Transport of virions through the Golgi apparatus results in the maturation of envelope proteins into N-glycosylated disulfide-linked multimers.

HBV is continuously released into the bloodstream, with a daily production of up to $10^{11}$ enveloped virions. HBV surface proteins are not only incorporated into virion envelopes, but they also bud very efficiently from intracellular post-ER pre-Golgi membranes. These envelopes lacking enclosed nucleocapsids can be secreted at more than a 100-fold excess over virions, resulting in very high serum concentrations of “surface antigen particles” or subviral particles. Subviral particles and virions have identical surface antigens (HBsAg), but their protein composition is not identical. Presumably the overproduction of HBsAg can influence the host immune response such that it is advantageous for the virus acting as immunological decoys (17–20). Extrahepatic symptoms may result from deposition of antigen–antibody complexes formed when these particles are neutralized by anti-HBsAg antibodies.
2.11. Immune System Evasion

Infection of a high percentage of hepatocytes is observed even in acute, resolved cases, suggesting a delayed immune response to HBV antigens. Patients recovering from acute HBV infection exhibit humoral response to pre-S and S antigens, as well as vigorous, polyclonal cytotoxic T lymphocyte (CTL) activity against multiple epitopes of HBV envelope, core, and polymerase proteins. This response involves human leukocyte antigen class II-restricted CD4+ helper T lymphocytes and CD8+ cytotoxic T lymphocytes. However, given the large quantity of hepatocytes infected and the rapid rate of clearance following transient infection, it has been proposed that HBV nucleic acids can be eliminated from infected hepatocytes by means other than direct CTL killing and replacement by noninfected cells. cccDNA may be lost from cell nuclei during mitosis. Additionally, the activation of an intracellular antiviral response by cytokines, such as TNF-α, interleukin-12, and interferons (IFNs) alpha and gamma, is known to induce noncytoidal loss of viral RNA and proteins. At high doses, TNF and interferons downregulate the core promoter.

Immunotolerance of HBV antigens may play a central role in viral persistence. Most infections in immunocompromised adults, and 90% of perinatal infections, progress to chronicity. These patients exhibit generally less severe liver disease than immunocompetent adults, despite higher viral load. The CTL and CD4+ T cell response in chronically infected patients is weak and restricted to relatively few epitopes. Hepatitis B e-antigen is not essential for infection in vivo, but appears to play an immunosuppressive role contributing to the maintenance of high-level viremia. HBeAg bears an N-terminal signal peptide and is actively secreted. It has been proposed to cross-react with antibodies to core or antagonize the inflammatory Th1 response, thereby suppressing the host’s ability to generate a sufficient CTL attack on infected hepatocytes. HBeAg may cross the placenta to establish T-cell tolerance to HBV e- and core antigens setting the stage for perinatally acquired chronic infection. Consistent with these proposed interactions, clearance of HBeAg during primary and chronic infections is frequently associated with ALT flare-ups. HBV mutant strains defective for e-antigen expression generally induce more severe immune-mediated liver injury than wild-type and are more likely to be cleared during the acute phase of infection.

2.12. Viral Mutation

The genetic stability of HBV is evidenced by the fact that each of the major genotypes exhibits only a few of the possible HBsAg sub-
types. The development of effective anti-HBV vaccines has been greatly facilitated by the high degree of sequence conservation in neutralization epitopes (e.g., the “a” determinant, amino acids 99–169) of the HBV envelope proteins. Even the most divergent genotype, F, retains approximately 85% genomic sequence identity with other genotypes. Despite this, viral reverse transcriptases lack a proofreading function and are inherently error prone, leading to frequency of HBV mutations estimated to be 1.4–3.2 × 10⁻⁵ nt substitutions per site per year.

The remarkably low tolerance for mutations is most likely a consequence of the overlapping nature of the HBV genome, which dictates that a single-point mutation can affect multiple coding sequences and/or regulatory elements. HBV quasispecies variability is prone to stronger conservatory constraints than RNA viruses that do not have nonoverlapping reading frames such as HCV and poliovirus. Nonetheless, mutations in each of the four HBV genes have been clinically isolated, and these variants frequently arise in response to immune system selection and antiviral therapy (21, 22). In several cases, the emergence of a particular mutant is known to be contingent upon nearby wild-type sequences; therefore, HBV genotyping is valuable for the prediction and assessment of the clinical implications of viral mutation. This quasispecies distribution implies that if a newly generated mutation leads to a selective advantage for the virus, this will allow the corresponding viral population to overtake the other variants in a Darwinian manner.

2.13. Immune-Escape Mutants

The emergence of HBV mutants has been implicated in vaccine failure and immune system evasion. Two major groups of mutations that lead to a reduction or a blockade of HBeAg expression have been identified. The common G-to-A transition at nucleotide 1896 results in a stop-codon mutation causing premature translation termination of the precore ORF, thereby preventing synthesis of HBeAg. Notably, this mutation is found in association with T1858, a sequence characteristic of HBV genotypes B–E, possibly because this pairing maintains stability of the pregenomic RNA encapsidation signal. This mutation is rarely found in genotypes A or F or in certain strains of HBV genotype C. The relative rarity of this mutation in genotype A may contribute to the observed low perinatal transmissibility of HBV from healthy HBsAg carrier mothers in Northwestern Europe where genotype A predominates. Precore mutants may replicate more efficiently than wild-type, but are most likely selected due to their ability to escape anti-HBe surveillance. Their pathogenic significance is unclear, as mixed
viremia brings about a complex set of dynamic interactions with the host immune response (22).

The second group of mutations resulting in alteration of HBeAg expression involves mutations affecting the basal core promoter occurring at nt 1762 and nt 1764. This results in reduced binding of liver-specific transcription factors, transcribing fewer precore and core mRNA transcripts and, therefore, less precore protein (HBeAg). These mutations, however, do not affect the transcription of pregenomic RNA or the translation of the core or polymerase. Genotype A-infected individuals often express this pattern of mutation and, therefore, have enhanced viral replication by suppression of precore and core mRNA relative to pregenomic RNA (23).

Mutations in the X region lead to alteration in the regulatory elements that control replication and can include mutations in the basal core promoter and enhancer II. The basal core promoter spans nt 1742–1802 and overlaps the reading frame of the X gene. The nt 1762 and the nt 1764 mentioned above are core promoter mutations that can cause changes in the X gene leading to production of truncated X proteins, which lack the amino acid domains required for transactivation activity of the HBxAg.

Most hepatitis B vaccines contain the major HBsAg protein and induce an immune response to the major hydrophilic region at residues 99–170. The anti-HBsAg response produces protective immunity. Mutations within this epitope have been selected during vaccination. Envelope gene mutations are detected in roughly half of all individuals who develop postvaccination HBV infection and are particularly prevalent in infants born to carrier mothers. The most common of these so-called “vaccine-escape” mutants is G145R in the “a” determinant of HBsAg. Mutations here can lead to conformational alterations affecting the binding of neutralizing antibodies. These HBV mutants have been shown to escape vaccine-induced or passively transferred neutralizing responses allowing the propagation of a true HBsAg-positive HBV infection despite the high titer of anti-HBsAb (23, 14). Mutations within this locus have also been found in orthotopic liver transplant recipients who developed reinfection despite human immunoglobulin prophylaxis. The presence of escape variants correlated with a high incidence of graft failure, nearly double the percentage observed for patients lacking variants. The S140 residue, unique to HBV E and F genotypes, may predispose patients to a vaccine escape mutation at an adjacent locus, as K141E has been observed only in West Africa, where genotype E predominates.

Pre-S1/S2 deletions and start codon mutants have been detected in patients with chronic and fulminant hepatitis, perhaps arising in
response to strong humoral and T-cell activity against this highly immunogenic region. Selection of emergent mutations in CTL epitopes of the envelope and core genes has been observed, though escape is incomplete due to the multispecific nature of the CTL response. Some of these mutant-derived core sequences act as antagonists of CTL response against the wild-type epitope and may contribute to viral persistence.

2.14. Polymerase Mutants

HBV integration typically yields replication-deficient integrated genomes. Therefore, inhibition of reverse transcriptase for a sufficiently long duration should permit eventual immune-mediated clearance of the preexisting cccDNA pool. The advent of treatment with nucleoside and nucleotide analogues has resulted in the production of minor quasispecies containing mutations in the HBV Pol gene (23). Studies have shown that long-term monotherapy with lamivudine or other polymerase inhibitors is often unsuccessful due to breakthrough infection by drug-resistant polymerase gene mutants after approximately 1 year of therapy (21, 25). Polymerase mutants are absent from untreated patients and exhibit generally lower virulence than wild-type, due to lower catalytic activity or possible missense disruption of the overlapping S gene. Clinical manifestations of breakthrough infection are variable: most patients sustain lower-than-pretreatment serum DNA and ALT levels; but, in rare occasions, emergence is accompanied by ALT flares and hepatic decompensation. Upon therapy withdrawal, the reemergence of wild-type HBV typically yields further bouts of hepatitis.

Lamivudine resistance increases progressively during treatment to 14–32% annually and can reach 70% after 48 months of treatment. Several mutations which confer resistance to lamivudine have been shown to cause substitutions within or adjacent to the “YMDD” motif at the catalytic site of the polymerase. A protein structure-based model by which these mutated residues block drug action has been proposed (24, 26). Famiciclovir-associated polymerase gene mutations, commonly affecting upstream domains of the protein, have been identified in immunocompromised patients. Upstream mutations are more likely to affect immune epitopes on HBsAg, although the effect on antigenicity has yet to be determined. Unfortunately, the two most common famciclovir-resistant mutations, L528M and V521L, are also observed with lamivudine treatment, suggesting that patients who develop lamivudine-resistant mutants may not respond favorably to famciclovir treatment and vice versa. HBV resistance to adefovir occurs less
frequently—around 20% after 2 years—than lamivudine resistance. Adefovir resistance is conferred primarily by a mutation at codon 236 in the D motif of HBV Pol gene as well as a B motif mutation at 181. Entecavir resistance has been observed in two patients who were resistant to lamivudine with mutations mapped to the B motif at 184, the C motif at 202, and the D motif at 250 (23). Recent in vitro and in vivo studies indicate that YMDD gene variants resistant to lamivudine remain sensitive to adefovir, suggesting that the antiviral effects of these drugs will be additive. However, mutations that confer resistance to lamivudine have been shown to give partial resistance to entecavir in vitro. The identification of such mutually exclusive resistance profiles may be useful in the design of effective sequential or combination therapy regimens.

3. HEPATITIS C VIRUS

One of the most remarkable features of hepatitis C virus is the frequency with which a chronic infection can be established, occurring in 55–85% of patients (27). In contrast to HBV, HCV exhibits extreme variability in nucleotide sequence. HCV, a member of the Flaviviridae family, has a single-stranded positive RNA genome with high genetic diversity. The diversity is due to defective repair activity of the RNA-dependent RNA polymerase, which results in nucleotide substitution, as well as the absence of 5’ to 3’ exonuclease activity causing a lack of editing (28). At least six HCV genotypes and a large number of subtypes have been identified, some with different clinical outcomes. More recently, chimeric viruses generated by intergenotypic homologous recombination events in the NS2 gene have been described. For a genotype 1a strain, the complete ~9600 nucleotide (nt) RNA genome has been verified by in vivo chimpanzee transfection, using transcripts derived from consensus cDNA clones. During the course of an infection, HCV mutations accrue at the rate of 1.4×10^3 to 1.9×10^3 substitutions per nucleotide per year. Variants reflecting slight modifications to the coding sequence, so-called “quasispecies”, are commonly found to coexist simultaneously within a patient, and any given serum-derived HCV inoculum contains a population of closely related viruses. The regions of the genome corresponding to essential viral functions such as those involved in translation or replication, as well as the noncoding 5’- and 3’-ends, are highly conserved. The most variable part of the genome is the region encoding the envelope glycoproteins E1 and E2, and certain strains have shown more than 50% variability (28). The observed “hypervariability” in HCV-envelope proteins is one explanation for the exceptional ability of HCV inocula to reinfect the same host following seroconversion and resolution of acute infection.
The absence of protective immunity against HCV continues to thwart vaccine development, and the emergence of drug-resistant strains will likely pose a future obstacle to long-term efficacy of inhibitors designed to target the enzymes responsible for viral replication.

3.1. Replication Cycle and Experimental Systems

Chimpanzee infection studies revealed the principal etiologic agent of HCV hepatitis to be an enveloped, 9.6-kb linear single-stranded positive-sense RNA virus and has been characterized extensively in vitro (29, 30). The genome serves as a template for translation as well as for replication and is composed of a 5′-noncoding region (NCR), which includes an internal ribosome entry site (IRES), a single open-reading frame that encodes structural and nonstructural proteins, and a 3′-NCR (31). The 5′-untranslated region (UTR) of virion RNA directs the cap-independent translation of a polyprotein, which is cleaved into structural (C, E1, E2, p7) and nonstructural (NS2, 3, 4A, 4B, 5A, 5B) viral proteins (32). Replication of the viral RNA is carried out by an HCV-encoded RNA-dependent RNA polymerase, through a full-length negative-strand RNA intermediate without evidence of DNA formation (33) (Fig. 3). Nascent positive-strand RNAs can be utilized for translation, subsequent rounds of RNA replication, or packaging into virions. Although development of hepatitis in immunocompromised individuals suggests that HCV proteins may be directly cytopathic, the replication process is generally noncytolytic. In most cases, chronic infection continues for many years without evidence of hepatocyte injury.

Since the discovery of the HCV in 1989, the difficulty in the development of a cell culture model has been a major obstacle for HCV research. Previous attempts using primary hepatocyte culture systems derived from resected liver tissue and inoculated with HCV-positive sera were only able to sustain low levels of viral RNA. One of the important milestones for HCV research was the development of replicon systems which supported HCV replication inside permissive cultured cells. Replicon systems have been used for screening antiviral compounds, studying host–viral interactions, RNA replication, and cellular innate immune responses. A shortcoming of this system is the lack of viral packaging and infectious viral release as would occur in infection. In 2005, several groups were able to establish complete viral cell culture systems. A genotype 1b HCV DNA was transfected into Huh7 cells, a cell line derived from a man with hepatocellular cancer. Another system developed included the JFH1 genotype 2a clone-based infectious cell culture system, which was cloned from a Japanese patient who had fulminant hepatitis caused by HCV. The viral replicons derived
from the JFH1 clone replicated much more efficiently without the need for adaptive mutation. It was later shown that infectious viral particles could be released from Huh7 cells when JFH1 full-length RNA was transfected into the cells and that these viral particles could reinfert other naïve cells (33).

Humans and chimpanzees are the only hosts able to support the complete viral life cycle, and the chimpanzee is still the only proven infection animal model. However, studies on chimpanzees are limited due to constraints on availability and cost. The development of other small animal models for HCV infection has been a priority. Tree shrews (T. belangeri) have been shown to be susceptible to infection with HCV. However, they must first be irradiated to reduce the immune response. Several lines of HCV transgenic mice have been established. These animals do produce circulating virus, but the lack of host immune tolerance to viral proteins does not simulate the normal immune response that
results in liver damage. A mouse model in which mouse hepatocytes were repopulated by human liver cells has also recently been demonstrated, and an even more successful model using a uPA-SCID transgenic mouse has shown potential applications in a model for HCV research (33). A rat model for HCV has recently been established through induction of tolerance of human hepatoma cell line Huh7. Rat livers transplanted with Huh7 cells were shown to develop HCV replication, gene expression, and biochemical as well as histological evidence of hepatitis. However, levels of virus were low, much lower than in average patients with HCV infection (34).

3.2. Viral Entry

Recent studies have identified viral and host cell components involved in HCV cell entry. Hepatocytes are the main target cells for HCV infection. However, HCV has also been found in B cells, dendritic cells, and other cell types. The HCV envelope integral membrane proteins are glycosylated noncovalent heterodimers of two polypeptides, E1 and E2. The latter of these is apparently responsible for viral attachment, as E2-specific antisera can prevent HCV binding to cultured cells. Circulating HCV virions have been shown to associate with β-lipoproteins. The capsid protein, core, colocalizes with apolipoprotein AII and intracellular lipid droplets; this interaction may contribute to hepatic steatosis observed in core transgenic mice. Notably, the expression of LDL receptor in some cell types was shown to induce HCV binding, which does not otherwise occur. The endocytosis of HCV particles appears to be mediated by LDL receptor, but may be assisted by other interactions. E2 was shown to bind a human cell surface membrane protein, CD81. This interaction may contribute to entry of HCV virions into hepatocytes, although expression of CD81 is a protein that has been found on the surface of many cell types. Other proposed HCV receptors in addition to LDL and CD81 include scavenger receptor class B type I (SR-BI), dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN), and more recently, claudin-1 which has been found to be essential for HCV entry into hepatic cells and has been termed a coreceptor (31).

HCV replication has been reported to occur in extrahepatic sites, most notably peripheral blood mononuclear cells (PBMCs) from chronically infected patients. High HCV RNA titers are observed in lymph nodes and approx. 50% of patients develop circulating HCV-associated cryoglobulins. An association with lymphoproliferative disorders such as
non-Hodgkin’s lymphoma has been reported. Lymphotropic variants of \textit{HCV} have been isolated from passage in cell culture and chimpanzee PBMCs. Comparison of \textit{HCV} RNA sequences isolated from viruses propagated in lymphocytes vs. hepatocyte cell lines revealed that the majority of amino acid positions which putatively influence cell tropism are located in E2.

Events following \textit{HCV} cell attachment remain to be elucidated. It is known that \textit{HCV} enters by clathrin-mediated endocytosis via transit through an endosomal low pH compartment and presumably by endosomal membrane fusion. The actual mechanisms involved in activating the low pH-induced fusion as well as the fusion peptides still remain unknown (31). The E2 glycoprotein is insufficient for membrane fusion, and a hydrophobic region of the E1 glycoprotein has been proposed to mediate fusion of the viral envelope to the cell membrane. Upon cell entry, core associates with 60S ribosomal subunits, which may contribute to uncoating of RNA and initiation of IRES-mediated \textit{HCV} translation and RNA replication (31).

### 3.3. Translation

There is no evidence that \textit{HCV} positive-strand RNA bears the 5’-methylguanidinyl cap and 3’-polyadenylate tail typical of eukaryotic messenger RNAs; but, it is nonetheless sufficient to direct translation of the \(\sim 3000\) amino acid-long \textit{HCV} open-reading frame. The 5’-UTR sequence folds into a complex secondary and tertiary RNA structure. Notably, it is one of the few regions of the genome exhibiting little sequence variation among all \textit{HCV} genotypes. The \(\sim 300\)-nt region upstream of the initiator codon functions as an internal ribosome entry site (IRES). It directly recruits 40s ribosomal subunits to the start codon and can do so even in the absence of any canonical eukaryotic initiation factors. The IRES–40S ribosome complex then recruits eukaryotic initiation factor eIF-3 followed by Met-tRNA-eIF2-GTP to produce a 48S rRNA complex at the AUG initiation codon, which is then converted into an 80S complex in a rate-limiting step involving GTP-dependent association of the 60S subunit (31). The formation of this complex is accelerated by the host NS1-associated protein-1 (NSAP-1), which binds to an adenosine-rich region within the \textit{HCV} RNA (35). The unique internal entry and factor independence of \textit{HCV} ribosome binding stand in marked contrast to cap-dependent translation initiation of host cell genes, making IRES a potential target for specific inhibition.

Sequences adjacent to the minimal IRES element are thought to influence its activity either by preventing disruption of RNA secondary structure or possibly by recruiting host cell factors. Various host
RNA-binding proteins have been isolated via their direct interaction with the HCV UTRs, although in many cases their identity and contribution to translation initiation remain obscure. Disruption of these interactions might destabilize the IRES structure or diminish ribosome entry and could be explored as a treatment strategy. Indeed, the inhibition of translation and/or the direct cleavage of positive-strand RNA may be essential components of an effective antiviral therapeutic regime. Because HCV translation does not require the preexistence of virally encoded factors, any remaining RNA is sufficient to initiate new rounds of replication, even if all HCV enzymes are simultaneously inhibited.

3.4. Protein Processing

Translation results in the production of a fusion protein which needs to be processed into each of its 10 proteins. Additionally an 11th protein is also produced by ribosomal frame-shifting within the core gene and is called alternative reading frame protein (ARFP) (35). Proteolytic processing of the HCV polyprotein is co- and posttranslational and mediated by both host and viral proteases (Fig. 4). The host signal peptidase, resident in the ER lumen, is responsible for cleavage between

![Fig. 4. Structure of the HCV gene. A long single open-reading frame encodes a polyprotein of ~3010 amino acids. The structural proteins are core, E1, and E2, the envelope proteins. The rest of the genome contains nonstructural proteins. Another protein known as ARFP (alternative reading frame protein) may be expressed from the core region of the genome as a result of translational frameshift.](image-url)
the HCV structural proteins and separation at the p7/NS2 junction. The E1 and E2 proteins are translocated into the lumen of the ER, with the C-terminal domains remaining embedded in the membrane. Non-
structural proteins are cleaved by the activity of two virally encoded proteases, NS2 and NS3-4A serine protease (35).

Enzymatically active viral proteases are essential for productive infection in the chimpanzee model (36). Autocatalytic cleavage at the NS2/3 site is carried out by the flanking protein domains, initially believed to function as a metalloprotease, but whose structural motifs are more indicative of a zinc-stabilized cysteine protease. All other principal cleavage sites within the HCV nonstructural region are hydrolyzed by a serine protease embodied in the N-terminal domain of the NS3 protein. (There is some evidence for further processing within the NS3 region by a cellular protease, which may contribute to proposed nonenzymatic functions of various NS3 domains discussed below.) Importantly, the stability and full activity of the NS3 protease requires association with the NS4A polypeptide, and the enzyme-active site may be subject to steric inhibition by the C-terminus of the NS3 protein. These properties, in addition to the known substrate specificity and crystal structure of the NS3/4A complex, will be important considerations for the design of HCV protease inhibitors.

The structural proteins include core, E1, E2, and p7. Core is the highly conserved viral capsid protein, which has been shown to play a role in the regulation of gene transcription, apoptosis, alteration of IFN signaling, cell transformation, and interference of lipid metabolism, all of which may give a survival advantage to the virus. The E1/E2 proteins are glycoproteins present on the viral surface and are potential targets for vaccine development. However, mutations in the hypervariable E2 region have posed problems for the development of effective neutralizing antibodies. It has also been shown that E1/E2 proteins can form pseudoviral particles capable of eliciting antibody responses. The p7 protein has been identified as an ion channel which appears to be essential in the viral life cycle as chimpanzee studies have shown that viruses with p7 mutations were unable to establish infections in these animals (33).

The remainder of the proteins are nonstructural proteins. NS2 is a cysteine protease which cleaves NS2/3 and has been shown to be involved in cell apoptosis and transcription modulation (33). NS3 is a bifunctional protein and has serine protease as well as RNA helicase activities. Fused NS3/4A is responsible for most of the nonstructural protein processing as stated above; it has also been shown to interfere with host innate immune response. NS4A has additional activity in that it targets NS3 to the ER via its N-terminal hydrophobic
amino acid stretch. NS4B is an integral membrane protein localized to the endoplasmic reticulum and plays a pivotal role in HCV replication (35). NS5A is a membrane-associated protein which interacts with other proteins and viral RNAs and has a role in cell transformation, transcription regulation, and apoptosis, as well has the suppression of IFN-induced antiviral efficacy. It has been found in association with p53, and its expression perturbs the Ras-ERK mitogenic signaling pathway as well as interferes with the function of the activator protein-1 (AP-1), a transcription factor involved in the activation of immediate-early genes (35). NS5B is an RNA-dependent RNA polymerase responsible for viral replication. It has been shown to associate with cyclophilin B resulting in enhanced RNA binding activity, and interestingly cyclosporine can inhibit HCV through this mechanism (33).

3.5. Immune System Evasion

Once synthesized, the various HCV proteins are subject to host-mediated proteolytic degradation and presentation on the cell surface as foreign antigens. Epitopes from all HCV proteins can be presented by multiple MHC Class I and II haplotypes and are susceptible to recognition by immunoglobulins and T-cell receptors (37). In acute and IFN-resolved cases, humoral, CD4+ T cell, and CD8+ CTL responses are directed against all viral antigens and are especially vigorous against epitopes on core and NS3. In comparison to HBV, viral antigen expression is relatively low in hepatitis C; but, unlike some other viruses, HCV does not appear to actively subvert antigen processing and/or presentation. To the contrary, the expression of molecules which mediate antigen recognition, such as MHC, intracellular adhesion molecules, TNF-α, and Fas antigen, is upregulated in HCV-infected cells. In chronic infection, the HCV-specific CTL response is effective enough to maintain some control over viral load; but, the majority of patients develop only a modest humoral response and are unable to establish a robust CTL activity sufficient to achieve clearance.

How HCV is able to persist in the face of an active, concerted immune response is unclear. The lack of a virus-associated reverse transcriptase activity precludes integration into the host genome. Given the quasispecies nature of HCV infection, it is likely that “escape mutation” arises from nucleotide misincorporation by NS5B RNA-dependent RNA polymerase activity. It has long been presumed that sequence heterogeneity in immunodominant epitopes allows immune-mediated selection of resistant quasispecies. There is some evidence that selection of humoral escape variants can occur during the course of HCV
infection in humans and chimpanzees. The principal neutralization epitope, hypervariable region-1 (HVR1, a 27–30-residue amino acid sequence located in the E2 N-terminus), exhibits extreme variation among known isolates. Quasispecies with mutated HVR1 have been shown to arise in response to antibody selection against the parent sequence. It is also possible that mutations modify a dominant CTL epitope, as has been observed in chimpanzee acute infection. Mutation may convert viral polypeptides to potent antagonists of CTL directed against the wild-type sequence. This scenario has been observed with an NS3 epitope as well as an NS5B epitope, resulting in persistent infection (38, 39).

The failure to clear HCV infection could conceivably result from a quantitative overwhelming of the T-cell response. The kinetics of viral replication may yield an insurmountably high ratio of infected hepatocytes to effector T cells. High viral load may also induce peripheral tolerance or T-cell “anergy,” wherein HCV-specific CTLs, activated in secondary lymphoid organs, are subsequently inactivated in the liver, because the viral antigen is presented in the absence of requisite costimulatory signals. HCV may exacerbate this scenario from within the host cell by debilitating the host cell response to cytotoxic mediators or other cytokines.

3.6. Inhibition of the Intracellular Antiviral Response

HCV is recognized by innate virus-sensing mechanisms and induces a rapid interferon (IFN) response. Several lines of evidence suggest that HCV-infected cells are deficient in various aspects of IFN induction or response. This may allow the virus to persist in the face of IFN-based antiviral therapy. Several HCV-encoded proteins actively subvert the intracellular antiviral response. NS3-4A protease acts on cellular sensors of viral RNA to block the initial induction of IFN-β (38). NS5A and E2 are known to inhibit the double-stranded RNA-activated protein kinase (PKR), an effector of the IFN-α pathway. Activation of PKR by double-stranded RNA substrates, such as viral RNA replication intermediates, causes phosphorylation of eukaryotic initiation factor 2, thereby shutting down translation of any host genes which might aid in viral replication. Binding by NS5A is thought to block dimerization of PKR, preventing its activation. The sequence of a relatively conserved 40 amino acid region within the putative PKR-binding domain of NS5A (the “interferon sensitivity-determining region,” ISDR) was previously shown to correlate with resistance to interferon treatment for Japanese patients infected with certain HCV genotypes. However, the correlation is weak or nonexistent when the analysis is extended to
other geographic regions or genotypes, and the selection of resistant ISDR sequences during the course of interferon treatment does not appear to be common. The NS5A protein may disrupt the interferon response in other ways, as the C-terminal region exhibits transcriptional activation properties and bears a nuclear localization signal. Although these functions are not evident in the full-length protein, liberation of the C-terminus by proteolytic processing raises the possibility that NS5A might influence the expression of other host genes involved in the antiviral immune response.

HCV core protein is known to inhibit apoptosis in various cell types. Although results from some experimental systems suggest that core actually sensitizes cells to TNF or Fas-mediated apoptosis, it is possible that this viral protein modulates intracellular signal transduction pathways so as to abrogate cell death signals. The interaction of core protein with the lymphotoxin-β receptor and TNF receptor-1 and concomitant effects on intracellular signaling have been demonstrated in vitro. It has been proposed that in this way, HCV disrupts host nuclear factor-κB-mediated signaling, so as to confer a survival advantage to infected hepatocytes or to prevent activation of B cells.

The NS3 protein may disrupt signal transduction pathways governing cell death and proliferation. The helicase domain includes a short amino acid sequence similar to autophosphorylation and inhibitor regulatory sites of cyclic AMP-dependent protein kinase (PKA). Polypeptides bearing this NS3 sequence bind to the PKA catalytic subunit, inhibiting its nuclear translocation and phosphorylation of host cell substrates. The NS3 protease domain suppresses actinomycin D-induced apoptosis in some cell types, apparently by sequestering or decreasing the expression of p53.

### 3.7. HCV Proteins and Hepatocarcinogenesis

In recent years, the effects of hepatitis C viral proteins on hepatocarcinogenesis have undergone intense investigation (40). The antiapoptotic effects of viral proteins, in addition to increased cellular turnover resulting from chronic inflammation, may contribute to the development of HCV-associated HCC (41). The prevalent HCC incidence in HCV carriers, estimated at 20%, suggests an important role for the virus in hepatocarcinogenesis. HCV proteins are almost invariably expressed in these tumors, and some chronically infected patients develop HCC even in the absence of cirrhosis.

The potentially oncogenic proteins implicated in HCC development in HCV-infected patients include core protein, NS3, and NS5A. Researchers have found relationships between subcellular localization,
concentration, specific molecular forms of the proteins, the presence of specific domains, and their effects on pathways leading to onco-
genesis. Each of the proteins has been associated with control of the cell cycle via interaction with p53, p21, cyclins, and proliferat-
ing cell nuclear antigen. Interactions with transcription factors, proto-
oncogenes, growth factors/cytokines and their receptors, and proteins related to apoptotic processes have also been described (40).

The involvement of the core protein in hepatocarcinogenesis is
probably the most recognized. Although the C-terminal hydrophobic
region of full-length HCV core renders the polypeptide membrane-
associated and localized to cytoplasmic membranes, the N-terminus
bears a nuclear localization signal. Some in vitro expression studies
suggest that core, and particularly its C-terminally truncated forms, can
be phosphorylated by protein kinases A and C and translocated to the
nucleus. Core may exhibit transcriptional regulatory activity on cellu-
lar promoters, so as to induce host cell proliferation. Accordingly, core
has been shown to activate the c-myc promoter and to suppress tran-
scription of c-fos, p53, Rb, and β-IFN. Additionally, it may interact
with heterogeneous nuclear ribonucleoprotein K, so as to depress the
human thymidine kinase gene promoter. In coexpression studies with
H-ras oncogene, core contributed to the tumorigenic transformation of
primary rodent fibroblast cells. HCC development reportedly occurs in
transgenic mice expressing core, and C-terminal mutations in the core
coding sequence have been observed in HCV RNA isolated from human
HCC tissue.

The serine protease domain of NS3 has also been reported to trans-
form murine cells and to induce HCC in transgenics. In HCV RNA
replicon-harboring cells, physical interaction between NS3 and p53 was
observed and p53-mediated transcriptional activation was suppressed
compared with HCV RNA-negative control cells (42).

Overexpression of HCV NS5A protein in cultured liver cells was
found to promote chromosome instability and aneuploidy. It was also
found to be associated with aberrant mitotic regulations such as a delay
in mitotic exit and other mitotic impairments (43). In addition to the
possible direct oncogenic effects of these proteins, neoplastic transfor-
mation likely arises as an inevitable consequence of chronic liver cell
proliferation, a condition which may be deliberately induced by HCV
in order to maximize viral replication.

3.8. RNA Replication

The RNA polymerase activity responsible for HCV RNA synthesis
is encoded in the NS5B region. This viral protein is sufficient for the
synthesis of full-length HCV RNA in vitro, although many host proteins are known to bind HCV 3'-terminal sequences and may influence template specificity in vivo. The HCV 3'-UTR contains a polypyrimidine (U/UC) sequence of variable length and terminates in a highly conserved 98-nt sequence, the “X region.” X is nearly identical in every isolate analyzed thus far, consistent with its essential role in replication. Deletion of X, or of the polypyrimidine sequence immediately upstream, renders RNA noninfectious in the chimpanzee model. Although the negative-strand 3'-terminus lacks a large polypyrimidine tract, the terminal 239 nt has been shown to function in vitro as a template for NS5B RDRP activity.

In vitro NS5B assays indicate that HCV RNA synthesis is initiated by a primer-independent de novo mechanism. Notably the 3'-terminal 45 nt of the positive strand fold into a thermodynamically stable stem-loop structure, which may require unwinding by an RNA helicase in order for the polymerase to gain access. Accordingly, NS3, found to colocalize with NS5B, bears an NTPase/helicase activity in its C-terminal domain. NS3 helicase binds HCV UTR sequences, unwinds RNA duplexes in a 3' to 5' direction and is essential for infection in the chimpanzee model (36). The helicase is thought to dissociate nascent complementary strands from the HCV RNA template, although NS3 activity has not yet been demonstrated in the context of viral RNA synthesis.

The C-terminal hydrophobic sequence of NS5B is necessary for its localization into membrane-associated complexes in the perinuclear region, the proposed site of HCV replication. A specific membrane alteration termed the membranous web was identified as the site of RNA replication. It is hypothesized that this web is derived from ER membranes (31). These complexes are thought to form by noncovalent protein–protein linkage of all HCV NS proteins with the exception of NS2, in association with one or more host-encoded factors. These protein aggregates are tethered to ER membranes by the NS4A peptide. Dissociation of replication complexes, or disruption of their anchorage to membranes, presents possible means to inhibit RNA replication. Also, the recently solved crystal structures of NS5B and the NS3 helicase domain will facilitate the rational design of inhibitors.

HCV RNA replication appears to be influenced in subtle ways by the NS5A protein. Two forms of NS5A, differing in the extent of serine phosphorylation, are generally present within infected cells. This phosphorylation may serve to regulate NS5A subcellular localization and putative transcriptional regulatory activity. However, the utility of NS5A hyperphosphorylation and the identity of the kinase responsible for it remain to be determined. Interestingly, mutation of serine
phosphorylation sites, or deletion of the ISDR, greatly enhances replication of subgenomic HCV replicons in cell culture (44).

Like the HBV polymerase, HCV NS5B lacks proofreading activity and exhibits a high rate of nucleotide misincorporation. To some degree, HCV may avoid replicating lethally mutated genomes by utilizing only the genomic RNA template from which NS5B is translated. Still, it is thought that the majority of serum HCV RNA is replication-defective, bearing one of more mutations prohibitive of viral replication. Because its coding sequence is nonoverlapping, HCV presumably exhibits a higher tolerance for mutation than HBV. Therefore, HCV escape variants may present an even greater obstacle for therapy than the HBV mutants. The oral nucleoside analogue, ribavirin, has shown promise as an inhibitor of HCV replication; but, its effects on HCV RNA levels are generally transient.

3.9. Assembly and Secretion

The details of HCV virion assembly are very poorly understood at present. But, the recent development of cell culture-based systems for the production of virus-like particles may soon shed light on the relevant mechanisms. Although core protein oligomerization and specific interaction with E1 have been demonstrated, core exhibits only nonspecific RNA binding. Core may preferentially bind positive-strand RNA; but, no discrete encapsidation signal is apparent. By analogy to HBV pregenomic RNA, which binds to both of its gene products to facilitate packaging and replication, the HCV-positive strand may bind core and NS5B cotranslationally. Binding to core might suppress IRES activity, leading to a transition from replication to assembly. Another event which may contribute to virion formation is the C-terminal proteolytic processing of the core and E2 proteins. At the time of translation, the cleavage between E2 proper and p7 is incomplete, giving rise to two forms of the envelope protein. An as yet unidentified protease, possibly signal peptide peptidase, is believed to trim the E1 signal sequence from the core C-terminus posttranslationally.

The E2 C-terminal domain acts as an ER retention signal; the glycoproteins are not translocated through the Golgi apparatus to the cell surface unless incorporated into virion envelopes. Therefore, cytoplasmic HCV core-encapsidated RNA particles are thought to bud through the ER membrane, rather than the cell membrane. In so doing, they acquire a coating of E1-E2 heterodimers. The complete virions progress through the Golgi apparatus and are released into the extracellular space by exocytosis. Interestingly, transgenic mice expressing HCV E1 and E2 exhibit salivary and lachrymal exocrinopathy resembling Sjogren’s syndrome, suggesting that these proteins may
disrupt cellular exocytosis and contribute to the pathogenesis of this disorder in chronically infected patients.

It is estimated that a typical infected liver releases $\sim 10^{12}$ HCV virions per day. Assuming 10% of hepatocytes are infected, this amounts to 50 particles per cell per day. Hepatocytes generally maintain a low level of intracellular RNA; approx. $3 \times 10^{11}$ RNA molecules are produced daily. Thus, as with HBV, there is evidence that a substantial portion of released particles are replication-defective virions, or naked core structures. With a virion half-life of just 4–7 h, most patients have an understandably low serum titer.

4. CONCLUSION: FUTURE TREATMENT STRATEGIES

It is apparent from the above discussion that, given the compactness of HBV and HCV genomes, conserved sites exhibiting multiple essential functions can be readily identified. It may be possible to design single inhibitors, or therapeutic regimes, which target these important viral functions simultaneously. Our expanding knowledge of hepatitis virus molecular biology has provided a wealth of novel strategies to achieve maximal genomic coding capacity, persistence of infection, and immune system evasion. Application of these concepts may be useful for the design of gene therapy constructs, perhaps even hepatotropic vectors based on HBV-or HCV-derived replicons.

ACKNOWLEDGMENTS

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REFERENCES


Epidemiology, Risk Factors, and Natural History of Chronic Hepatitis C

Nalini K. Sharma, MD and Averell H. Sherker, MD FRCP (C)

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EPIDEMIOLOGY
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Key Principles

- Approximately 3% of the world’s population is infected with the hepatitis C virus (HCV) with the highest prevalence rates noted in Africa and Asia.
- In the United States, the incidence of HCV infection is declining secondary to effective blood donor screening adopted in the early 1990s and changing practices of intravenous drug users due to an increased awareness of HIV and hepatitis.
- Hepatitis C can be categorized into six genotypes and 50+ sub-types. Genotypes 1a and 1b are the most common, accounting for about 60% of global infections.

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- Hepatitis C is transmitted primarily via parenteral routes. Mucosal exposures to blood or serum-derived fluids and environmental sources also play a role in HCV transmission.
- Controversy exists regarding the natural history of hepatitis C. While many experts view hepatitis C as a progressive disease with a high likelihood of advancing to cirrhosis, hepatocellular carcinoma, and death, others consider this virus to be more variable in nature, with the majority of patients “dying with the disease, not of the disease.” The CDC estimates that up to 20% of chronic HCV infections lead to cirrhosis over a period of 20–30 years.
- Several factors influence the rate of progression of hepatitis C. Progression is hastened when acquired via blood transfusion; at an older age; in males; in non-African-Americans; in the setting of excessive alcohol (and possibly tobacco) use; and, in persons with HIV and/or HBV coinfection or features of metabolic syndrome. It is controversial if HCV viral load and genotype affect the evolution of disease.

1. EPIDEMIOLOGY

1.1. Global Prevalence and Incidence

The World Health Organization (WHO) reports that approximately 3% of the world population, or 170 million persons, is infected with the hepatitis C virus (HCV) with between 3 and 4 millions new infections each year (1). Limitations of global HCV epidemiological data include lack of data submission from numerous countries, variations in the population groups studied, and differing methods of data collection and interpretation. The majority of studies extrapolate data from blood donors alone, which is not representative of the entire population and is likely an underestimate.

Figure 1 illustrates the global prevalence of HCV. Africa and Asia have the highest reported prevalence rates, in contrast to the low rates of HCV in North America, Western Europe, and Australia. Egypt has an unusually high prevalence of hepatitis C even by the standards of other developing nations with 20% of Egyptian blood donors positive for HCV antibody. Previous parenteral therapy for Schistosomiasis with inadequate sterilization techniques has been implicated as the cause for this high prevalence rate (2). Similar epidemiological events explain other countries’ spike in infection rates. For example, Italy’s HCV prevalence rate of 12.6% is attributed to the improper use of glass
Fig. 1. Global prevalence of hepatitis C. Source: WHO, 2003.

syringes for administration of the Salk vaccine to prevent polio in the 1960s (3). Japan’s prevalence rate of 2.6% is thought to be secondary to the use of injection methamphetamines in the 1950s (WHO).

1.2. United States Prevalence and Incidence

HCV is the most common bloodborne infection in the United States. The estimated prevalence of HCV seropositivity in the United States general population is 1.6% or 4.1 million persons. Of those who are anti-HCV positive, 79.7%, or 3.2 million persons, have chronic infection. The prevalence is highest among non-Hispanic black males between 40 and 49 years of age. This data is derived from the most recent National Center for Health Statistics (NHANES) survey (1999–2002) of 15,079 random participants whose serum samples were tested for HCV antibody and HCV RNA (4). This information is consistent with results from NHANES III (1988–1994), except that peak prevalence data have shifted from ages 30–49 to 40–49 (representing similar birth-year cohorts) (5). Figure 2 illustrates this data.

A drawback to analyzing the NHANES population is the exclusion of incarcerated, institutionalized, and homeless persons. The Centers for Disease Control (CDC) estimates that if the incarcerated population is included, 3.5 million persons have chronic HCV infection (6). Cheung et al. (7) performed a retrospective analysis of homeless veterans admitted to a domiciliary facility at the VA Palo Alto Health Care
System and found a 41.7% prevalence of HCV seropositivity, suggesting that homeless persons represent a significant pool of chronic HCV infection.

Studies focusing on specific populations have shown varying prevalence rates. For example, Dominitz et al. (8) reported a prevalence rate of 4.0% among a Department of Veteran Affairs (VA) patient population randomly selected from 20 VA centers. This is lower than prior estimates that have ranged from 6.6 to 35% (9–11). Among 10,000 active duty military personnel, Hyams et al. (12) found an overall HCV seropositivity prevalence rate of 0.48%; this rate was lower (0.1%) in persons aged lesser than 30 as compared to those aged greater than 40 (3.0%). The lower prevalence among active duty members compared to the general VA population can be attributed to mandatory illicit drug testing throughout military service since 1971. Recently, Tabibian et al. (13) reported a seropositivity prevalence of 38% among 129 veterans in a psychiatric ward. Among health-care workers at Johns Hopkins Hospital, a prevalence of 0.7% was reported, comparable to that observed in local blood donors (14).

In the United States, the incidence of HCV infection is declining secondary to effective blood donor screening adopted in the early 1990s and changing practices of intravenous drug users due to an increased...
Fig. 3. Incidence of acute hepatitis C.

awareness of HIV and hepatitis. This decline is illustrated in Fig. 3 (15). The CDC estimates that while there were 180,000 new cases per year in the 1980s, this declined to 20,000 new cases in 2005 (15). This data is based on cases reported voluntarily to the CDC by state and territorial health departments via the National Electronic Telecommunications System for Surveillance (NETSS). Figure 3 illustrates the declining incidence of acute hepatitis C.

1.3. Genotype

Hepatitis C can be categorized into six genotypes and 50+ subtypes. It is important to note an infected person’s specific genotype as it affects treatment dose, duration, and response. NHANES III reported that 73.7% of the US population is infected with genotype 1 (5).

Genotypes 1–3 have a worldwide distribution. Genotypes 1a and 1b are the most common, accounting for about 60% of global infections. They predominate in Northern, Southern, and Eastern Europe; North America; and Japan. Genotype 2 is less frequently represented than genotype 1 and is often associated with the risk factor of prior blood transfusion (16). Type 3 is common in Southeast Asia and is variably distributed in different countries. In Western countries, it is often associated with a history of illicit drug use (16, 17). Genotype 4 is principally
found in the Middle East, Egypt, North and Central Africa. Type 5 is found almost exclusively in South Africa, and genotype 6 is distributed throughout Asia (39, 58, 94, 103) (Table 1).

<table>
<thead>
<tr>
<th>Country</th>
<th>Predominant genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States (5)</td>
<td>1a, 1b</td>
</tr>
<tr>
<td>Egypt (2)</td>
<td>4</td>
</tr>
<tr>
<td>England (171, 172)</td>
<td>3</td>
</tr>
<tr>
<td>Chile (37, 173)</td>
<td>1b</td>
</tr>
<tr>
<td>Japan (174, 175)</td>
<td>1b</td>
</tr>
<tr>
<td>China (176)</td>
<td>2</td>
</tr>
<tr>
<td>Thailand (177)</td>
<td>3a</td>
</tr>
<tr>
<td>India (178, 179)</td>
<td>3</td>
</tr>
<tr>
<td>Saudi Arabia (180)</td>
<td>4</td>
</tr>
</tbody>
</table>

2. RISK FACTORS

Hepatitis C is transmitted primarily via parenteral routes. Mucosal exposures to blood or serum-derived fluids and environmental sources also play a role in HCV transmission. Currently the predominant risk factor for HCV in the United States is intravenous drug use. Prior to 1990, blood transfusions contributed the majority of cases; however, with the adoption of effective blood donor screening programs and eventually universal serological screening, this method of transmission has been virtually eliminated. According to a large case–control study of US blood donors, injection drug use, history of blood transfusion, and sex with an injection drug user are the three commonest risk factors for HCV, whereas drug inhalation and high-risk sexual activity are not independently associated with hepatitis C (18). NHANES III reported an association between HCV and marijuana and/or cocaine use, in addition to high-risk sexual behavior (5). Dominitz et al. conducted a study involving veterans with hepatitis C at 20 Veteran Affairs medical centers and found that 78% reported intravenous drug use or blood transfusion as a risk factor. Among patients with HCV infection, 20–40% of patients do not have an identified risk factor for transmission. Karmochkine et al. surveyed 450 HCV patients without a history
of intravenous drug use or blood transfusion and 757 anti-HCV-negative controls. Multivariate analysis illustrated 15 independent risk factors for transmission including gastrointestinal endoscopy, intravenous or intramuscular injections, varicose vein sclerotherapy, acupuncture, contact sports, beauty treatments, and intranasal cocaine use (19). Kamili et al. conducted an experimental study that showed that HCV in blood is infectious even after exposure to drying and storage at room temperature (20). This study supports the notion that environmental sources are a potential route for HCV transmission.

In 1998, the CDC recommended routine screening in persons at high risk for HCV infection (21). This recommendation was also endorsed by the National Institutes of Health (22) and Veterans Administration (VA) (23). In 2004, the US Preventive Services Task Force (USPSTF) recommended against screening for HCV in the general population and found insufficient evidence to advocate HCV screening of high-risk patients (24). Drs. Alter, Seeff and colleagues responded to the USPSTF recommendations by stating that HCV screening for high-risk patients is necessary to provide infected persons with counseling and potential treatment (25). Mallette et al. studied the clinical characteristics and outcomes of HCV patients diagnosed via a screening program based on risk factors. Results showed that 7.3% of patients who underwent screening were anti-HCV positive and 4.3% were viremic. Of the patients who tested positive for anti-HCV, 47% were considered treatment candidates. This study supports a policy of HCV screening for persons with identified risk factors to provide appropriate counseling and identify candidates for hepatitis A and B vaccination as well as antiviral therapy (Table 2 and Fig. 4).

![Risk factors for HCV](image)

**Fig. 4.** Risk factors for HCV.
<table>
<thead>
<tr>
<th>Testing recommended</th>
<th>Uncertain need for testing</th>
<th>Testing not recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current and former injection drug users</td>
<td>Recipients of transplanted tissue</td>
<td>Health care workers</td>
</tr>
<tr>
<td>Recipients of clotting factors before 1987</td>
<td>Intranasal cocaine users</td>
<td>Pregnant women</td>
</tr>
<tr>
<td>Hemodialysis patients</td>
<td>Persons with tattoos</td>
<td>Household contacts</td>
</tr>
<tr>
<td>Recipients of blood and/or solid organs before 1992</td>
<td>Persons with body piercings</td>
<td>General population</td>
</tr>
<tr>
<td>Persons with undiagnosed liver test abnormalities</td>
<td>Persons with multiple sexual partners/STDs</td>
<td></td>
</tr>
<tr>
<td>Infants born to infected mothers after 12–18 months</td>
<td>Long-term monogamous relationship with person infected with HCV</td>
<td></td>
</tr>
<tr>
<td>Health care/public safety workers with a known exposure</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.1. Intravenous Drug Use

Currently, the majority of cases of HCV transmission can be attributed to intravenous drug use through the sharing of needles, syringes, and other paraphernalia (26–28). The CDC estimates that intravenous drug use accounts for up to 60% of new cases of HCV. The annual incidence of HCV transmission among intravenous drug users ranges from 15 to over 30% (29). Furthermore, the CDC reports that 60–80% of persons who have used intravenous drugs for 5 or more years are infected. Villano et al. conducted a prospective study of active intravenous drug users from Baltimore, MD and found that 30.3% participants became anti-HCV positive, mostly within the first 2 years of surveillance (30). Similarly, the Hepatitis C European Network for
Cooperative Research (HENCORE) group reported an 80% prevalence rate among intravenous drug users (31).

2.2. Blood Products

The CDC reports that prior to 1989, blood transfusions were associated with a 7–10% risk of HCV transmission. As a result of the routine implementation of sensitive anti-HCV serological testing in 1992, the risk of hepatitis C acquisition via blood transfusion is currently 0.001% per unit transfused (32) as compared to 0.02% per unit transfused prior to effective blood donor screening (33). This minimal risk is attributed to the potential for transmission during the window period of infection, which occurs after transmission, but prior to detectable HCV antibody. The introduction of nucleic acid testing will further reduce this risk (34, 35). There have been no transfusion-associated HCV cases in the United States since 1992 among an NIH blood recipient population (28, 36). Although transfusion-associated HCV has declined in the United States, the incidence still remains high in certain parts of the world. In a recent study, 147 Chilean patients with chronic HCV were interviewed and blood transfusion was found to be the most common risk factor associated with infection: 54% of HCV-infected persons gave a history of blood transfusion, while only 5% had a history of intravenous drug users (37).

Prior to the implementation of procedures to inactivate viruses from pooled plasma in 1987, persons who received clotting factor concentrates were at high risk of HCV transmission. In fact, prevalence rates in the setting of hemophilia were as high as 90% (38, 39). An outbreak of HCV occurred among recipients of intravenous immune globulin that was not inactivated for viruses in 1993 (40, 41). Although administration of intramuscular immune globulin has not been implicated as a risk factor for HCV transmission, inactivation of viruses is now required for both intravenous and intramuscular immune globulin in the United States (21). Two large outbreaks of HCV were linked to contaminated anti-rhesus D preparations given to female recipients during childbirth (42, 43).

2.3. High-Risk Sexual Activity

Several case reports and studies have provided evidence that sexual activity is independently associated with transmission of HCV (44-47). Rates of transmission of hepatitis C are lower than those of hepatitis B and HIV (48, 49). CDC surveillance data in 2005 indicated that 15–20% of persons with acute HCV infection reported a history of sexual exposure as the only identifiable risk factor. Persons with a history
of sexually transmitted diseases, multiple sexual partners, vigorous sexual practices, and male homosexual activity are at greatest risk of HCV acquisition. Among heterosexuals, male to female transmission is more efficient than female to male transmission (50). On the contrary, several case–control studies did not find a link between acquisition of hepatitis C and either homosexual activity (44, 45, 51) or sexual promiscuity (51, 52). In the NHANES III study, the number of sexual partners and age of first sexual intercourse correlated with HCV seroconversion. Thomas et al. studied 1257 persons who denied intravenous drug use at an STD clinic in Baltimore, MD, and found that 9.7% were positive for anti-HCV (53). Alter postulated that HCV is more likely to be transmitted via sexual intercourse in the setting of acute infection, high viral load, and a lack of antibody to complex with antigen (26). Several studies have found an increased risk of HCV transmission among heterosexual partners in the setting of coinfection with HIV (49, 54). Terrault reports that among high-risk individuals with hepatitis C, there is a 1% annual risk of HCV transmission (50) (Table 3).

### 2.4. Intrafamilial and Monogamous Sexual Transmission

Intrafamilial transmission of hepatitis C has been reported (55). Oshita et al. performed a study of 219 family members including spouses, children, and others who lived with 99 HCV-infected individuals. These family members were tested for ALT, anti-HCV +/− HCV RNA PCR and compared to a control population with similar demographic characteristics. Results showed that 26 family members (12%) tested positive for anti-HCV, specifically 18/75 (24%) spouses, 5/110 (5%) children, and 3/34 (9%) others, compared to 6 (2%) persons in the control group. These rates were similar to previous studies (56, 57). Of the family members with HCV seropositivity, 81% were HCV RNA positive. The higher rate of HCV seropositivity among spouses could have been secondary to sexual transmission. The rate of anti-HCV positivity increased with age and decades of marriage (55). More recently, a systematic review was performed to examine the risk of HCV transmission between infected patients and household contacts and stable heterosexual partners. The prevalence of HCV seropositivity among 4250 stable sexual contacts of patients with hepatitis C was 13.48%. Of 580 sexual contacts of patients who had acquired HCV via blood transfusions, the rate of HCV seropositivity was lower at 2.41%. Among household contacts, there was a 4.0% risk of transmission compared to 0% in contacts of anti-HCV-negative controls (58). Diago et al. reported an overall 4.5% prevalence of anti-HCV among household contacts with 30/394 (7.6%) of heterosexual stable partners and 35/1057 (3.3%)
### Table 3

Reported Rates of Chronicity of HCV Infection

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient population</th>
<th>Mode of transmission</th>
<th>% Chronic infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas et al., 2000 (93)</td>
<td>919 persons with a history of intravenous drug use</td>
<td>Intravenous drug use</td>
<td>90</td>
</tr>
<tr>
<td>Alter HJ, 1997 (28)</td>
<td>248 asymptomatic blood donors</td>
<td>Blood transfusion, intranasal drug use, intravenous drug use (50%), sexual contact, male ear piercing</td>
<td>86</td>
</tr>
<tr>
<td>Villano et al., 1999 (94)</td>
<td>43 persons with acute HCV</td>
<td>Intravenous drug use</td>
<td>86</td>
</tr>
<tr>
<td>Seeff et al., 2001 (96)</td>
<td>90 blood recipients</td>
<td>Blood transfusion</td>
<td>77</td>
</tr>
<tr>
<td>Seeff et al., 2000 (95)</td>
<td>17 military recruits with stored blood samples from 1948–1954</td>
<td>Unknown</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>62</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td>54</td>
</tr>
</tbody>
</table>

of nonsexual contacts affected. Although the prevalence of anti-HCV among children of HCV-infected individuals was lower than other household contacts, the risk was higher when the mother was the index case (3%) as compared to the father (0.6%). Again, an increased prevalence was seen with increasing age (59). Saliva has also been cited as a possible route of HCV transmission (60, 61).
On the other hand, Caporaso et al. reported that after adjustment for confounders, sexual transmission does not play a role in intrafamilial spread of HCV infection (62). In addition, Stroffolini et al. found HCV transmission between spouses is likely secondary to parenteral exposures, and less likely sexual transmission (63). Kao et al. performed a prospective study of 112 anti-HCV-positive patients and their anti-HCV-negative spouses. There was one seroconversion occurrence at 2 years over a follow-up period of 45.9 months. The annual interspousal transmission of HCV was 0.23%, with the possibility of a cumulative risk (64). Vandelli et al. reported the results of a prospective study in which 7879 HCV-infected persons and their anti-HCV-negative spouses were followed for 10 years. Three HCV seroconversions were observed for an incidence rate of 0.37 per 1000 person-years (65). Terrault reports a 0–0.6% annual risk of transmission and a 2.7% prevalence rate among monogamous partners of patients with hepatitis C (49). The National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) reports an annual transmission rate of <1% per year and does not recommend a change in sexual practices among monogamous couples.

Tahan et al. were the first to quantify HCV seroconversions in anti-HCV-negative spouses of HCV-infected persons in terms of sexual encounters. Six hundred persons with spouses who were anti-HCV positive were tested for anti-HCV. Twelve (2%) persons were anti-HCV positive and 11 were HCV-RNA positive. Persons positive for anti-HCV engaged in a greater number of sexual encounters (434 ± 295 vs. 307 ± 333). This population was followed prospectively for 3 years. There was no evidence of new seroconversion for 3 years and 257.9 ± 72.2 sexual occurrences (66).

Collectively, this data suggests that both household contacts and sexual partners may be at increased risk – albeit small – of transmission of hepatitis C, with sexual contacts being at higher risk. Terrault advocates that family members should not share items that may be contaminated by blood (50).

### 2.5. Mother to Infant Transmission

Vertical transmission is a known risk factor for HCV infection. Coinfection with HIV and increased viremia are thought to contribute to an even greater risk. Ferrero et al. prospectively studied 170 anti-HCV-positive women and their 188 newborn babies to determine the rate of vertical transmission. Babies were followed until clearance of anti-HCV or diagnosis of HCV infection. Results revealed a 2.0% rate of HCV transmission among HIV-negative women, which increased to 5.4% with HIV coinfection. Increased maternal viremia was associ-
ated with an increased risk of transmission. With regard to clearance of anti-HCV among noninfected infants, babies born to viremic mothers have a slower rate of clearance of anti-HCV as compared to non-viremic mothers (67). Another prospective study of 86 infants born to anti-HCV-positive mothers showed similar results and also reported that HCV transmission is not affected by mother’s age, route of delivery, or genotype, but may be increased in the setting of active intravenous drug use and HCV/HIV coinfection (68). Hunt et al. reviewed studies from 1988 through 1996 and suggested that rates of vertical transmission may be higher among mothers with acute HCV infection as compared to chronic HCV infection (69). Although most studies do support an increased risk of mother to child transmission of HCV in the setting of HIV coinfection, Manzini et al. reported slower clearance of passively acquired anti-HCV among HIV-positive mothers, without an increase in vertical transmission of HCV (70). Airoldi has suggested that highly active antiretroviral therapy may significantly decrease the risk of vertical transmission of HCV (71).

2.6. Breastfeeding

Several studies (69, 72, 73) and the CDC report no evidence linking HCV transmission to breastfeeding; therefore, HCV infection is not a contraindication to breastfeeding. However, if a mother’s nipples are cracked and bleeding, she should temporarily refrain from breastfeeding as a precautionary measure (72). Kumar performed a prospective study of 65 HCV-RNA-positive women and 42 anti-HCV-negative controls to assess breastfeeding as a mode of HCV transmission. All infants studied were breastfed. Five mothers with chronic HCV developed symptomatic liver disease and three of their infants were found to be HCV-RNA positive during a 12-month follow-up period. The authors concluded that women with symptomatic chronic HCV and high viral loads should not breastfeed (74). In the setting of HCV/HIV coinfection, breastfeeding is discouraged (71).

2.7. Hemodialysis

Although hemodialysis is a well-known risk factor for HBV transmission, the CDC only recently cited it as a risk factor for HCV transmission. Recommendations for the prevention of hepatitis B in hemodialysis centers were initially published in 1977, with implementation of vaccination among patients and staff since 1982. These strategies led to a sharp decline in HBV transmission.

With regard to HCV, limited data from US studies since 1990 have reported a 3% annual incidence of HCV transmission in the setting of
hemodialysis without a history of intravenous drug use or blood trans-fusions (75, 76). In 1999, the national prevalence rate of anti-HCV was 8.9%, with a greater than 40% rate among some hemodialysis centers (unpublished CDC data, 2001) (77). Higher rates of anti-HCV seroconversion were seen among persons on hemodialysis for 5 years or more, with rates increasing from 12% among persons on hemodialysis for less than 5 years to 37% among persons on hemodialysis for 5 or more years (75, 78, 79). During 1999–2000, the CDC studied three HCV out-breaks in hemodialysis centers and found that transmission occurred because of inadequate infection control practices. Seroconversion was associated with patients receiving hemodialysis immediately following a patient with chronic HCV, use of equipment and supplies that were not disinfected between patient use, shared utilization of medication carts and medication vials, and blood spills that were not promptly cleaned.

In 2001, the CDC recommended the implementation of infection control practices specific to hemodialysis units, infection control training and education, and routine surveillance of hepatitis C for hemodialysis patients. Monthly ALT assessment and biannual anti-HCV anti-body testing were suggested as initial testing modalities, with HCV PCR reserved for those with elevated transaminases of unknown etiology. Anti-HCV testing was not recommended for hemodialysis staff members. In addition, isolation of patients with hepatitis C was not con-sidered necessary (77).

The above recommendations resulted in an increase in the num-ber of hemodialysis centers testing for HCV from 56 in 1999 to 64% in 2002. The prevalence rate of HCV decreased from 8.9 in 1999 to 7.8% in 2002. This decline in prevalence was attributed to a decline in new infections due to an increased awareness of HCV transmission in hemodialysis settings. In April 2008, the Kidney Diseases Improving Global Outcomes Foundation developed the first international clinical practice guidelines to address the prevention of hepatitis C in patients undergoing hemodialysis. Although several nephrology organizations recommend mandatory semiannual testing of anti-HCV in hemodialysis patients, the Centers for Medicare and Medicaid Services (CMS) do not reimburse providers for the cost of this testing unless there is a suspicion that the patient has been exposed to HCV-infected blood (80).

2.8. Intranasal Drug Use

Although several studies have reported that intranasal drug use is not independently associated with an increased risk of HCV transmission (81, 82), others have noted an association (28). The CDC has described it as a potential risk factor, primarily through sharing of pipes and
straws. The National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) states that intranasal cocaine use is associated with a *slightly* increased risk of hepatitis C. McMahon et al. reviewed the epidemiological evidence of HCV transmission in the setting of oral and intranasal drug use and found that although current studies do not dismiss these modes of transmission, further research is needed (83).

### 2.9. Tattoos

The concept of tattooing as an independent risk factor for HCV transmission is controversial with various studies relaying discordant results. Ko et al. studied 213 healthy young men who denied participation in intravenous drug use or high-risk sexual activity. Results revealed that 11/187 (12.6%) men with tattoos as opposed to 3/126 (2.4%) tattoo-free men were positive for anti-HCV. The risk of HCV seropositivity was higher with an increased number of tattoos and non-professional status of the tattooer (84). Hellard et al. conducted a survey and found tattooing during incarceration to be an independent risk factor for HCV transmission among a group of prisoners in Australian correctional facilities (85). Neumeister et al. studied the risk factors and prevalence of HCV among native Americans and found tattoos ≥ 5 years old to be a strong risk factor for transmission (86). Haley et al. reported a higher risk of HCV acquisition with commercial tattooing as compared to intravenous drug use (87). Haley also postulated that tattooing may be associated with subclinical HCV infection, while intravenous drug use may lead to an acute infection (88). The CDC acknowledges that although several studies have found an association between tattooing and HCV transmission in select populations, they may not pertain to the general population. As less than 1% of persons with hepatitis C reported a history of tattooing in the CDC’s surveillance system, the CDC believes that further studies are needed to determine if tattooing is a risk factor for HCV transmission.

### 2.10. Health Care Setting

Hauri et al. reported that up to 40% of worldwide HCV infections can be attributed to contaminated health care injections in developing countries (89). Contaminated glass syringes for Schistosomiasis treatment have been implicated as a mode of HCV transmission in Egypt (2), while multiple injections for kala-azar treatment have been associated with HCV seropositivity in India (90). The World Health Organization has found the highest rates of therapeutic needle reuse in Southeast Asia, Middle East, and the Western Pacific. In order to combat unsafe therapeutic injection practices, the WHO implemented the
Safe Injection Global Network (SIGN), a partnership of various governments, international health agencies, and corporations that advocate safer worldwide injection practices (27).

Regarding occupational transmission of HCV, health-care workers exposed to hollow-bore or deep tissue needle stick injuries from an HCV-positive source have a 1.8% risk of transmission, which is lower than that of HBV and HIV (26, 91). The prevalence of HCV infection among health-care workers is similar to the general population (26).

3. NATURAL HISTORY OF HCV

Effective investigation of the natural history of a disease requires specific information including the identification of disease onset, method of differentiating between acute and chronic disease, and the ability to track disease morbidity and mortality without the influence of comorbid conditions and/or treatment (92). With regard to hepatitis C, the onset of disease is rarely known and patients often have comorbid conditions and available treatment options that can alter the natural course of the infection.

Controversy exists regarding the natural history of hepatitis C. While many experts view hepatitis C as a progressive disease with a high likelihood of advancing to cirrhosis, hepatocellular carcinoma, and death; others consider this virus to be more variable in nature, with the majority of patients “dying with the disease, not of the disease.” These discordant opinions arise from the presence of variable types of studies that have been reported, including retrospective, prospective, and retrospective–prospective studies. Additionally, diverse patient populations with regard to host-related factors (age, gender, race, route of transmission, comorbidities) and virus-related factors (genotype, viral load, quasispecies) have been studied – often with discordant results.

3.1. Chronic Hepatitis C

Acute hepatitis C is rarely symptomatic, thus most patients are unaware of the fact that they even contracted the illness, unless they can identify a recognized exposure. Chronic hepatitis C is defined as the persistence of HCV RNA viremia for at least 6 months. Potential clinical outcomes for persons with chronic hepatitis C include cirrhosis, hepatocellular carcinoma, and death.

Progression from acute to chronic HCV occurs in 54–90% patients (28, 93–99). This variability is likely due to diverse study designs and patient populations. Thomas et al. reported that viral clearance was significantly more common in persons of non-black race and negative
HIV status. An association (although not statistically significant) was noted between viral clearance, female gender, age under 45 years, and coinfection with hepatitis B. There was no association between persistent viremia and weekly alcohol use or the frequency of intravenous drug use (93). In an analysis of 43 acute HCV infections, Villano and colleagues also reported that viral clearance was more common among non-black individuals, in addition to persons with jaundice and a lower peak viral load. Persons with undetectable viral load had no evidence of biochemical disease after clearance of infection. It should be noted, however, that persons with chronic hepatitis C often had normal transaminases at some point during the course of chronic infection (94). Rodger et al. compared HCV-RNA-positive persons with HCV-RNA-negative persons to determine factors linked to chronic infection. They found that a longer duration and increased frequency of intravenous drug use, as well as an increased average lifetime consumption of alcohol were inversely associated with clearance of infection (99). Vogt et al. found that younger age at time of infection may be related to viral clearance (98). This factor may have contributed to the relatively low rate of chronic HCV in the population studied by Rodger, as the mean age of the study subjects at the time of infection was less than 22 years (99).

3.2. Cirrhosis, Hepatocellular Cancer, and Liver-Related Death

While some studies show frequent progression of chronic HCV to cirrhosis, hepatocellular carcinoma, and death (95, 100–106), others report these outcomes as less common occurrences (28, 93, 98, 99, 107, 108). The CDC estimates that up to 20% of chronic HCV infections lead to cirrhosis over a period of 20–30 years. Poynard et al. performed a study using serial biopsies or a single biopsy with a presumed date of infection based on identified risk factors and reported that persons infected with HCV can be differentiated into three categories with regard to fibrosis progression without treatment: rapid fibrosers (33% of persons) who progress to cirrhosis in <20 years; intermediate fibrosers (36% of persons) who develop cirrhosis over 30 years; and slow fibrosers (31% of persons) who may progress to cirrhosis after >50 years of infection (109).

Numerous studies have been performed to evaluate risks of HCV-related morbidity and mortality. These evaluations are dependent on the type of study performed, host-related factors, and virus-related factors. This point is illustrated in a review by Freeman and colleagues, which analyzed 33 liver clinic series; 5 posttransfusion cohorts; 10 blood
donor cohorts; and 9 community-based cohorts. Development of cirrhosis after 20 years of infection was 22% for the liver clinic series; 24% for posttransfusion studies; 4% for blood donor studies; and 7% for community-based studies. Older age at the time of HCV infection, male gender, and heavy alcohol intake also correlated with a more rapid progression to cirrhosis (110). Limitations of studies on fibrosis progression include sample variability of liver biopsies, referral bias with inclusion of patients with severe liver disease, and inaccurate estimates of duration of infection.

3.2.1. STUDY DESIGN

Retrospective Studies

Initial studies of chronic hepatitis C natural history involved retrospective analysis of patients referred to academic centers. The advantage of these studies is that a long duration of infection can be studied and the roles of various factors can be evaluated. Disadvantages of these studies include an estimated date of exposure and a strong selection bias as this patient population is more likely to have clinically evident liver disease, resulting in referral to an academic liver center. Several retrospective studies have included patients with a presumed 20–30 years of infection and have shown that 20–51% of patients with chronic HCV progress to cirrhosis, 2–11% develop hepatocellular carcinoma, and 4–15% die of liver-related causes (100, 102–104). Bjoro and colleagues studied 17 Norwegian patients with primary hypogammaglobulinemia who contracted HCV infection as a result of transfusion with contaminated immune globulin from 1982 to 1986. Six patients had histological evidence of cirrhosis, and two went on to die of liver failure. The authors suggest that chronic HCV in the setting of primary hypogammaglobulinemia was associated with a greater morbidity and mortality when compared to a “healthy population” with chronic HCV (103).

- Tong and colleagues followed 131 patients who acquired HCV via blood transfusion, an average of 22 years prior to referral to a tertiary care center. Based on liver biopsy or abnormal coagulation parameters, 67 patients (51%) were cirrhotic. Upon entry into the study, seven patients (5.3%) had hepatocellular carcinoma, while an additional seven patients (5.3%) developed hepatocellular carcinoma during an average 4-year follow-up. Nineteen patients (14.5%) died of liver-related causes, including eight from cirrhosis complications and 11 from hepatocellular carcinoma. Persons who contracted HCV before age 50 developed cirrhosis and hepatocellular cancer at a slower rate as compared to persons who received blood transfusions after age 50 (100).
• Yano and colleagues from Japan studied liver biopsies of 70 patients with chronic HCV, acquired either sporadically or via blood transfusion. During follow-up, 35 patients (50%) developed histological evidence of cirrhosis.

• Niederau and colleagues followed 838 patients with a history of chronic HCV referred to an academic hepatology clinic for potential antiviral treatment. Of 580 patients who had undergone liver biopsy, 130 had histological evidence of cirrhosis at entry, while an additional 11 unbiopsied patients had clinical evidence of cirrhosis. During a follow-up of approximately 4 years, an additional 26 patients developed manifestations of cirrhosis, while 17 patients developed hepatocellular carcinoma and 31 patients died of liver-related disease (13 of whom had hepatocellular carcinoma). The authors noted an increased mortality in patients with chronic HCV who had cirrhosis, or greater than 15 years of infection. Long disease duration, excessive alcohol use, history of intravenous drug use, and old age also contributed to a decreased complication-free survival. A drawback to the study was the inclusion of patients who had been treated with interferon, thus making it difficult to study the natural history without confounding factors (104).

Prospective Studies

Prospective studies involve patients with recognized acute hepatitis who are followed to assess for the development of liver-related morbidity and mortality. Advantages include a known time of exposure and probable mode of infection, whereas disadvantages include a shorter duration of follow-up. Among patients followed in several prospective studies, 5–25% progressed to cirrhosis, 0–1.2% developed hepatocellular cancer, and 1.2–6% died of liver-related disease over a follow-up of 13–24 years (28, 101, 105, 106).

• Di Bisceglie and colleagues prospectively studied 33 non-A, non-B hepatitis patients who received blood transfusions perioperatively at the time of cardiac surgery between 1972 and 1983 at the National Institutes of Health (NIH), as well as six additional NIH patients who contracted acute hepatitis from blood transfusions. Ninety percent of these patients had chronic hepatitis C. Eight patients (20.5%) had histological evidence of cirrhosis with an average of 4.2 years after blood transfusion.

• Mattsson and colleagues studied stored blood samples from 39 of 61 patients who had acute non-A, non-B hepatitis in Sweden in 1978. Sixteen patients had chronic hepatitis C. Liver biopsies were performed in eight patients within 13 years of the acute episode, and two patients had histological findings of cirrhosis. One patient died of liver-related disease but was not included in the analysis. Drawbacks to the study
included a short duration of follow-up and the possibility of underestimation of patients with cirrhosis, as not all were biopsied (106).

- Koretz and colleagues prospectively studied 80 patients with non-A, non-B hepatitis secondary to blood transfusions in the 1970s. Sixty-four of these patients were diagnosed with hepatitis C, 50 of whom were chronic. This study suggested that, based on life-table analysis, 20% of patients with chronic HCV could develop hepatic failure, defined by variceal bleeding, ascites, hepatic encephalopathy, coagulopathy, hyper-
splenism, or hypoalbuminemia. Liver biopsies were not performed in these patients. Drawbacks of this study were the variable duration of follow-up and the possibility of underestimation, based on exclusion of patients without clinically evident cirrhosis (101).

- Alter and colleagues prospectively studied 248 asymptomatic blood donors positive for anti-HCV. Liver biopsies were done in 81 HCV RNA-positive persons and 5% demonstrated cirrhosis. Three of these cirrhotic patients were discovered 27 years after their presumed exposure and one patient was found to have hepatocellular carcinoma 53 years after a blood transfusion. More severe disease was seen in patients with ALT elevations greater than twice the upper limit of normal (28).

**Retrospective–Prospective Studies**

Retrospective–prospective studies involve the identification of patients who previously developed a known acute hepatitis. These patients were studied retrospectively, enrolled, and followed prospectively for the development of liver-related morbidity and mortality. Over a follow-up of 15–45 years, these studies have generally shown lower rates of progression to cirrhosis (0.4–18.1%), hepatocellular carcinoma (0%), and death from liver-related causes (0.2–9.1%) in comparison to purely retrospective studies (93, 95, 98, 99, 107, 108).

- Vogt et al. compared 458 children under the age of 3, who underwent cardiac surgery in Germany prior to 1991, with 458 controls. When stored blood samples were tested, 67 patients (14.6%) who underwent cardiac surgery and three (0.7%) controls were anti-HCV positive. Thirty antibody-positive children (45%) had undetectable HCV RNA at a mean of 19.8 years after their first operation. Seventeen patients had liver biopsies, of which one, who had also been coinfected with HBV, was found to be cirrhotic. The authors concluded that clearance of HCV viremia was more common among children and progression of chronic HCV-related liver disease acquired at a young age was slower than older adults (98).

- Kenny-Walsh and colleagues evaluated 376 women with chronic HCV is likely secondary to transfusion of contaminated anti-D immunoglob-
ulins. Three hundred and sixty-three infected women underwent liver
biopsies. Cirrhosis was found in 7 women, or 2% of biopsies, an average of 17 years after the initial transfusion. Three of these cirrhotic participants reported excessive alcohol consumption (108).

• Seeff et al. studied 8568 stored blood samples taken from healthy military recruits between 1948 and 1954 for evaluation of group A streptococcal infection and acute rheumatic fever. Seventeen persons were anti-HCV positive and 11 were HCV RNA positive. Of these 11 patients, one person had been diagnosed with cirrhosis and another had died of liver-related disease, 42 years after the blood sample was taken. Liver-related morbidity and all-cause mortality were slightly higher in the anti-HCV-positive group. There were no deaths attributed to hepatocellular carcinoma. Lower rates of cirrhosis, HCC, and liver-related death may have related to the fact that this was a young and healthy study population. Advantages of this study were an extended observation period in a healthy population, without comorbidities that could alter the course of HCV-related liver disease. Disadvantages included the possibility of false-negative anti-HCV results secondary to an extended period of serum specimen storage and a small cohort of patients with hepatitis C (95).

• Rodger and colleagues compared long-term outcomes of 98 persons with acute hepatitis C to 202 persons with acute hepatitis unrelated to HCV. Anti-HCV testing was performed on stored sera from 1971 to 1975 in Australia. The presumed route of HCV transmission was intravenous drug use, as opposed to other studies that focused on transfusion-associated HCV. Fifty-one patients with anti-HCV positivity were found to have chronic HCV based on HCV RNA testing. Normal liver tests were associated with a lower risk of long-term complications, a finding which has been observed in other studies as well (28, 111, 112). The authors concluded that outcomes of cirrhosis, hepatocellular cancer, and liver-associated death in the setting of chronic HCV after a follow-up of 25 years were less common than previously perceived. Although patients with anti-HCV positivity had a higher rate of mortality compared to the anti-HCV-negative group, these deaths were not related to HCV complications. A drawback to this study was that only 14% of anti-HCV-positive persons underwent liver biopsy, although hyaluronate levels were measured as a surrogate for fibrosis to reduce an underestimation of cirrhosis (99).

• Thomas and others studied 1667 patients with a history of intravenous drug use within 10 years of recruitment and anti-HCV positivity. Forty patients were categorized as having end-stage liver disease (ESLD), defined by clinical documentation of varices, ascites, or hepatic encephalopathy. Of these patients, 35 had died while one had received a liver transplant. Of patients without documented ESLD, 374 had died of non-liver-related causes. Liver biopsies were performed on
210 participants without clinical evidence of ESLD and cirrhosis was found in two persons. In addition, five patients who died without previously identified ESLD had evidence of cirrhosis on autopsy. ESLD was associated with age greater than 38, male sex, and heavy alcohol use. It did not correlate with HIV status, HBV status, moderate alcohol use, race, viral load, or viral genotype. The low incidence of ESLD in this cohort may be attributed to the restrictive definition of ESLD, without considering laboratory results suggestive of cirrhosis, as well as a relatively short duration of follow-up (93).

- An endemic outbreak of hepatitis C in Germany in 1979 secondary to administration of contaminated anti-D immunoglobulin provided a patient population of 1016 women who were prospectively studied for 20 years by Weisse and colleagues. Of these women, 55% had evidence of chronic HCV infection (7% had not responded to interferon), while 42% had spontaneous clearance of virus and 3% responded to interferon. Four participants had clinical evidence of cirrhosis; it was not seen in any of the 220 biopsy specimens obtained. Two patients died of liver disease; one patient had fulminant hepatitis B and the other was a chronic alcohol abuser. There were no cases of hepatocellular carcinoma (107).

3.2.2. HOST-RELATED FACTORS

Age

Several studies have suggested that younger age at the time of infection may protect against progression to cirrhosis, HCC, and liver-related mortality (93, 95, 98, 100, 109, 110, 113). As discussed previously, Vogt et al. evaluated young children who underwent cardiac surgery during their first 3 years of life and found that only 5.4% of children had histological evidence of cirrhosis after a follow-up of 17 years (98). Furthermore, two studies of young women who received contaminated immunoglobulin at median ages of 24 and 28 years showed that a relatively low number (0.4–2.0%) had cirrhosis after a follow-up of 17–20 years (107, 108). Even among Seeff’s study of young (age < 25) military recruits with chronic hepatitis C, two of 11 patients were cirrhotic after a 45-year follow-up (95). Ryder and colleagues performed serial liver biopsies in 214 patients with chronic hepatitis C to assess progression of fibrosis. Results showed that older age of patients and presence of fibrosis on the initial biopsy were associated with progression of fibrosis (114). These results were similar to Svirитель et al., who performed liver biopsies in 144 patients with chronic hepatitis C and found that age over 40 years at the time of liver biopsy was associated with an increased Ishak fibrosis score (115). What is not known is whether the rate of progression increases as these young persons age over time,
possibly explained by the inability of the immune system to contain the virus as it ages (109).

**Gender**

HCV is more commonly eliminated by women (107–110, 116). Poynard and Freeman conducted separate studies and found that male gender, in addition to alcohol consumption and older age at infection, was associated with fibrosis progression (109, 110). Yamakawa and colleagues conducted a study to compare progression to chronic hepatitis C in men and women in a town in Japan. They found that although anti-HCV positivity did not differ between genders, the proportion of anti-HCV-positive participants who were also HCV-RNA positive was higher in men (78.2%) than in women (67.3%) (116). Several studies have postulated that this may relate to the anti-fibrotic effects of estrogen (117–119). Yasuda and colleagues induced hepatic fibrosis in male and female rats with administration of dimethylnitrosamine and found that estradiol inhibited fibrogenesis by preventing proliferation of hepatic stellate cells which are responsible for collagen synthesis, as well as contributing antioxidant activity (118).

**Race**

Relative to Caucasians, African-Americans are 2–3 times more likely to be anti-HCV positive and less likely to undergo spontaneous clearance of HCV viremia (5). In addition, African-Americans are more prone to develop complications of cirrhosis, including hepatocellular carcinoma, and have lower response rates to antiviral treatment (120–122). However, their rates of fibrosis progression are slower than Caucasians (120, 123, 124). Bonacini and colleagues studied the histological progression of 291 chronic HCV patients (53 African-American, 116 Caucasian) with regard to duration of infection and race. The estimated rate of fibrosis among African-Americans was 0.055 stages per year compared to 0.096 stages per year among Caucasians (120). This disparity may be explained by variable immune responses between both groups (123).

**HIV Coinfection**

In the United States, 30% of HIV-positive patients are also infected with HCV (125). In the setting of HIV infection, HCV fibrosis is accelerated (126, 127). Benhamou and colleagues compared the rates of fibrosis progression in 122 anti-HIV positive, anti-HCV-positive persons and 122 anti-HIV negative, anti-HCV-positive persons. They found a Metavir fibrosis score of 2–4 in 60% of coinfected patients as compared to 47% of HCV-monoinfected patients. The median fibrosis
progression rate (ratio between fibrosis stage and HCV duration) was 0.153 fibrosis units per year in the former and 0.106 fibrosis units per year in the latter. Multivariate analysis showed that HIV seropositivity, CD4 count <200, age >25 at the time of HCV infection, and alcohol >50 g/day were all associated with a faster rate of fibrosis progression (126). Fibrosis progression may be linked to a weaker CD8+ T-cell response to HCV antigens in the setting of HIV (128). HAART therapy slows the progression of fibrosis through immune reconstitution, and possibly, HIV viral load suppression (129, 130).

**HBV Coinfection**

Several studies have reported that HBV infection in the setting of chronic HCV leads to a worsening of liver disease and increased risk for hepatocellular carcinoma (131–133). However, Senturk et al. evaluated 51 patients with HCV and HBV coinfection and found that clinical or histological findings did not differ between the group with HBV/HCV coinfection as compared to the groups with single infections. The authors did find that HCV was the dominant infection (134). Other studies have shown that hepatitis C tends to reduce HBV virus replication (131, 135, 136). The AASLD recommends that non-HBV immune patients with chronic HCV should be vaccinated against hepatitis B (137).

**Metabolic Syndrome**

The metabolic syndrome is defined as a constellation of factors including hypertension, abdominal obesity, diabetes mellitus, dyslipidemia, and nonalcoholic fatty liver disease (NAFLD). NAFLD comprises a histological spectrum of disease ranging from steatosis alone to steatohepatitis to fibrosis, and in some cases, cirrhosis. Extensive research has been conducted to examine correlations between the metabolic syndrome and hepatitis C. The metabolic syndrome can promote progression of hepatic fibrosis and a decreased response to antiviral therapy (138). The prime mechanism responsible for the metabolic syndrome is insulin resistance (139). Insulin resistance leads to hepatic steatosis and stimulates inflammation, resulting in steatohepatitis and possibly fibrosis (138). Other theories describe oxidative stress as a necessary “second-hit” in the development of steatohepatitis. Oxidative stress initiates lipid peroxidation, resulting in stellate cell activation and synthesis of type I collagen (138, 140). Leptin, a peptide primarily produced by adipose tissue, may also play a role in fibrinogenesis (139). Of note, hepatic steatosis itself may cause activation of stellate cells (139). The AASLD Practice Guidelines state that patients with a BMI
>25 kg/m² in the setting of chronic HCV should make an effort to lose weight (137).

**Alcohol Consumption**

Heavy alcohol intake hastens progression to cirrhosis (93, 109, 110, 124, 141–144). Harris and colleagues found a fourfold increased risk for cirrhosis in the setting of increased alcohol consumption in a population of patients with transfusion-associated HCV (124). Ostapowicz et al. studied 234 patients in Australia with chronic hepatitis C and found that greater alcohol use during HCV infection and an increased cumulative consumption of alcohol were associated with progression of liver disease (142). Pessone et al. found a dose–response relationship between self-reported past and present alcohol consumption and HCV viremia (143). They also reported a link between fibrosis and prior alcohol use (143). Corrao noted a synergistic effect of alcohol on chronic HCV at doses of 75–100 g/day and suggested that patients without signs of liver disease could consume <50 g alcohol daily (141). Poynard and colleagues found that daily consumption of alcohol >50 g is a risk factor for advanced liver disease (109), while Hezode reported that even 21–50 g/day can have a negative impact on women with chronic HCV (145). The physiological basis for the detrimental effects of alcohol include immune dysregulation, proinflammatory and profibrotic cytokine stimulation, oxidative stress, and steatosis (146). Seeff advises against “alcoholism” in the setting of chronic HCV (147). AASLD guidelines recommend that patients with chronic HCV should minimize alcohol consumption, and not exceed 50 g/day (137).

**Tobacco Use**

Tobacco use is suspected to promote fibrosis in the setting of chronic HCV (148, 149). Pessone and colleagues identified smoking as an independent risk factor for fibrosis in a study of 310 patients with chronic HCV who underwent a liver biopsy. In addition, smoking has been linked to hepatocellular carcinoma in the setting of chronic hepatitis (150, 151). Hepatotoxic compounds in cigarettes may account for this progression (152).

### 3.2.3. Virus-Related Factors

**Mode of Transmission**

Several studies have suggested that the mode of transmission of HCV may affect progression to complications, with blood transfusions associated with increased risk of cirrhosis possibly related to a higher initial inoculum (99–101, 105, 106, 153). Transfusion studies have shown a 20% rate of progression to cirrhosis at 20 years (100, 147, 154), while
studies of intravenous drug users show lower rates of serious sequelae (99). Gordon and colleagues evaluated 463 patients at an nonurban medical center with chronic hepatitis C and available liver histology, of which 215 (45%) were transfusion recipients, 195 (42%) acquired HCV mainly through intravenous drug use, and 53 (13%) were without identified risks. With a median follow-up duration of 21 years, 173 (37%) patients were cirrhotic and 118 (68%) of them were transfusion recipients. The Kaplan–Meier cumulative risk for cirrhosis 25 years after HCV acquisition was 52.5% among transfusion recipients and 35% among intravenous drug users (155).

Viral Load

While some studies have suggested that an increased viral load is indicative of more advanced liver disease (156, 157), others have not revealed similar findings (142, 158–161). Adinolfi et al. studied liver biopsies of 298 patients with chronic hepatitis C and found that viral load was linked to grade of inflammation and stage of fibrosis in noncirrhotic patients. However, once patients advanced to cirrhosis, their viral loads declined (157). Fanning and colleagues examined liver biopsies of 77 young, healthy women with chronic HCV genotype 1b of 17 years duration with contaminated anti-D immunoglobulin as a source for infection. The authors discovered a weak but statistically significant correlation between viral load and hepatic inflammation. However, viral load was not associated with the degree of liver fibrosis (161).

Virus Genotype

There are conflicting data regarding the effect of virus genotype on fibrosis progression. While some studies have suggested that specific genotypes are associated with progression of fibrosis (162–164), others have not found such a correlation (109, 165, 166). Kobayashi and colleagues evaluated 136 patients with chronic hepatitis C who had two liver biopsies performed 5 years apart. Ninety-six patients were infected with genotype 1 virus, while 40 patients had genotype 2 virus. Genotype 1 infected patients had a higher initial viral load and repeat liver biopsy showed worsening of liver histology in terms of grade and stage of disease. The authors suggested that genotype 1b disease was more “pathogenic” than genotype 2 disease (167). In contrast, Mahaney and colleagues reported more severe liver disease in patient infected with genotype 2 virus (168). Mihm et al. examined 90 liver biopsies of patients with chronic HCV genotypes 1a, 1b, or 3a and found that fibrosis was more common in genotype 1b infection, while steatosis was seen more frequently with genotype 3a infection. The authors
Table 4
Factors that influence the progression of chronic HCV

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect on progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at infection</td>
<td>Younger age, slower rate of progression</td>
</tr>
<tr>
<td>Sex</td>
<td>Female, slower rate of progression</td>
</tr>
<tr>
<td>Race</td>
<td>AA, slower rate of progression</td>
</tr>
<tr>
<td>HIV or HBV coinfection</td>
<td>More rapid progression</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>More rapid progression</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>More rapid progression</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>More rapid progression suggested</td>
</tr>
<tr>
<td>Mode of transmission</td>
<td>Transfusion: higher inoculum, more rapid rate of</td>
</tr>
<tr>
<td></td>
<td>progression</td>
</tr>
<tr>
<td>Virus concentration</td>
<td>Inconclusive evidence</td>
</tr>
<tr>
<td>Virus genotype</td>
<td>Inconclusive evidence, 1b may be associated with</td>
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<tr>
<td></td>
<td>more severe disease</td>
</tr>
<tr>
<td>Virus quasispecies</td>
<td>No evidence</td>
</tr>
</tbody>
</table>

speculated that the increase in fibrosis seen in genotype 1b disease could be attributed to an older age population and a longer duration of disease (169).

Virus Quasispecies

The presence of HCV quasispecies has been compared in patients with resolution of acute hepatitis and the development of chronic hepatitis. Human studies suggest that the development of heterogeneous quasispecies is linked to progression to chronic hepatitis (170). There is no evidence that quasispecies affect advancement of liver disease in the setting of chronic hepatitis C (92) (Table 4).

4. CONCLUSIONS

Chronic hepatitis C is associated with progression to cirrhosis, hepatocellular carcinoma, and liver-related death. It is difficult to clearly define morbidity and mortality risk as variable study types and patient populations yield conflicting results. It is therefore important to takes these factors into consideration when analyzing studies appropriately.
Retrospective studies show the highest rates of morbidity and mortality, followed by prospective studies and retrospective–prospective studies. The actual rates likely lie somewhere in between the extremes of these three categories of study. Progression of HCV is hastened when acquired via blood transfusion; at an older age; in males; in non-African-Americans; in the setting of excessive alcohol (and possibly tobacco) use; and, in persons with HIV and/or HBV co-infection or features of metabolic syndrome. It is controversial if HCV viral load and genotype affect the evolution of disease.

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Current and Future Therapy of Chronic Hepatitis C

Mohammad Ashfaq, MD and Gary Davis, MD

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Key Principles

• The current standard of care for treatment of chronic hepatitis C is once weekly pegylated interferon and daily oral ribavirin. The optimal treatment regimen is determined by viral genotype, patient weight, and the initial virus response to therapy, termed early virologic response (EVR). Non-responders can be identified after the first 12 weeks of treatment so that therapy can be stopped.
• Treatment of chronic hepatitis C with antiviral therapy requires close monitoring since about 30% of patients require dose modifications for cytopenia or side effects.
• Approximately half of treated patients are cured of their infection. Treatment response, termed sustained viral response (SVR), is associated with resolution of inflammation and fibrosis regression. Patients with cirrhosis who achieve an SVR have no further risk of liver failure and the risk of liver cancer is markedly reduced, though not eliminated.

• Some groups of patients have a lower chance of response to treatment than others. Recent studies suggest that higher doses of antiviral drugs might be of benefit in these cases. Patients with advanced cirrhosis or extrahepatic manifestations of hepatitis C infection are best treated by a physician, who has considerable experience with these drugs.

• New virus-specific antiviral agents are in development but it appears that they will need to be given in conjunction with interferon and ribavirin. It is hoped that these new drugs will improve efficacy and perhaps shorten the required duration of treatment. These drugs, if approved, will probably not be available before 2010.

1. INTRODUCTION

The global prevalence of hepatitis C virus (HCV) infection is estimated to be 130–170 million persons (1). The prevalence varies widely in different regions of the world (2). In the United States, it is estimated that between 3 and 5 million persons have chronic infection (3, 4). The identification of the virus responsible for the disease in 1989 led to increased attention to risk factors for transmission and screening of blood donors which caused a dramatic fall in new infections (5). Currently, the incidence of new HCV infections is only about 25,000 to 30,000 cases per year (6). Nonetheless, the prevalence of infection has remained quite stable since most (50–90%) acute infections result in chronic infection and hepatitis. Modeling studies have predicted that the prevalence will gradually decline over the next two decades as the infected population ages, but the likelihood of cirrhosis and disease complications such as decompensated cirrhosis and hepatocellular carcinoma (HCC) will increase (7,8). Thus, there is a great need for the medical community to address this problem with increased efforts to identify infected patients and deliver effective therapy.

2. CURRENT STANDARD OF CARE

The current standard of care for treatment of chronic hepatitis C is the combination of pegylated interferon and ribavirin. This is a regimen that has evolved over the last 18 years. Recombinant interferon was first approved by the FDA for the treatment of non-A, non-B hepatitis in 1991 as monotherapy, but it was effective in a relatively small proportion of patients (9, 10). Ribavirin was added to the regimen after it was noted that it reduced the chance of relapse when treatment was stopped (11). Pegylated interferons replaced the standard formulation
in 2001 and offered the convenience of once a week dosing (12, 13). Improved outcomes have accompanied these changes (Fig. 1).

![Sustained Virological Response](image_url)

**Fig. 1.** Outcomes of treatment regimens for chronic hepatitis C.

### 2.1. Current Agents

Interferons are naturally occurring glycoproteins that are produced in vivo by cells, particularly leukocytes, in response to viral infection. Pharmacologic doses of interferons were first produced by stimulation of cultures of buffy coat lymphocytes collected from blood donors, but commercially available interferons today are recombinantly produced. Interferons inhibit the replication of many viruses, including hepatitis viruses, through a variety of mechanisms, including direct antiviral (inhibition of virus attachment and uncoating, induction of intracellular proteins and ribonucleases) and by amplification of specific (cytotoxic T-lymphocyte) and nonspecific (natural killer cell) immune responses (14). In the late 1980 s, interferons were among the first agents studied for treatment of what was then called chronic non-A, non-B hepatitis (9). Although IFN suppresses the level of HCV replication, the exact mechanisms of action in HCV infection are not known. Nonetheless, it is generally believed that clearance of the HCV is at least in part mediated by IFN-related enhancement of the host immune response to the virus.

Initially, standard recombinant interferons were used for treatment of chronic HCV infection. These had a limited half-life that required dosing three times per week. Long-acting pegylated interferons replaced standard interferons after their approval by FDA in 2001. Pegylation involves the attachment of a large inactive molecule (polyethylene gly-
col; PEG) to the interferon molecule in order to reduce its renal clearance. This process results in some variable loss of activity of the native protein that is dependent on the size and site of attachment of the PEG molecule but is also associated with a tenfold increase in drug half-life and a corresponding decrease in clearance (15–17). The PEG molecule is cleaved after binding of the complex to the interferon receptor and cleared. The longer half-life allows large doses of the drug to be administered less frequently (once weekly instead of three times per week), increases host exposure to interferon, and doubles the response seen with standard interferon preparations (18–20). Pegylated interferons are more effective than standard interferon and there is a clear dose response with increasing doses of the PEG-interferons (9, 12, 13, 21).

Ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) is a synthetic nucleoside analogue which structurally resembles guanosine and has in vitro activity against many viruses, including flaviviridae which closely resemble HCV (22, 23). The mechanism of action in HCV infection is not clear, but the predominant current opinion is that ribavirin induces lethal mutations in the viral genome, a mechanism known as viral error catastrophe (22–24). Early studies of ribavirin alone found that serum ALT levels fell to within the normal range in 40% of treated patients, but virus levels did not significantly change (25–27). However, when combined with interferon, the combination both improves response during treatment and reduces subsequent relapse. Thus, there is a dramatic improvement in the sustained virologic response rate (11, 28–30). The results of two similarly designed large randomized controlled trials comparing combination therapy to interferon monotherapy showed sustained viral-negative responses in 41 and 33% of subjects treated with 12 or 6 months of combination therapy, respectively, compared to 16 and 6% in those treated with interferon alone for 12 or 6 months (31). Furthermore, the combination of pegylated interferon with ribavirin (the current standard of care) showed an even higher viral response rate (12, 13). PEG-interferon alfa-2b 1.5 μg/kg once weekly plus 800 mg daily ribavirin led to a sustained virologic response rate of 54%, even though the dose of ribavirin in this study was suboptimal (12). Sustained response was 42% in patients infected with genotype 1 and 82% in those with genotype 2 or 3. Similarly, PEG-interferon alfa-2a 180 μg once weekly plus 1000–1200 mg of ribavirin per day resulted in a sustained virologic response rate of 56% (13). Sustained response was 46% in patients infected with genotype 1 and 76% in those with genotype 2 or 3.
2.2. Optimizing Treatment Regimens

The sensitivity of different HCV genotypes to interferon-based therapies varies considerably and this determines both the drug doses and the treatment duration required (Table 1). Thus, the determination of the virus genotype before treatment remains the initial critical step in evaluating a patient with chronic hepatitis C. Patients infected with genotype 1 should receive 1 year of one of the two available pegylated interferons (PEG-IFN) plus ribavirin. The two pegylated interferons are, for all intents and purposes, bioequivalent. Although the approved dose of ribavirin is 1000–1200 mg/day for those with weight less than or greater than 75 kg, respectively (32), an extended weight-based dose ranging from 800 to 1400 mg/day is commonly used (Table 1) (33). It is not known whether such dosing improves response or is appropriate for other genotypes. Recently, some investigators have suggested that “rapid viral response” (RVR; undetectable HCV RNA after 4 weeks of treatment) in patients with genotype 1 identifies a small subgroup (about 20% of treated patients) who may be treated for only 24 weeks and still achieve an SVR rate of 73–91% (34). Others have found a lower SVR rate with 24 weeks of treatment, so this needs to be confirmed before becoming standard practice (35).

Patients with genotype 2 or 3 respond as well with doses of 800 mg/day and just 6 months of treatment as they do with higher doses and a longer duration (32). Recently, studies have suggested that some patients with genotype 2 or 3 infection who respond rapidly to treatment (RVR) can be treated for as little as 12–16 weeks with excellent outcomes if weight-based dosing of ribavirin is utilized (36–38). Shortening the course of treatment is not recommended regardless of the early viral response if a standard dose (800 mg per day) of ribavirin has been used (39).

Patients who are infected with genotype 4 have viral response rates similar to or perhaps slightly better than genotype 1 and, like genotype 1, some achieve good responses with only 24 weeks of therapy (40). Genotypes 5 and 6 have SVR rates approaching those achieved with genotypes 2 and 3, although they require a full year of therapy (41, 42).

2.3. Patient Selection, Drug Administration, and Monitoring Therapy

All HCV-infected patients should be counseled about routes of virus transmission, the natural history of the infection, the detrimental effects of alcohol and cannabis use on the course of disease, treatment options,
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Interferon Dose (per week)</th>
<th>Ribavirin Dose (mg/day)</th>
<th>Duration (weeks)</th>
<th>SVR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>180 μg PEG alfa-2a or 1.5 μg/kg PEG alfa-2b</td>
<td>800–1400 mg/day&lt;sup&gt;a&lt;/sup&gt; weight-based</td>
<td>48</td>
<td>41–42%</td>
</tr>
<tr>
<td>2</td>
<td>180 μg PEG alfa-2a or 1.5 μg/kg PEG alfa-2b</td>
<td>800 mg/day&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24</td>
<td>66–75%</td>
</tr>
<tr>
<td>3</td>
<td>180 μg PEG alfa-2a or 1.5 μg/kg PEG alfa-2b</td>
<td>800 mg/day&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24</td>
<td>66–75%</td>
</tr>
<tr>
<td>4, 5, 6</td>
<td>180 μg PEG alfa-2a or 1.5 μg/kg PEG alfa-2b</td>
<td>1000–1200 mg/day&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48</td>
<td>55–64%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Weight-based dosing of ribavirin is 800 mg daily in a divided dose for weight less than 65 kg, 1000 mg for weight 65–84 kg, 1200 mg for weight 85–104 kg, and 1400 mg for weight of 105 kg or more.

<sup>b</sup> Ribavirin dose should be weight-based if shorter duration of therapy is anticipated in patients with rapid viral response.
possible treatment risks especially including the risk of teratogenicity of ribavirin, and outcomes regardless of what intervention, if any, is ultimately decided upon. If treatment is not considered, the importance of long-term follow-up must be emphasized. Valuable patient resources are available on the websites for the Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/diseases/hepatitis/) and the American Liver Foundation (http://www.liverfoundation.org/education/info/hepatitisc/). Up-to-date physician information on epidemiology and treatment is available from several different online resources including Clinical Care Options (http://www.clinicaloptions.com/Hepatitis.aspx) and subscription services such as Up-To-Date and WebMD.

Rationale treatment management decisions are based on a clear understanding of the epidemiology and natural history of chronic hepatitis C, as well as the factors that influence the response to treatment. The potential benefits in terms of freedom from risk of progression, longevity, and quality of life must be weighed against the current effectiveness, cost, tolerability of therapy, other comorbid conditions that the patient might have, financial impact, and desire for therapy. The balance of these factors defines the treatment threshold for a particular patient.

If the physician and patient agree to proceed with treatment, assessment of the status of the disease (as determined by liver biopsy) and infection (as determined by viral genotype and HCV RNA level) should be made. This allows a more accurate estimate of prognosis and chance of response to available treatment. It also allows the physician to use these patient characteristics that may independently influence treatment response to personalize the treatment strategy to achieve the optimal response (see above). Although most hepatologists use only viral genotype and histology to choose the best treatment duration, others have recommended a more complex “a la carte” method that also incorporates gender, age, and viral level into Equation (43). Retrospective analysis suggests that such algorithms might improve sustained viral response rates to 50–83%. However, the wisdom of complicated algorithms that would, in effect, treat a higher proportion of infected cases with a longer and more costly regimen is controversial and has not been confirmed prospectively.

Baseline assessment of liver tests, complete blood counts, and HCV RNA level are important in order to later determine treatment response and drug-related toxicity. The patient should be instructed in injection techniques. Treatment tolerance is improved if the patient is educated about the potential side effects of therapy and what they might expect. Some easy measures such as evening dosing, exercise, adequate
hydration, and use of acetaminophen at the time of each interferon dose will reduce anxiety, side effects, and non-compliance. Physician extenders such as nurses, pharmacists, and commercial pharmacy support services are extremely helpful in this respect. Reinstitution of antidepressants should be considered in patients with active depression or a significant past history of depression.

Monitoring response and potential drug toxicity is essential (Fig. 2). Symptoms related to treatment rarely necessitate dose adjustments. However, hematologic alterations, particularly anemia, can be significant and clinically important during the first few weeks and may require dose adjustments (Table 2). Therefore, blood counts including hemoglobin, white count, differential, and platelet count should be repeated 2 and 4 weeks after starting therapy. Transient interferon dose reduction is indicated only for a neutrophil count less than 750 per mL or platelets to less than 50,000 per mL. Ribavirin should be reduced if the hemoglobin falls to less than 10 g/dL. The amount of dose reduction required to reverse cytopenia has not been established and this has led to differences in the recommendations for the two interferon preparations (Table 2). Although the labeling of the drugs calls for reducing doses by approximately half, this is usually not necessary. Temporary dose modifications are common in patients treated with combination therapy. In fact, in large controlled trials dose modifications were required at least transiently in 34 and 42%, respectively (12, 13). Ribavirin causes a hemolytic anemia that is accompanied by a vigorous reticulocytosis. Although this usually serves to maintain stable levels of hemoglobin after the first few weeks of treatment, about 8–13% of patients will require reduction of the ribavirin dose for anemia, usually during the first 4 weeks of treatment (44). Ribavirin-induced anemia is dose-dependent and therefore anemia usually stabilizes or improves with dose reduction. Occasionally transfusion or support with erythropoietin is necessary, though it generally takes 4–8 weeks before the hemoglobin increases after erythropoietin is started (45). About 15–20% of patients treated with pegylated interferon require dose reductions for neutropenia (12, 13). Growth factor support is rarely required for neutropenia. Significant thrombocytopenia necessitating dose reduction is uncommon since the anemia caused by ribavirin induces a reactive thrombocytosis. Thus, platelet counts tend to remain relatively stable throughout combination therapy, even when the pre-treatment count is low. Interferon should be permanently stopped only if symptoms are incapacitating, the absolute neutrophil count is less than 500 per mL, or the platelet count is less than 25,000 per mL. Discontinuation of therapy for cytopenia is uncommon if patients have been monitored and dose adjusted appropriately. It is extremely important that treatment not be
<table>
<thead>
<tr>
<th>Date</th>
<th>HCV Status</th>
<th>Labs</th>
<th>Sustained Response</th>
<th>Reducer</th>
<th>Non-Responder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>Baseline</td>
<td>WBC</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 1</td>
<td></td>
<td>ANC</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 2</td>
<td></td>
<td>Hemoglobin</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 3</td>
<td></td>
<td>T-Bilirubin</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 4</td>
<td></td>
<td>AST</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 5</td>
<td></td>
<td>ALT</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 6</td>
<td></td>
<td>HCV RNA</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 7</td>
<td></td>
<td>Creatinine</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 8</td>
<td></td>
<td>Glucose</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 9</td>
<td></td>
<td>FSH</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 10</td>
<td></td>
<td>Liver Enzymes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 11</td>
<td></td>
<td>Grade</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Week 12</td>
<td></td>
<td>Liver Fx Stage</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Fig. 2. Monitoring of antiviral treatment for chronic hepatitis C (sample data sheet).
Table 2  
Modification of Doses of Antiviral Drugs (from Product Labeling Except Where Noted)

<table>
<thead>
<tr>
<th>Laboratory Values</th>
<th>Pegylated IFN alfa-2a</th>
<th>Pegylated IFN alfa-2b</th>
<th>Ribavirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC ≥750/mm³</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>ANC &lt;750/mm³</td>
<td>Reduce to 135 µg</td>
<td>Reduce by 50%</td>
<td>No change</td>
</tr>
<tr>
<td>ANC &lt;500/mm³</td>
<td>Discontinue permanently</td>
<td>Discontinue permanently</td>
<td>No change</td>
</tr>
<tr>
<td>Platelet &lt;80,000/mm³</td>
<td>No change</td>
<td>Reduce by 50%</td>
<td>No change</td>
</tr>
<tr>
<td>Platelet &lt;50,000/mm³</td>
<td>Reduce to 90 µg</td>
<td>Discontinue permanently</td>
<td>No change</td>
</tr>
<tr>
<td>Platelet &lt;25,000/mm³</td>
<td>Discontinue permanently</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 gm/dL if no cardiac disease</td>
<td>No change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No change&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>≥2 gm/dL fall if history of stable cardiac disease</td>
<td>No change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No change&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Laboratory Values</td>
<td>Pegylated IFN alfa-2a</td>
<td>Pegylated IFN alfa-2b</td>
<td>Ribavirin</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------------------</td>
<td>------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>&lt;8.5 gm</td>
<td>Stop</td>
<td>Stop</td>
<td>Stop</td>
</tr>
<tr>
<td>Moderate side effects (symptoms)</td>
<td>Decrease dose to 135 μg (in some cases reduction to 90 μg may be needed).</td>
<td>Decrease dose by 50%</td>
<td>No recommendation</td>
</tr>
<tr>
<td>Severe side effects (symptoms)</td>
<td>Discontinue permanently</td>
<td>Discontinue permanently</td>
<td>Stop</td>
</tr>
</tbody>
</table>

\(^a\) Authors’ note: Interferon suppresses bone marrow function and modest dose reductions may allow the hemoglobin to stabilize and/or recover if ribavirin reduction alone is insufficient. Also consider iron deficiency of ribavirin reduction does not stabilize the hemoglobin level.

\(^b\) Recommendation with Copegus (Roche) is to reduce dose to 600 mg/day. Recommendation with Rebetol (Schering) is to reduce in 200 mg/day increments until hemoglobin is stable.
stopped prematurely or for decreases in blood counts that do not meet the criteria stated above. Inappropriate dose reduction and discontinuation significantly reduces the likelihood of a treatment response. Early discontinuation of treatment can reduce the likelihood of a sustained treatment response by 80% (46).

The safety and tolerability of combination therapy have been reviewed in detail elsewhere and will only be highlighted here. These reviews are highly recommended for physicians who have not used these drugs before. Overall, interferon-based therapies are reasonably well tolerated. Most patients experience flu-like side effects including fatigue, fever, headache, myalgia, and arthralgia (44, 47). These are most severe during the first few weeks of therapy and often abate to a large degree as treatment is continued. Gastrointestinal symptoms including nausea, vomiting, or diarrhea occur in about a third of patients but are rarely severe. Psychiatric symptoms such as depression, impaired concentration, irritability, and insomnia occur in about a third of cases, but are also common in untreated patients with chronic hepatitis C. Dermatologic signs and symptoms occur in about a quarter of patients. Injection site erythema is most common and occurs more frequently with pegylated interferons. A faint morbilliform rash can be seen from the ribavirin.

As described above, ribavirin causes a predictable dose-related hemolysis. Thus, the drug should be used with great caution or avoided completely if there is pre-existing anemia, a hemolytic disorder, coronary artery disease, or hypoxia. Since ribavirin is renally excreted, it can cause profound hemolysis in patients with renal failure and should generally be avoided. Careful consideration should be given to the potential effects of an acute anemia in each patient in whom combination treatment is considered. The mean fall in hemoglobin is 2–3 gm/dL (11–13, 28). The decline occurs gradually during the first 4 weeks of treatment and the hemoglobin level usually remains relatively stable thereafter. Finally, ribavirin has embryotoxic and teratogenic effects in animals and should be avoided in patients of child-bearing potential unless adequate contraception is assured.

Severe adverse events, including severe psychiatric symptoms, suicide attempts, and profound cytopenia are extremely uncommon being reported in fewer than 1 in 1000 treated cases (47). Development of immune-mediated disorders such as thyroid disease, diabetes, dermatologic conditions, neuropathy, and other autoimmune-like signs was seen in about 1% in a large retrospective series (47). Development of autoantibodies is not necessarily associated with autoimmune disease. Autoantibodies are common in patients with HCV infection and may be more common during interferon treatment (48, 49).
2.4. Assessing Treatment Response

Treatment responses are defined by changes in the HCV RNA level during and after treatment (Table 3). HCV RNA should be measured with a sensitive quantitative assay such as real-time PCR, for example the Roche Taqman. Serum ALT is not part of the definition of response since its level does not always reflect on treatment response. Ribavirin may cause the ALT to normalize in the absence of a virologic response and ALT may occasionally be elevated despite a virologic response, especially in those receiving pegylated products (12, 13, 27).

Early virologic response (EVR) is defined as a fall in the HCV RNA level by at least 2 logs (99%) within the first 12 weeks of treatment. EVR is based on the concept that the slope of the second phase decline in HCV RNA levels during treatment correlates with the likelihood of eventual virus clearance (50). Thus, EVR is used to assess responsiveness to treatment during the first weeks of therapy. Genotype 1-infected patients who fail to achieve an EVR have less than a 1% chance of reaching an SVR with continued therapy (51). This justifies discontinuation of therapy after 12 weeks in the 20% or so of patients without EVR. Patients with genotype 2 or 3 almost always reach an EVR, so it is not usually helpful to assess HCV RNA levels during treatment in them.

Measurement of HCV RNA at the end of therapy is helpful in identifying those who have cleared virus (ETR) and require subsequent screening to confirm a durable response. About 15% of patients with ETR will relapse during the first few months after treatment is stopped. Sustained viral response (SVR) is confirmed by the absence of detectable HCV RNA by a sensitive molecular test 6 months after completing therapy. Occasionally studies will report “SVR-3 months” since almost all relapses occur within 12 weeks of stopping treatment. SVR is the major goal of treatment and is durable. It implies viral eradication and is associated with histologic improvement, regression of inflammation and fibrosis, and in patients with cirrhosis, a marked reduction in risk for hepatocellular carcinoma and elimination of the risk of decompensation (52–54).

The implications of rapid viral response (RVR) are described above, but this is a new concept and requires confirmation before influencing treatment duration in most patients. RVR identifies those patients who are most sensitive to antiviral treatment and might tolerate dose reductions or early treatment discontinuation. Importantly, failure to reach an RVR does not connote treatment failure and it is not a reason to stop treatment.
<table>
<thead>
<tr>
<th>Milestone</th>
<th>Week of Treatment</th>
<th>Definition$^a$</th>
<th>Implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid viral response (RVR)</td>
<td>4</td>
<td>HCV RNA undetectable by rtPCR or TMA</td>
<td>Higher chance of SVR may respond as well if treatment needs to be shortened</td>
</tr>
<tr>
<td>Early virologic response (EVR)</td>
<td>12</td>
<td>HCV RNA decreased by $\geq 2$ logs from baseline or HCV RNA undetectable</td>
<td>Failure to achieve EVR associated with $&lt;1%$ chance of SVR and treatment can usually be stopped</td>
</tr>
<tr>
<td>End-of-treatment response (ETR)</td>
<td>End of treatment</td>
<td>HCV RNA undetectable by rtPCR or TMA</td>
<td>On treatment response. Observe for SVR or relapse</td>
</tr>
<tr>
<td>Sustained virologic response (SVR)</td>
<td>24 weeks after treatment</td>
<td>HCV RNA undetectable by rtPCR or TMA</td>
<td>Eradication of virus (cure)</td>
</tr>
</tbody>
</table>

$^a$ rtPCR = real-time polymerase chain reaction such as TaqMan; TMA = transcription mediated amplification.
3. TREATMENT OF SPECIFIC PATIENT GROUPS

Treatment of most patients with chronic hepatitis C is done by hepatologists, gastroenterologists, and infectious disease physicians. A physician’s experience and comfort with these patients and the medications required for their treatment determines one’s treatment threshold. Some patients are more complicated and require more experience with the complexities of treatment. Such cases should generally be referred to an experienced treater or managed in collaboration with one.

3.1. Fibrosis or Cirrhosis

Bridging fibrosis or cirrhosis is present in 20–30% of patients with chronic hepatitis C. The rationale for treating patients with advanced fibrosis is clear since these patients have a greater risk of progressing to develop hepatocellular carcinoma or complications of cirrhosis. These complications are significantly reduced if the patient clears virus (see above). Although the presence of significant fibrosis negatively impacts treatment response to interferon-based regimens, it does not preclude response or necessarily increase the complexity of treatment. Patients with stage 3 (bridging fibrosis) or 4 (cirrhosis) fibrosis have about a 10% lower SVR rate than patients without fibrosis (41–44% compared to 54–55% in those with stage 0–2) (12, 13).

Higher doses of interferon or ribavirin may improve the response to treatment in patients with fibrosis. In one trial using a dose of 3.0 μg/kg of pegylated interferon alfa-2b (twice the normal dose), the SVR rate was identical in patients with and without advanced fibrosis (55). Continuous interferon, so-called maintenance therapy, has not been shown to be effective in preventing fibrosis progression or disease complications (56, 57).

Treatment of patients with decompensated cirrhosis can be considered in hopes of eradicating infection prior to liver transplantation. SVR in this group usually prevents recurrence of HCV infection after transplant (58, 59). However, such treatment is extremely difficult. Most patients have pre-existing cytopenia that prevents them from receiving full doses of medications and severe complications, often related to the liver disease rather than the treatment, may require dose reductions or discontinuation of therapy. Cytopenia may be partially avoided by use of growth factors or by initiating treatment at low doses and escalating as tolerated (58). Despite the problems, SVR is possible in about 25% of patients. Treatment in this group of patients is best managed by experienced transplant hepatologists.
3.2. African-Americans

SVR rates have consistently been lower in African-Americans than in Caucasians in trials of interferon-based therapy. Recent studies with pegylated interferon and ribavirin in previously untreated patients with genotype 1 infection observed SVR rates of 19–26% in African-Americans vs 39–52% in Caucasians (60, 61). Several factors including the higher proportion with genotype 1, higher body weight, and a slower decline in HCV RNA in viral kinetic studies might at least partially explain this observation. However, the apparent ability of higher doses of interferon or ribavirin to improve responses suggests that the explanation may lie in an impaired ability of intracellular antiviral mechanisms to turn on at standard doses of these medications (55).

3.3. HIV–HCV Coinfection

HCV infection is common in HIV-infected individuals (62). Coinfected patients tend to have high HCV RNA levels and some studies suggest that they have more severe liver disease, more rapid disease progression, and a higher prevalence of hepatic fibrosis (63–65). Liver disease due to hepatitis B, hepatitis C, or alcohol is second only to AIDS as a cause of death in HIV patients (66). Several clinical trials of pegylated interferon and ribavirin have recently been reported (67–69). Treatment responses vary considerably in these studies due to differences in design and compliance. However, SVR in genotype 1 patients was low (14–29%) while it was reasonably intact in genotypes 2 and 3 subjects (62–73%). Overall, SVR rates appeared to be 10–15% below what would be expected in an HIV-negative population, but this may be in part related to the lower doses of ribavirin that were used in these studies because of concerns about potential drug interactions. Such interactions were not observed and full weight-based doses of ribavirin are now appropriate.

It is important to educate HIV-coinfected patients about the endpoints of antiviral therapy for chronic hepatitis C since they differ from HIV. Viral eradication, not chronic suppression, is the goal. Thus, failure to achieve EVR reliably predicts lack of response and justifies stopping treatment. Patients receiving highly active anti-retroviral therapy (HAART) or with low CD4 counts appear to be more likely to experience adverse effects from treatment, including drug hepatotoxicity, and must be monitored for this. Severe hepatotoxicity, especially related to ritonovir and didanosine (ddI), is almost four times higher in HIV patients who are coinfected with HCV with a risk of about 12% (70).
3.4. Obesity and Fatty Liver Disease

Obesity and hepatic steatosis are both associated with a reduced response of HCV-infected patients to interferon-based treatments (12, 13, 71). For genotypes 1 and 2, hepatic steatosis is associated with a fall in SVR from 57 to 25% and 96 to 86%, respectively (71). Genotype 3 infection induces steatosis in and of itself and this is not associated with reduced SVR. The mechanism(s) by which obesity or steatosis reduce SVR is(are) not known, but it has recently been suggested that it may be related to insulin resistance and hyperinsulinemia (72). Other mechanism may also be involved. It is not yet known if higher doses will overcome the lower response, but pilot studies suggest that this might be the case (73).

3.5. Extrahepatic Manifestations

Mixed cryoglobulinemia is the most common extrahepatic manifestation in patients with chronic hepatitis C. It typically manifests as cutaneous vasculitis, glomerulonephritis, neuropathy, or systemic vasculitis (74, 75). Interferon alone, or in combination with ribavirin (if renal function is intact), is able to suppress cryocrits in the majority of patients with mixed essential cryoglobulinemia and treatment may be indicated based solely on the presence of clinical complications of cryoglobulinemia, regardless of the presence or severity of the liver disease. The fall in cryocrit observed during treatment usually correlates with a drop in serum HCV RNA levels, normalization of complement levels, and clinical improvement. However, polyneuropathy appears to be relatively resistant to treatment (76, 77). Although results in these small series vary considerably, sustained virologic response following discontinuation of interferon-based treatment appears to be lower than in patients without cryoglobulinemia (78).

Glomerulonephritis (GN) has been associated with chronic HCV infection. Most of these cases present with cryoglobulinemia and proteinuria that may reach the nephrotic syndrome range (79). The histologic lesion is usually membranoproliferative GN, so-called cryoglobulinemic GN, although other histologic forms such as mesangial proliferative GN (usually IgA nephropathy) and membranous GN may also occur. In contrast to membranoproliferative GN, the latter two histologic lesions are typically not associated with cryoglobulinemia (79–81). Membranous GN responds poorly, if at all, to interferon (80). Furthermore, a recent report cautions that glomerulonephritis not caused by HCV may worsen under interferon treatment (82). Finally, ribavirin is renally excreted and should be used with great caution, if at
all, in patients who have developed significant renal insufficiency as a consequence of their GN.

3.6. Post-transplantation

Complications of chronic hepatitis C including hepatocellular carcinoma and decompensated cirrhosis are the most common indication for liver transplantation accounting for about 40% of transplants performed in the United States and Europe. If HCV RNA is detectable at the time of transplantation, infection will always recur in the graft. HCV RNA levels increase rapidly following transplant as a consequence of immunosuppression. These patients often have very high HCV RNA levels which may sometimes result in rapidly progressive chronic hepatitis or, less commonly, an aggressive cholestatic hepatitis which leads to liver failure (83). Treatment of such patients is predictably difficult since most patients have genotype 1 infection, high viral loads, have previously failed to respond or tolerate interferon-based therapy, often have pre-existing cytopenia and renal insufficiency, and have drug interactions that increase the risk of cytopenia or symptoms. While antiviral treatment is successful in some patients with recurrent hepatitis C, it is extremely difficult to administer and fraught with hazards that may threaten the patient or the graft. Thus, these patients should be managed by an experienced transplant hepatologist.

Interferon-based antiviral therapy should generally be avoided in recipients of other (not liver) solid organ transplants because of the risk of rejection (84). However, treatment appears to be safe in autologous and most allogeneic bone marrow transplant recipients (85).

3.7. Viral Non-Responders

Perhaps the most difficult group of patient to manage are those who have failed to respond to a previous course of antiviral therapy. These patients fall into several categories including those who were unable to tolerate therapy, those who had the dose of one or both drugs reduced (appropriately or not) or received less than the optimal duration of therapy, those who received an older treatment regimen (for example, standard interferon and ribavirin), and those who simply did not sufficiently inhibit viral replication. Thus, non-responders are a very heterogeneous group. The first step in managing these patients is to find out why they failed treatment and decide if that problem can be overcome with retreatment. If it cannot, retreatment is not an option with current regimens. Another issue to consider in deciding whether to restart antiviral therapy is if the patient needs therapy or can wait for regimens that might offer a greater chance of response.
The best strategy is to optimize therapy to have the best chance of an SVR during the initial course because responses to retreatment are not very good. Approximately 10–20% of viral non-responders to interferon monotherapy or standard combination therapy achieve a sustained response when retreated with pegylated combination therapy (Table 4) (86). Factors that predict a better response to retreatment include a genotype other than 1 (65% vs 14% for genotype 1), HCV RNA level less than 1.5 million IU/mL (27% vs 15%), absence of cirrhosis (23% vs 11%), Caucasian rather than African-American (20% vs 6%), age less than 60 years (19% vs 6%), and achievement of EVR during the previous course of treatment (34% vs 1%) (87).

<table>
<thead>
<tr>
<th>Prior Therapy</th>
<th>Retreatment Regimen</th>
<th>SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-responders</td>
<td>IFN monotherapy</td>
<td>Peg-IFN + RBV</td>
</tr>
<tr>
<td></td>
<td>IFN + RBV</td>
<td>Peg-IFN + RBV</td>
</tr>
<tr>
<td>Relapers</td>
<td>IFN + RBV</td>
<td>Peg-IFN + RBV</td>
</tr>
</tbody>
</table>

Virologic relapse occurs in 45–80, 35–40, and 15% of those who lose detectable HCV RNA during interferon monotherapy, standard combination therapy, or pegylated combination therapy, respectively (9–13). There is little or no benefit in retreating patients with the same treatment regimen (88). However, treatment of relapsers to monotherapy with higher doses or a longer duration of interferon, or combination therapy may achieve a sustained response (Table 4). A recent study found that half of patients who relapsed after standard interferon and ribavirin achieved SVR when retreated with pegylated interferon and ribavirin (86).

4. THERAPEUTICS AGENT IN DEVELOPMENT

The next decade should see the introduction of specifically targeted antiviral therapy for hepatitis C (STAT-C). These are agents that are specifically directed at HCV replication mechanisms including
viral entry, genome attachment to intracellular structures such as the endoplasmic reticulum, viral polymerase, viral protease, and assembly of viral components required for virus export and function. The viral protease inhibitors are furthest along in clinical development (Table 5). Many of these drugs have now entered phase 2 or 3 human clinical trials. It is possible that some of these drugs may be approved for clinical use by 2010 or soon thereafter. However, it is clear from the studies to date that they will need to be given in combination with interferon and ribavirin. Thus, patients who are intolerant of interferon-based therapy will not be candidates for these new agents. It is not yet clear how these new agents will be combined with the current regimen of pegylated interferon and ribavirin to provide the best outcome, but it is hoped that such combinations will allow shorter courses of therapy with greater efficacy. This appears to be an achievable goal.

Table 5
New Agents in Development in Man as of January 2008

<table>
<thead>
<tr>
<th>Mechanism of Action</th>
<th>Drug</th>
<th>Investigation Phase</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease inhibitor</td>
<td>VX-950 (Telaprevir)</td>
<td>III</td>
<td>Vertex</td>
</tr>
<tr>
<td></td>
<td>SCH 503034</td>
<td>III</td>
<td>Schering-Plough</td>
</tr>
<tr>
<td></td>
<td>MK-7009</td>
<td>II</td>
<td>Merck</td>
</tr>
<tr>
<td></td>
<td>GS3192/ACH806</td>
<td>I</td>
<td>Gilead</td>
</tr>
<tr>
<td></td>
<td>ITMN B</td>
<td>I</td>
<td>InterMune</td>
</tr>
<tr>
<td></td>
<td>BILN 1931</td>
<td>I</td>
<td>Boeringer</td>
</tr>
<tr>
<td>Polymerase inhibitor</td>
<td>R1626(prodrug)/ R1479</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RO 5024048</td>
<td>II</td>
<td>Roche</td>
</tr>
<tr>
<td></td>
<td>R7128</td>
<td>II</td>
<td>Pharmasset</td>
</tr>
<tr>
<td></td>
<td>BILB1941</td>
<td>I</td>
<td>Roche</td>
</tr>
<tr>
<td></td>
<td>GSK625433</td>
<td>I</td>
<td>Boeringer</td>
</tr>
<tr>
<td>Cyclophilin B</td>
<td>DEBIO-025</td>
<td>L</td>
<td>Debiopharm</td>
</tr>
<tr>
<td>inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helicase inhibitor</td>
<td>None in clinical studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPR agonist (thiazolide)</td>
<td>Alinia (nitazoxanide)</td>
<td>I</td>
<td>Romark Laboratories</td>
</tr>
</tbody>
</table>
REFERENCES


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Hepatitis C in Special Populations

Douglas Dieterich, MD, Marie-Louise Vachon, MD and Damaris Carriero, MD

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Key Principles

- “Special” HCV-infected populations are those in whom specific aspects relating to the clinical presentation, diagnosis, and/or treatment can be identified and targeted to improve the health of a patient and a population.
- The populations described in this chapter include the following: those co-infected with the human immunodeficiency virus (HIV)/hepatitis B virus (HBV), specific ethnic groups (Latinos/African Americans), those with insulin resistance and those with chronic kidney disease.

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• Approximately 30% of those infected with HIV have concomitant HCV. HIV appears to accelerate the progression of HCV. The standard of care in this population is pegylated interferon and ribavirin. All patients in whom the potential benefit of therapy outweighs its risk should be offered therapy.
• HBV co-infection is unusual but may cause hastened disease progression in HCV. Treatment options would depend on which viral infection is believed to predominate.
• Ethnic differences exist in the epidemiology, natural history, and treatment responses of HCV in Latinos and African Americans.
• Latinos infected with hepatitis C are more likely to be males, co-infected with HIV, and have acquired HCV at a younger age compared to Caucasians. The Latino population has a lower chance of SVR compared to Caucasians.
• Multiple studies show a slower progression of liver disease in African Americans, who also have a lower chance of response to peg-IFN + RBV therapy compared to Caucasians.
• There is an increased prevalence of insulin resistance and diabetes in HCV. Patients with insulin resistance and diabetes in chronic HCV have more severe liver disease and decreased response to treatment compared to the general population.
• There is a higher prevalence of HCV infection in patients with chronic kidney disease (CKD), especially in those on hemodialysis (HD), than in the general population. Interferon-based therapy does produce comparable response rates to those with preserved renal function, but there is lower tolerability to therapy and a high dropout rate.
• When evaluating a member of a special population, efforts should be made to customize treatment both to the patient and to the virologic response in order to maximize chances of success.

1. INTRODUCTION

What makes a population special? As medical care givers, what should prompt us to consider a population differently? In this chapter, we consider a population special when specific aspects relating to the clinical presentation, diagnosis, and/or treatment can be identified and targeted to improve the health of a patient and a population. Considering a population special is recognizing that diversity exists and needs to be addressed as individualized care. In the case of hepatitis C (HCV), populations can differ according to the prevalence of the infection, risk factors for acquisition, access to care and acceptance of treatment, presentation and evolution of disease, comorbidities, development of
complications, and safety and efficacy of treatment. In this chapter, we will review hepatitis C in six different populations: the co-infected with HIV, the co-infected with hepatitis B, Latinos, African Americans, the insulin resistant/diabetic, and the patient with chronic kidney disease.

2. CO-INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV)

2.1. Prevalence and Epidemiology

HCV/HIV co-infection is prevalent. In the United States, it is estimated that 300,000 persons are co-infected with hepatitis C and HIV. The overall prevalence of HCV in HIV individuals is about 30% which is higher than the 1.8% reported in the general population (1–3). From the hepatitis C perspective, around 8% of this population is co-infected with HIV (1). The high rate of co-infection rests on the shared risk factors for transmission which, moreover, determine the prevalence of co-infection. Intravenous drug use (IVDU) is still the number one cause of HCV transmission (independent of the HIV status). Studies have reported co-infection rates of 75–90% in HIV seropositive IVDUs (4, 5); more recent data suggest that HCV prevalence is decreasing in this population (6). If we examine more closely patients with HCV who are IVDUs, their risk of co-infection with HIV climbs to 33% (7). Heterosexual transmission of HCV is a subject of discussion. We can conclude from multiple studies that HCV is not transmitted efficiently between men and women if at all (8). However an increasing number of HCV infections are observed in HIV+ men who have sex with men (MSM). This latter group have recently been identified as part of an outbreak of acute HCV. In those reports, acquisition of acute HCV is associated with high-risk sexual behavior, ulcerative sexually transmitted infections and use of recreational, non-IV drugs such as methamphetamine. This drug may enhance hepatitis C virus replication in human hepatocytes (9). It is also noteworthy that HCV RNA has been detected more frequently (38 vs 18%) in the semen of co-infected patients compared to HCV mono-infected (10). There are reports of needlestick exposure where HIV and HCV have been simultaneously transmitted (11, 12). Vertical transmission of HCV is also of particular matter in this special population. The perinatal transmission of HCV is estimated to be two- to fourfold higher among HIV-infected women (13, 14). In the mono-infected patients, the risk of transmission is increased at a higher HCV RNA level. One study compared HCV transmission in mono- and co-infection and found a similar risk at higher RNA levels (>6 log₁₀IU/ml). But, the risk was increased in
the co-infected patients at lower RNA levels (15). Vertical transmission is not affected by the mode of delivery (16–18). Elective caesarean section is generally not indicated to avoid the transmission of hepatitis C; it is the HIV viral load that determines the mode of delivery (19). Breastfeeding is not an option in co-infected women because HIV infection contraindicates it in the developed world.

2.2. Natural History

Co-infection exacerbates the natural history of HCV. HCV mono-infection may not be progressive in all patients. The same assumption may not be true in HIV-co-infected patients. This special population has a more aggressive form of disease characterized by rapid fibrosis progression, increased incidence of cirrhosis and end-stage liver disease (ESLD), and development of hepatocellular carcinoma (HCC) and death. This is more evident as the antiretroviral therapy (ART) era leads to a lower mortality from AIDS, permitting the concomitant viral hepatitis to evolve. Serum and hepatic HCV RNA levels are higher in co-infected individuals (20, 21). The correlation between higher viremia and more advanced liver disease, however, has been inconsistently reported (21–23). HCV RNA levels further increase after initiation of ART, especially in patients with low pre-treatment CD4 cell count (<350 cells/mm³) (24). Another distinctive feature of co-infection is the lower likelihood of patients to spontaneously clear HCV after the acute infection. The rate of spontaneous viral clearance is about 25–30% in HCV mono-infected patients following acute hepatitis C (25). In one study, HIV-negative patients cleared HCV two times more often than HIV-positive ones and in this latter group, clearance was less likely to occur at a lower CD4 cell count (<200/mm³) (7). The method of acquisition could also influence the rate of spontaneous clearance: it has been suggested that sexual transmission is more likely to lead to spontaneous clearance in co-infected individuals (26). Interestingly, hepatitis B virus co-infection also increases the likelihood of HCV clearance in co-infected individuals (7, 27).

HIV infection accelerates the progression of liver fibrosis in co-infected individuals (23, 28). More advanced liver fibrosis is associated with lower CD4 cell count (<500 cells/mm³) and HIV viremia (29, 30). Those findings suggest that early intervention with ART may help to slow HCV progression in co-infected patients. This hypothesis is supported by some investigators (31), but rejected by others (32–34). While early findings suggested that higher CD4 cell counts predicted a more successful response to HCV treatment (35), results from large trials did not find any association. Besides the CD4 cell count, other factors that
have been associated with more rapid hepatic fibrosis progression rates in co-infected patients are high alcohol intake (>50 g/d), age at HCV infection (over 25 years), male gender and, recently, acute HCV infection (36, 37). In this last study, 11 HIV-positive patients without any pre-existing liver disease presented with acute hepatitis C, and underwent a liver biopsy. The histology showed moderately advanced fibrosis (Metavir score stage 2 fibrosis) in nine of them (82%). Fibrosis can lead to cirrhosis and once HIV-co-infected patients present with decompensated liver disease, the chance of survival at 3 years is about 30%, half what is expected for their mono-infected counterparts (38–40). The expected time after HCV exposure to develop cirrhosis in mono-infected patients is about 30 years. Studies looking at the rate of fibrosis progression have estimated that this period is reduced by half in co-infected individuals (41). HCC is also more likely to develop in this population, and do so at a younger age, and after a shorter interval of time from acquisition of HCV infection (42).

Whether HCV has a deleterious effect on HIV is unclear (43–45). Co-infection with HCV does not seem to affect the risk of HIV disease progression (3, 43).

The effect of antiretroviral therapy (ART) on the evolution of liver disease in co-infection is controversial. The concept that keeping HIV undetectable, there by improving cellular immunity, would stop or slow liver injury makes perfect sense. ART has been found to be related to lower liver-related mortality (39). However, ART can cause liver injury by different mechanisms and HCV, in fact, increases the risk of ART-related hepatotoxicity (46, 47). Investigators from the D:A:D study reported an increased risk of liver-related deaths with long-term ART-exposure. These data should be interpreted with caution. ART results in lower AIDS-related deaths (hence, increased rate of liver-related mortality) which allows co-infected individuals to live longer and develop liver-related complications (48). On the other hand, one study reported that successful treatment of hepatitis C reduces the risk of ART toxicity (49). This suggests that in co-infected patients with stable untreated HIV infection, HCV therapy should be addressed first. In any case, when there is an indication to treat HIV, therapy should be instituted because benefit clearly outweighs risk.

Every HIV-infected individual should be screened for the presence of HCV antibody (hepatitis A and B serologies are also indicated). The two infections share the same transmission risks. Hence, the prevalence of co-infection is high and justifies screening (50). A positive result may indicate past/resolved infection or current/chronic disease. This distinction is made by measurement of the quantitative serum viral load and viremia indicates chronic disease. A negative result
can, however, represent a false-negative in this immunosuppressed population (mostly when CD4 cell count is <100 cells/mm³) or in the presence of chronic kidney disease and warrants confirmation with serum viral load. Viremia establishes the diagnosis of chronic HCV infection. A negative result can also reflect the window period during acute infection and testing should be repeated if clinically indicated. Apart from the first visit screening test, anti-HCV antibody testing should be performed when a patient presents with liver enzyme elevation, but should also be part of a periodical blood check-up in patients with high-risk sexual behavior (mostly MSM) or drug exposure. Some experts believe that yearly HCV testing is appropriate in HIV-infected individuals. Once the diagnosis is established, the standard test to stage the disease is the liver biopsy. It is also helpful to guide the decision to initiate treatment, inform patients and physicians of prognosis, and provide an accurate diagnosis when the etiology is multifactorial or diagnosis is uncertain. It is not an absolute prerequisite to treatment initiation. The invasive nature of this technique aroused interest among researchers in finding alternative non-invasive strategies to evaluate liver fibrosis. It is not in the scope of this chapter to focus on the different assays available. Serum biomarkers (HCV FibroSURE™, FIB-4, APRI score, others) can help differentiate earlier stages of fibrosis from cirrhosis but are unprecise in the evaluation of intermediate stages of fibrosis (51, 52). Transient elastography (FibroScan) estimates the extent of hepatic fibrosis by measuring liver stiffness (53, 54). Until now, results from studies in co-infected individuals do not significantly differ from mono-infected ones and further research is underway to determine the role of those noninvasive tests in this population.

2.3. Treatment of Co-infection

SVR rates are lower in co-infected patients. Multiple studies report a lower rate of hepatitis C treatment initiation among co-infected patients when compared to their mono-infected counterparts (55). This is explained by physician’s lack of trust in patient’s adherence, reluctance because of potential drug interactions or adverse events with ART, and perception of poor treatment outcomes. However, the accelerated course of liver disease prompts initiation of treatment in many cases. This famous sentence in the treatment of coronary artery disease: “time is muscle” can certainly be used here: “time is liver”. The decision to treat is based on the evaluation of benefits against risks associated with the treatment’s toxicity.

Which baseline factors are associated with sustained virological response (SVR)? As in HCV-mono-infection, the two major ones
are the HCV genotype and the baseline viral load (HCV RNA). Findings from the APRICOT trial revealed that a baseline viral load \( < 400,000 \text{ IU/ml} \) has an odds ratio (OR) of 4.77 in predicting SVR, while the genotype has one of 2.87 (56, 57). Other factors can help to predict SVR and should be taken into account in an effort to increase response (Table 1). Patients who are more likely to respond to treatment are those with genotype 2 or 3, genotype 1 with low baseline viral load \( (< 400,000 \text{ IU/ml}) \), no, or minimal fibrosis. The likelihood of response does not represent an indication to treat or not. This is an independent decision.

<table>
<thead>
<tr>
<th>Potentially Modifiable</th>
<th>Non-modifiable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low BMI</td>
<td>Low baseline viral load</td>
</tr>
<tr>
<td>No polysubstance abuse</td>
<td>Genotypes 2–3</td>
</tr>
<tr>
<td>Use of adjuvant growth factors during treatment if needed</td>
<td>RVR and EVR</td>
</tr>
<tr>
<td>Optimal peg-IFN and RBV doses</td>
<td>Host genetic (Caucasian)</td>
</tr>
<tr>
<td>Length of treatment</td>
<td>Younger age</td>
</tr>
<tr>
<td>Adherence to treatment</td>
<td>No or minimal liver fibrosis stage</td>
</tr>
<tr>
<td>No ddI or AZT use</td>
<td>No psychiatric comorbidity</td>
</tr>
<tr>
<td>Lack of IR(^*)</td>
<td></td>
</tr>
<tr>
<td>Lack of hepatic steatosis(^*)</td>
<td></td>
</tr>
</tbody>
</table>

SVR, sustained virologic response; BMI, body mass index; peg-IFN, pegylated-interferon; RBV, ribavirin; ddI, didanosine; AZT, zidovudine; RVR, rapid virologic response; EVR, early virologic response; IR, insulin resistance.

\(^*\)Potentially modifiable: No studies have shown yet that improving those factors lead to a higher SVR.

The ultimate goal of therapy is eradication of the virus in view of stopping or even reversing liver injury and its later complications (58). In practice, this is assessed by an undetectable level of plasma HCV RNA at the end of the treatment (end of treatment response, EOTR) followed by the sustained undetectability after 6 months off-treatment (sustained virologic response, SVR). SVR correlates with long-term “cure”, measured at 5 years, in co-infected as in mono-infected patients in 99% of cases (48, 59).
When considering treatment for a patient, “First, do no harm” (see Table 2). Every co-infected patient who is not considered a candidate for treatment at one point should be followed closely as the dynamic interactions between the viruses, the host, and the environment can sooner or later favor the benefit over the risk of treatment. Experts consider a liver biopsy every 2–3 years in this population (60).

### Table 2

<table>
<thead>
<tr>
<th>Absolute Contraindications</th>
<th>Relative Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergy to peg-IFN or RBV</td>
<td>Active drug use</td>
</tr>
<tr>
<td>Active opportunistic infection</td>
<td>HIV suboptimally controlled</td>
</tr>
<tr>
<td>Decompensated liver disease (Child-Pugh B or C)</td>
<td>Renal insufficiency</td>
</tr>
<tr>
<td>Severe cardio-pulmonary disease</td>
<td>Depressive disorder</td>
</tr>
<tr>
<td>Sarcoïdosis</td>
<td>Hematologic abnormalities*</td>
</tr>
<tr>
<td>Uncontrolled autoimmune diseases</td>
<td></td>
</tr>
<tr>
<td>Pregnancy or not willing to use</td>
<td></td>
</tr>
<tr>
<td>birth control</td>
<td></td>
</tr>
</tbody>
</table>

*Correction of hematologic abnormalities may allow treatment.

Active drug use is changed from an absolute to a relative contraindication (61). The reduced chance of response in this population is in part due to poorer adherence and higher discontinuation rate. Efficacy of treatment is reduced by the loss of cellular immunity associated with chronic drug abuse. The decision to start treatment must be individualized, and is based on patient commitment, and a relation of trust between patient and caregiver.

Is there a CD4 cell count threshold over which treatment is effective and safe? The answer is no. Over the past few years, different thresholds have been proposed.

However, the AASLD guidelines do not specify any. Many experts agree that treatment should be individualized. CD4 cell count should not prevent treatment in a patient for whom benefit outweighs risk. Liver fibrosis progresses more rapidly in co-infected patients, and to a greater extent in those with lower CD4 cell counts. Treating patients offers the opportunity to stop liver injury and prevent progression to ESLD. In the major HCV treatment studies in HIV (where in some, patients could be enrolled with a CD4 cell count as low as 100
cells/mm³), SVR was not related to the CD4 cell count. HIV viremia, however, is an important factor particularly in patients with lower CD4 cell count. In clinical trials, the safety and efficacy of treatment could not be established at a count lower than 200.

HCV therapy can exacerbate a pre-existing mental illness or lead to irritability, anxiety, and depression in people without any underlying psychiatric condition. Patients must be aware of this possibility so they can recognize early symptoms and seek help for proper management. Early intervention can improve the patient’s quality of life during treatment, prevent more severe psychiatric manifestations following interferon administration (e.g., suicidal attempt), and maintain adherence to treatment. We recommend a thorough psychiatric evaluation prior to treatment and a low threshold for prophylactic antidepressant use. A psychiatry referral and follow-up during therapy is sometimes needed (62).

The standard of care for the treatment of co-infected patients is pegylated-interferon (peg-IFN) and weight-based ribavirin (RBV). The duration of treatment is 48 weeks independent of genotype. There are several different studies on which the recommendations are based (see Table 3). The reader is referred to the guidelines for HCV treatment:

- Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents (63)
- Diagnosis, management, and treatment of HCV (50)
- Care of patients co-infected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV–HIV International Panel (60)
- Short statement of the first European consensus conference panel of the treatment of chronic hepatitis B and C in HIV-co-infected patients (64).

APRICOT is the largest study (multicentered) with 868 patients enrolled. Co-infected patients (all genotypes) not previously treated were randomized to one of the three arms to receive either (1) peg-IFN alpha-2a 180 μg + RBV 800 mg, (2) peg-IFN alpha-2a 180 μg + placebo, or (3) standard interferon (3 M IU 3X/wk) + RBV 800 mg. Treatment duration was 48 weeks. With an overall SVR of 40% (compared to 20% in the peg-IFN alone arm and 12% in the standard IFN arm), peg-IFN alpha-2a and RBV 800 mg was found as the combination of choice. For genotype 1 compared to genotypes 2 and 3, the SVR rate was 29 and 62%, respectively. This trial also found that (1) overall, and with genotype 1 infection, patients with higher baseline HCV RNA levels (>800,000 IU/ml) have lower rates of SVR compared to low pretreatment levels, (2) the CD4 cell count decreases during therapy, but the mean percentage of CD4+ lymphocytes increases, (3) the high negative predictive value of the absence of an EVR to predict virologic
Table 3
Summary of the five Pivotal Studies of Peg-IFN + RBV in Co-Infected Patients

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<thead>
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</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>868</td>
<td>133</td>
<td>412</td>
<td>95</td>
<td>389</td>
</tr>
<tr>
<td>Peg-IFN (dosage)</td>
<td>alpha-2a</td>
<td>alpha-2a</td>
<td>alpha-2b</td>
<td>alpha-2b</td>
<td>alpha-2a</td>
</tr>
<tr>
<td></td>
<td>(180 µg/wk)</td>
<td>(180 µg/wk)</td>
<td>(1.5 µg/kg/wk)</td>
<td>(100 vs 150 µg/wk)</td>
<td>(180 µg/wk)</td>
</tr>
<tr>
<td>RBV dosage</td>
<td>800 mg</td>
<td>600–800 to 1000 mg dose-esc.</td>
<td>800 mg</td>
<td>600–1200 mg (w-based)</td>
<td>1000 vs 1200 mg (w-based)</td>
</tr>
<tr>
<td>Duration (weeks)</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48–72</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>61%</td>
<td>77%</td>
<td>59%</td>
<td>55%</td>
<td>49%</td>
</tr>
<tr>
<td>SVR Genotype 1</td>
<td>29%</td>
<td>14%</td>
<td>17%</td>
<td>38%</td>
<td>35%</td>
</tr>
<tr>
<td>SVR Genotypes 2–3</td>
<td>62%</td>
<td>73%</td>
<td>44%</td>
<td>53%</td>
<td>72.4%</td>
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<tr>
<td>SVR All genotypes</td>
<td>40%</td>
<td>27%</td>
<td>27%</td>
<td>44%</td>
<td>49.6%</td>
</tr>
<tr>
<td>SVR Control arm</td>
<td>12%</td>
<td>12%</td>
<td>20%</td>
<td>21%</td>
<td>–</td>
</tr>
</tbody>
</table>

APRICOT, AIDS Pegasys Ribavirin International Co-infection Trial; ACTG, AIDS Clinical Trials Group; PRESCO, Peginterferon Ribavirin ESpaña Co-infection, w-based, weight-based; dose-esc., dose-escalation; SVR, sustained virological response.
failure applies to co-infected as well as mono-infected patients, and (4) safety issues are mostly hematologic in this population and comparable to HCV mono-infected patients.

The RIBAVIC study had a similar design to the APRICOT trial, but was meant to compare standard interferon (3 MU three times a week) with peg-IFN alpha-2b (1.5 µg/kg/wk) (65); 412 patients were enrolled. SVR rates of 27% (17% for genotypes 1 and 4) in the peg-IFN compared to 20% in the regular interferon arm were reported. Compared to the APRICOT trial in which the SVR rate was 40%, the RIBAVIC population included a higher proportion of patients with bridging fibrosis and cirrhosis (40%), which is a negative prognostic factor for SVR. Many patients had adverse events not related to treatment, discontinued treatment and could not achieve SVR. However, SVR rates in the control arm were 20% in both studies. The number of serious adverse events, although similar in both groups, was higher than reported in the mono-infected population with 11 cases of pancreatitis/hyperlactatemia in patients taking didanosine (ddl) as part of their ART regimen.

Another major study, ACTG 5071, randomized 133 subjects to receive either peg-IFN alpha-2a (180 µg/wk) or standard interferon alpha-2a + RBV in a dose-escalation schedule: 600 mg for 4 weeks, then 800 mg for 4 weeks, then 1000 mg for 48 weeks (66). They again demonstrated the superiority of peg-IFN formulation over regular interferon with a SVR rate of 27% (14% in genotype 1 vs 73% in other genotypes) compared to 12% in the standard interferon arm. A liver biopsy was performed at week 24 of treatment in patients with no virologic response, and histologic improvement was present in 35% of those patients in both arms. This suggests that benefits from the therapy extend beyond virologic control. This study also pinpoints the importance of the cumulative dose of ribavirin early in the treatment of hepatitis C. The inadequate dosage of RBV may have explained the low SVR (high relapse) rates observed.

The fourth important study published the same year was the one by Laguno et al. Standard interferon (3 MU 3 times a week) was compared to peg-IFN alpha-2b (100–150 µg/wk), but with weight-based RBV 800–1200 mg for 48 weeks (67). Ninety-five patients, of whom 30% had bridging fibrosis or cirrhosis, were randomized. Forty-four percent achieved a SVR, 38% of those with genotype 1, and 53% of those with genotypes 2 and 3 (genotype 2 or 3 infections with HCV RNA <800,000 IU/ml were treated for 24 weeks only). Safety analyses also showed a higher rate of side effects compared to studies in mono-infected patients, particularly depressive symptoms in 43% patients. They also diagnosed nine cases of mitochondrial toxicity in patients receiving ddi/d4T.
The most recent trial is PRESCO (2007) aimed at determining the role of weight-based RBV and extended duration of therapy in co-infected patients (68). It enrolled 389 patients who received peg-IFN alpha-2a (180 μg/wk) plus ribavirin (1000 or 1200 mg according to weight). Genotype 1-infected participants were treated for 48 vs 72 weeks while the length of therapy in genotype 2 or 3 was 24 vs 48 weeks. They achieved an overall SVR rate of 49.6%; 35% for genotype 1 and 72.4% for genotype 2 or 3. SVR rates were significantly greater in the 72 weeks arm, but 36/45 (80%) patients in the genotype 1 group withdrew from treatment. Therefore those results must be interpreted cautiously. High-dose ribavirin was well tolerated. These studies underlie guideline’s recommendations for HCV treatment in HIV-co-infected patients.

Every patient for whom the benefit outweighs the risk should be offered treatment. The standard of care is peg-IFN alpha (2a or 2b) + weight-based RBV in genotype 1-infected patients, and fixed-dose of 800 mg in genotypes 2 and 3 infections, all for 48 weeks. It is reasonable to use weight-based RBV in overweight genotypes 2- and 3-infected individuals. The expected SVR rates are 14–38% with genotype 1 infection and 44–73% with genotype 2 or 3. Traditionally, response is assessed at week 12 (EVR); this measure correlates with SVR in mono- as in co-infected patients (57, 68, 69). In patients with less than a 2 log_{10}IU/ml drop from baseline HCV RNA load, treatment should be discontinued since SVR can be achieved in only 2% of patients without EVR. More recently, a substudy from RIBAVIC found that when the viral load cannot be suppressed below 460,000 IU/ml at week 4, the chance of achieving SVR is null (negative predictive value of 100%) (70). Following this report, another substudy, this time from APRICOT, demonstrated that RVR is the best predictor for SVR while the absence of an EVR is the best predictor of the lack of SVR (71) (Table 4).

<table>
<thead>
<tr>
<th>Table 4</th>
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<tbody>
<tr>
<td>Major Differences in Treatment of HCV in Co-Infection vs Mono-Infection</td>
</tr>
<tr>
<td>Higher Pre-treatment HCV RNA levels</td>
</tr>
<tr>
<td>Lower kinetic of RNA decrease</td>
</tr>
<tr>
<td>Lower rate of SVR</td>
</tr>
<tr>
<td>More side effects</td>
</tr>
<tr>
<td>More treatment discontinuation</td>
</tr>
</tbody>
</table>
Once treatment is initiated in co-infected patients, be alert for adverse events. This population may experience more side effects from treatment. Hematologic abnormalities are frequent and growth factors can correct anemia (recombinant human erythropoietin) and neutropenia (filgrastim) and should be used in order to avoid dose reduction which compromises chances of response. There is no FDA-approved treatment to increase platelets although some new agents are under study. Other more common side effects in these patients are psychiatric (see previous section) and gastrointestinal such as nausea and diarrhea. Higher rate of side effects translates into decreased adherence to therapy and discontinuation of treatment. Second, there is a risk for drug–drug interactions with the use of antiretroviral agents. The use of didanosine (ddI) and stavudine (d4T) is now contraindicated with RBV therapy because of the cumulative mitochondrial toxicity that causes hyperlactatemia, pancreatitis, hepatic failure, and even death in some patients (65, 72). When it comes to HCV treatment, ddI means “don’t do it.” The newer nucleoside reverse transcriptase inhibitors (NRTIs) are much less prone to cause such side effects, but the risk exists and physicians have to be aware of the clinical presentation of hyperlactatemia: nausea and vomiting, extreme fatigue, abdominal and muscle pain. The use of zidovudine (AZT) should also be avoided because of the risk of worsening anemia (73). Finally, the choice of ART may be related not only to safety issues, but also to efficacy. Co-infected patients on ART including tenofovir (TDF) have a better response to peg-IFN + RBV compared to abacavir (ABC)-based regimens. SVR rates of 45 vs 29% have been reported in one study and 36.4 vs 7.1% in another one (74–76).

Consider early treatment of acute hepatitis C. The recent outbreaks of acute hepatitis C in MSM have increased our knowledge of the natural history of hepatitis C and its treatment in HIV individuals. We know that the likelihood of spontaneous clearance is lower in mono-infected patients. Treatment of HCV in the acute stage is associated with a lower rate of SVR compared to mono-infected patients (77, 78). However, when compared to chronic disease, treatment of acute HCV infection in HIV-positive patients leads to a higher SVR if treatment is initiated early (78–80). To improve the chance of response to treatment, initiation should be considered before infection becomes chronic, but after 12 weeks from acquisition to give the patient the chance to spontaneous clear (81). We have found it helpful to closely monitor the serum viral load during the first months (every 1–2 weeks) to determine if spontaneous clearance is occurring. Treatment recommendation is peg-IFN plus weight-based RBV for 24 weeks, independently of genotype. Factors associated with SVR in the acute infection differ from the chronic stage (Table 5) (60).
Table 5
Factors Associated or Not with SVR in Acute HCV in HIV

<table>
<thead>
<tr>
<th>Predictors of SVR</th>
<th>Factors not Associated with SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes 2 and 3</td>
<td>Age</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>CD4 cell count</td>
</tr>
<tr>
<td>RVR</td>
<td>Baseline HCV viral load</td>
</tr>
<tr>
<td></td>
<td>HIV viremia</td>
</tr>
<tr>
<td></td>
<td>Symptomatic infection</td>
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</table>

3. **CO-INFECTION WITH HEPATITIS B (HBV)**

The population of patients dually infected with HBV and HCV warrants special consideration. Both viruses are transmitted parenterally. The prevalence of co-infection varies, with an estimated 7–30% HCV antibody in HBsAg-positive patients (82, 83). In HCV seropositive individuals, HBsAg is found in 2–10% (83, 84). In a study of hepatitis B core antibody (HBeAb)-positive patients, up to 50% had HCV antibody (85). The prevalence of co-infection varies, depending on endemicity of both viruses and risk factors for transmission. Populations at risk for co-infection are IVDUs, patients who received blood transfusions, dialysis patients, renal transplant patients, and others (86, 87).

HBV and HCV influence each other. Usually, their interaction is characterized by the dominance of one or the other, frequently, HCV (88, 89). The dominance may reverse over time (89). In vitro, HCV suppresses HBV replication (90–92). In co-infected patients, high HCV viremia is often observed with low HBV DNA titers (93–95). Dominance of HBV and dual reciprocal interactions has also been demonstrated (96–98). One of the factors underlying the dominance of one virus is the timing of acquisition. Simultaneous infection with both viruses has been reported. In one small series of five cases, HBsAg appeared later, and at lower level and serum ALT was less than HBV mono-infection (99). In endemic countries where HBV is mostly transmitted vertically, hepatitis C presents as a superinfection (100). It has been linked to 23.1% chance of fulminant hepatitis in the setting of co-infection compared to 2.9% in mono-infected HCV individuals (101). In chronic HBV, superinfection with HCV can lead to HBeAg seroconversion and HBsAg loss (102, 103). When those patients were followed-up over a 20-year period, they observed higher rates of hepatic decompensation (48 vs 34%) and HCC (32 vs 10%) compared to
HBV mono-infection. When hepatitis B is the superinfection (rarer), fulminant hepatitis can also be seen (104). In one study, encephalopathy or ascites formation was present in 29 vs 0% in acute HBV mono-infection (105).

Many studies focused on the accelerated liver disease progression in the HBV/HCV co-infection. Dual infection results in an accelerated rate of fibrosis (96). This leads to a higher risk of cirrhosis and decompensated liver disease (88, 96). Co-infection is an independent risk factor for HCC development (106, 107). In a study of individuals with compensated cirrhosis, the risk of developing HCC was 2.0 (per 100 person years) in HBV, 3.7 in HCV, and 6.4 in co-infection. In the HBV/HCV co-infected group, there was a 45% chance of HCC occurrence at 10 years. Up to a ninefold increase in risk compared to HCV mono-infection has also been reported (108, 109). This study and others support synergism between HBV and HCV in carcinogenesis (106, 107). Patients should be followed closely every 3–6 months with imaging and alpha-fetoprotein levels. Recently, investigators evaluated the cause of death in patients with chronic HCV and/or HBV (110). All-cause mortality was significantly increased in the three groups. In the co-infected, standardized mortality ratio (SMR) was 8.5 compared to 2.3 in HBV and 5.8 in HCV. Excess liver-related mortality was, respectively, 46.2, 21.7, and 35.5.

There is no established standard of care for co-infection with HCV and HBV (111). Many studies evaluated standard interferon-alpha monotherapy, which has activity against both viruses (112, 113–117). The results for HCV were comparable to HCV mono-infected (SVR rates of 17–44%). Response of HBV and loss of HBeAg were very different between studies. The combination of interferon plus RBV was then assessed and HCV SVR rates were increased (43–69%) (118–120). In one study, 42 patients were enrolled (120). At 6 months, HBV DNA was suppressed in 31%, 50% had an HBeAg seroconversion, and 14% lost HBsAg. One of the observations made during those trials is the frequent HBV rebound in viremia once HCV is cleared (hepatitis flare). One trial investigated the combined use of standard interferon (12 months) and lamivudine (12 + 6 months) in eight patients (121). Half achieved an SVR. Only three had HBV DNA undetectable at month 18 (5 at month 6) and two seroconverted their HBeAg.

Peg-IFN and RBV is the standard of care for HCV mono-infection and for chronic HBV, peg-IFN monotherapy is an option. Two case reports of the use of peg-IFN-alpha-2b + RBV in co-infection have been published (122, 123). The two patients achieved SVR. One had an HBeAg seroconversion while the other had a HBsAg seroconversion. Based on these data, the Hep-Net B/C Co-infection Study Group
initiated a trial to prospectively evaluate the efficacy of this regimen for 48 weeks. Nineteen patients (HCV dominant) were enrolled. Ten had genotype 1, and nine had genotypes 2 and 3 infections. Seventy four percent achieved an SVR. Two out of six patients with HBV DNA viremia at the start were suppressed (33%). HBV viremia was detected in 4 out of 13 patients with HBV DNA undetectable at the start of treatment. One of them required treatment with an HBV polymerase inhibitor. Safety did not differ from the mono-infected patients. This trial suggested that peg-IFN + RBV should be the treatment of choice for co-infection (HCV dominant) and that response to HCV in co-infected patient is equivalent to that of mono-infected patients.

The first step in evaluating a co-infected patient with HBV/HCV is to assess which virus is dominant to determine treatment needs (111). This is done by ordering a complete serological and virological panel for both infections. A threshold of $10^4$ IU/ml is used for HBV DNA levels. The three scenarios below can help to guide treatment.

*First scenario:* High HCV RNA level and HBV DNA $<10^4$ IU/ml: standard of care for HCV treatment (peg-IFN + RBV) and follow HBV DNA closely.\(^1\)

*Second scenario:* High HCV RNA level and high HBV DNA $>10^4$ IU/ml: peg-IFN + RBV X 3 months. If HBV DNA does not respond add entecavir or adefovir. Continue treatment for HCV according to genotype.\(^2\)

*Third scenario:* High HBV DNA and undetectable HCV RNA: Start HBV treatment with HBV polymerase inhibitors.

4. ETHNIC VARIATIONS IN HCV

4.1. Latinos and African Americans\(^3\)

Latinos are the largest minority in the United States with, in 2004, 40.5 million people representing 14.2% of the total U.S. population (124). According to the U.S. Census Bureau, “Hispanic” or “Latino” refers to persons who trace their origin or descent to Mexico, Puerto Rico, Cuba, Spanish-speaking Central and South America countries, and other Spanish cultures (124). Healthcare is more difficult to access for this population because of cultural and linguistic barriers,

\(^1\) Our preference would be to use peg-IFN + RBV for 3 months, then add entecavir or tenofovir, complete HCV treatment and continue polymerase inhibitor after peg-IFN + RBV stop.

\(^2\) See Foot note 1.

\(^3\) In the text, comparison between Latinos and African Americans is made with Caucasians because reference data are more complete for this population.
socioeconomic factors (lack of medical insurance), and possible discrimination from the medical care community. Few data exist on the prevalence of hepatitis C in the U.S. Latino population and derive mostly from the third National Health and Nutrition Examination Survey (NHANES III) in which only Mexican Americans were represented. From 1988 to 1994, the prevalence of anti-HCV antibodies was 2.1% in this group compared to 1.5% in the non-Hispanic whites and 3.2% in the non-Hispanic black population (125). In the same cohort, but from 1999 to 2002, the prevalence of hepatitis C was 1.3% compared to 1.5% in non-Hispanic whites and 3.0% in non-Hispanic blacks (126). Even though Mexican Americans constitute about 2/3 of the U.S. Latino population, those data can hardly be applied to this very diverse community living in the United States. For example, a random seroprevalence survey in 21-to-64-year-old adults from Puerto Rico in 2001–2002 found a prevalence of anti-HCV antibodies as high as 6.3% (127, 128). The principal risk factor for acquisition of hepatitis C in the United States is IVDU as it is for the general population. However, more risky behavior was observed among Mexican Americans and Puerto Ricans who injected more often, were more prone to share paraphernalia, and not disinfect skin prior to injection (129, 130).

In the U.S. Census Bureau survey of March 2004, African Americans constituted 12.5% of the U.S. population with 36.1 million people (124). To compare with Latinos (Mexican Americans in this cohort), data from the NHANES III showed in non-Hispanic blacks, a prevalence of anti-HCV antibody, from 1988 to 1994, of 3.2% compared to 1.5% in the non-Hispanic whites (125) and of 3.0% in the survey of 1999–2002 (125, 126). Hence, in this population, prevalence of hepatitis C is, at least, double that of Caucasians. Non-Hispanic black men between 40 and 49 years old had the highest prevalence with 9.8%. Americans are mostly infected with genotype 1 virus, but an even greater proportion of African Americans carry this genotype (90%) compared to Latinos (74%) and Caucasians (76%) (131). Another peculiarity of this group is the higher occurrence of viremia with seropositivity. This suggests a less propensity to spontaneously clear the infection compared to other populations. Non-Hispanic blacks had 86.2% positivity of HCV RNA vs 73.6% in Mexican Americans vs 67.6% in non-Hispanic whites. Also, there was a difference according to gender in this population. Men had viremia in 97.8% compared to 70.2% in women (125).

Latinos infected with hepatitis C are more likely to be males, co-infected with HIV and have acquired HCV at a younger age compared to Caucasians (132). By themselves, all those characteristics have a negative impact on the prognosis of liver disease in hepatitis
C which has been demonstrated to be more aggressive in Latinos overall (129, 133). They have higher serum ALT, AST, and bilirubin and lower serum albumin levels compared to Caucasians, Asians, or African Americans (134). The majority of studies in this population report higher necroinflammatory grades on liver biopsy, faster progression of liver fibrosis, higher frequency of cirrhosis, and accelerated time to cirrhosis (129, 135–137). It is noteworthy that chronic alcohol use is also a cause of more rapid fibrosis progression and is frequent in this population (129). Data on HCC prevalence is sparse in Latinos. In one report from Florida, Latinos were twice as likely as Caucasians or African Americans to be diagnosed with HCC; there was no mention of the underlying liver disease in this population (138). Apart from the faster rate of fibrosis and cirrhosis, other underlying conditions in this population such as diabetes and obesity have been independently associated with HCC (139, 140). Insulin resistance, diabetes, obesity, and steatosis are more prevalent in this population (130, 141). In fact, Latino ethnicity is an independent risk factor for hepatic steatosis which leads to faster progression of fibrosis (see section on IR and diabetes) (137, 142).

Multiple studies show a slower progression of liver disease in African Americans (143–145). They report lower mean serum ALT values, necroinflammatory activity at liver biopsy, and liver fibrosis scores. Slower progression of fibrosis is also found (145). Contradictory results were also published where investigators found no difference between those two populations (135). However, mean alpha-fetoprotein (AFP) values are higher in cirrhotic African Americans compared to Caucasians, and the risk of developing HCC, which is rising in the United States, is further increased in this population by a factor of 3 (146, 147). Those patients also have a two to three times higher mortality rates when HCC is diagnosed compared to non-Hispanic whites.

The Latino population has a lower chance of SVR compared to Caucasians. In a few retrospective studies, Latino ethnicity is associated with lower SVR (148, 149). One of them aimed at determining factors associated with treatment outcome found a SVR rate of 23% in Latinos compared to 39% in Caucasians and 61% in Asians (only 14% in African Americans) (149). Latinos are underrepresented in all major prospective clinical trials that evaluated the efficacy of the actual treatment: peg-IFN + RBV. There is only one prospective trial designed to evaluate the standard of care in Latinos vs non Latino Caucasians infected with genotype 1 HCV: The Latino Trial (150). Emerging data show that, as hypothesized, SVR rates are lower in the Latinos: 33.5 vs 49.3% in Caucasians. Investigators reported a higher rate of treatment discontinuation in the Latino group, but for efficacy rather than safety
reasons. Many factors are thought to be responsible for the lower chance of response to treatment: higher stages of fibrosis, cirrhosis, comorbidities such as IR and diabetes, obesity, steatosis, and genetics.

African Americans have a lower chance of response to peg-IFN + RBV therapy compared to Caucasians (151–153). Contrary to the Latinos, many studies have been conducted that studied treatment response in African Americans. When this observation was first reported with standard interferon treatment, investigators suggested that it was the result of the greater proportion of genotype 1 in this group (156). Prospective trials were then undertaken. In 2004, two trials were published and each demonstrated a lower SVR in African Americans. One used peg-IFN alpha-2b + RBV (98% genotype 1) and SVR rates were 19 vs 52% in Caucasians (153, 154). A second trial used peg-IFN alpha-2a + RBV and SVR rates were 26 vs 39% (155). In 2005, a trial evaluated double dose peg-IFN alpha-2b + weight-based RBV in treatment-experienced patients (156). African Americans needed double dosing to have a SVR rate equivalent to other groups. The VIRAHEP-C study in 2006 (peg-IFN alpha-2a + RBV for 48 weeks) confirmed a real difference in treatment response, with SVR rates of 28% in African Americans vs 52% in Caucasians (151). This is not exclusive to genotype 1 infection: another study reported a SVR rate of 44% in African Americans treated for HCV genotype 2 or 3 infections compared to 84% in Caucasians (157). In 2007, the WIN-R trial evaluated the role of weight-based RBV plus peg-IFN alpha-2b (155, 158). Weight-based dosing was associated with a higher chance of response (SVR 21 vs 10%), but still lower than the 37% in Caucasians. Overall, treatment is well tolerated in this population. One trial reported a higher rate of neutropenia (37 vs 18%), but a lower rate of anemia necessitating dose reduction (24 vs 32%) (155). The possible factors that have been suggested to underlie this lower treatment response in African Americans, apart from the genotype, are genetic immune response to interferon, different HCV kinetics, insufficient medication dosages and adherence (159).

5. HCV AND INSULIN RESISTANCE/DIABETES

There is an increased prevalence of insulin resistance and diabetes in HCV. Insulin resistance (IR) is a state in which a given concentration of insulin is associated with a subnormal glucose response (160). In the normal state, insulin inhibits liver gluconeogenesis and increases glucose uptake by muscle and fat tissues in order to bring back the blood glucose to an optimal level. Insulin resistance is characterized by a failure to respond to insulin secretion resulting in hyperglycemia which
further stimulates the production of insulin by pancreatic beta-cells, leading to hyperinsulinemia. The most frequently used tests to estimate insulin resistance are the Homeostatic Model Assessment (HOMA) and the Quantitative Insulin Sensitivity Check Index (QUICKI) score which are far more convenient to perform than the hyperinsulinemic-euglycemic clamp, the gold standard (161, 162). See Fig. 1.

<table>
<thead>
<tr>
<th>HOMA-IR</th>
<th>QUICKI</th>
</tr>
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<tbody>
<tr>
<td>$I_0$ (uU/mL) X $G_0$ (mmol/L)</td>
<td>1</td>
</tr>
<tr>
<td>22.5</td>
<td>$\log (I_0 \text{ uU/ml}) + \log (G_0 \text{ mg/dl})$</td>
</tr>
</tbody>
</table>

**Fig. 1.** HOMA-IR and QUICKI calculation.

HOMA-IR, Homeostatic Model of Assessment of Insulin Resistance; QUICKI, Quantitative Insulin sensitivity Check Index score; $G_0$, fasting glucose; $I_0$, fasting insulin.

Insulin resistance and diabetes are two frequent comorbidities in the general population and their prevalence is increasing (163). In patients chronically infected with the hepatitis C virus, the prevalence has been found to be even higher with 40–70% fulfilling the criteria for diagnosis of IR (164). Moreover, in patients over 40 years of age, HCV has been associated with a two-fold increase in the incidence of diabetes type II (165). Multiple mechanisms have been proposed for the association between IR and HCV, but are not yet well understood (166). The risk factors associated with the development of diabetes mellitus in the general population apply to patients with HCV, but there are also some findings specific to HCV (165). One study found that hepatitis C virus is associated with IR, independently of visceral fat (167). Virus-specific factors influence the development of the IR state associated with chronic hepatitis C. More than one study emphasized the relationship between IR and high viral load (167–170). Insulin resistance is commonly found in genotypes 1 and 4 compared to other genotypes (168).

Steatosis is more common in chronic HCV and can be related to IR. Compared to seronegative patients, HCV patients are two to four times more likely to present with liver steatosis (166). Steatosis can manifest with any of the HCV genotypes, but the inherent pathophysiology for its development appears to differ in genotype 3-infected patients compared to non-genotype 3 infections. In the first case, steatosis is thought to result from direct viral cytopathic effects on the liver, independent of IR, and without metabolic risk factors (142, 171). In other genotypes, steatosis is closely associated with IR and can be referred
to as “metabolic steatosis” (172). Those two types of steatosis behave differently, at least in terms of impact on treatment response. Apart from genotype 3 and IR, other factors such as older age (over 40 years), high body mass index (BMI), diabetes mellitus, liver inflammation, fibrosis, and chronic alcohol use have been independently correlated to steatosis (173). In genotypes other than 3 with metabolic steatosis, IR is an independent predictor of advanced hepatic fibrosis. In genotype 3, the presence of steatosis is not associated with progressive liver disease, but the liver fibrosis is related to IR (174). In another study, in all genotypes, IR was a predictor of liver fibrosis, independent of steatosis (168).

Patients with insulin resistance and diabetes in chronic HCV have more severe liver disease and decreased response to treatment compared to the general population. This points out the need for targeted interventions. Numerous studies have established a link between IR and liver fibrosis, which is a prerequisite to cirrhosis (171, 175–178). As mentioned earlier, HCC risk is correlated to obesity and diabetes, two states characterized by IR.

IR and steatosis are both associated with a decreased chance of SVR. For a complete review of studies in this field, the reader is referred to Harrison, S.A. (166). In genotype 1-infected patients, pre-treatment IR, as measured by the HOMA-IR score, inversely correlated with SVR rates. In one study, peg-IFN + RBV led to an overall SVR rate of 43.4%. When SVR rates were analyzed according to HOMA-IR scores, investigators found a 60.5% SVR in patients without IR vs only 32.8% in those with an HOMA-IR greater than 2. SVR rates steadily decreased at higher HOMA-IR scores (179). In patients who responded to treatment, HOMA-IR scores decreased significantly during treatment; others reported the same results (180). In a study of genotypes 2 and 3 patients, those with a HOMA-IR of less than 2 had a 6.5-fold increase in SVR. In addition, BMI and hepatic fibrosis were independently correlated to IR (181). We know from a few studies that IR influences virological response from the beginning of treatment. For example, HCV RNA decreases significantly less at 24 hours in patients with higher HOMA-IR scores. With a HOMA-IR score of 4 or more, no one attained a complete early virological response (EVR) (182). In another study, every patient with HOMA-IR of less than 2 achieved an EVR (partial or complete) compared to only 61.5% of those with higher scores. In HIV co-infected patients undergoing treatment, IR also affected chances of RVR and EVR (183, 184). Independent from IR, steatosis decreases chances of SVR. The role that genotypes play in steatosis is still unclear at present (185–187).

Until now, the majority of studies focused on IR in relation to treatment response. IR does decrease the chance of SVR. At least one ques-
tion remains: does improving baseline IR result in higher SVR rates? There are no available data at the moment to answer this question, and guidelines do not yet address screening for IR or how to manage it. Trials are underway to determine the effectiveness of insulin sensitizers (metformin and thiazolidinediones) in lowering IR in patients with HCV, and the impact on SVR. Results from one study, the INSPIRED-HCV, were recently published (188). Patients with HCV and IR who previously failed treatment were enrolled to receive the standard of care + pioglitazone 15 mg. Five patients were enrolled and the study was stopped because of no satisfactory EVR. Explanations for this failure are multiple. First, pioglitazone was started simultaneously with peg-IFN + RBV and no single patient had a satisfactory HOMA-IR score (<2) at week 12 of treatment. Allowing for an optimal HOMA-IR score prior to treatment makes more sense, and the pioglitazone dose was probably too low. Patients had advanced liver disease: two patients had cirrhosis and two others had a stage 3 fibrosis (Metavir score) at liver biopsy 3 and 4 years earlier. So those results clearly do not discourage further studies with different approaches.

Based on expert opinion, patients should be tested for IR at baseline when treatment is considered, at least to serve as an indicator of treatment response. We also encourage patients to lose weight, exercise and get better control of their diabetes, if needed, prior to treatment. When IR is present, insulin sensitizers such as metformin or thiazolidinediones appear to be safe in this population and are an interesting option (189). They should be used with the goal of improving IR before peg-IFN + RBV treatment is instituted until we have more data from the ongoing trials.

6. HCV IN CHRONIC KIDNEY DISEASES (CKD)

There is a higher prevalence of HCV infection in patients with CKD, especially in those on hemodialysis (HD), than in the general population. Anti-HCV seroprevalence among HD patients ranges between 7 and 40% in developed countries and between 8 and 10% in the United States (190, 191). Conversely, the reported decline in HCV prevalence in recent years is associated with reduced blood product requirements through routine use of erythropoietin in patients with chronic kidney disease (CKD) and anemia, successful serologic screening of blood donors and universal precautionary measures to prevent nosocomial HCV transmission within dialysis units (190).

The natural history of HCV in CKD is unclear because these patients have comorbid conditions such as diabetes and cardiovascular disease that reduce long-term survival. Serious complications of ESLD may
not manifest during the shortened life span of a patient maintained on HD due to the usual slow progression of HCV (192, 193). Nonetheless, several studies suggest that HCV-associated liver disease is a major cause of morbidity and mortality in patients with CKD (191). A meta-analysis that studied the effect of HCV on mortality showed that the summary estimate for adjusted RR of all-cause mortality with anti-HCV was 1.34 (95% CI, 1.13–1.59) (190). Data from international renal transplantation registries indicate that the death rate for dialysis patients with cirrhosis is 35% higher than in patients without it (194). Additionally, a multi-center, multinational study reported an excess of hepatocellular carcinoma (HCC) in CKD patients, most likely attributable to an increased prevalence of chronic viral hepatitis (195). Finally, there is data to suggest that HCV infection is a risk factor in the development of diabetes mellitus in pre- and post-transplant patients (196).

A number of small clinical trials have evaluated standard IFN monotherapy in HCV-infected patients on maintenance HD. A meta-analysis of 24 trials indicates a summary estimate of the SVR rate as 39% (95% CI, 32; 46), and 33% (95% CI, 19; 47) for genotype 1. Despite higher SVR rates in HD patients than in patients with normal renal function, there is lower tolerability to therapy and a high dropout rate of 19% (95% CI, 13; 26) (197).

Improved response rates with peg-IFN in large trials showed promise of possible use in CKD patients. There are no recorded significant differences in peg-IFN alpha-2a clearance between patients with normal and impaired kidney function (creatinine clearance >100 ml/min vs 20–40 ml/min) (198). However, the pharmacokinetics of peg-IFN alpha-2a during HD may vary due to permeability and dialyzer pore size (199). Longer half-life with pegylation and the high rate of adverse events with treatment have yielded limited data from several small, uncontrolled studies (190).

Lack of information about appropriate ribavirin dosing and concerns about side effects have precluded use of ribavirin in dialysis patients (200–202). Ribavirin undergoes renal clearance and causes dose-related hemolysis that can be severe in patients with baseline anemia. Furthermore, HD does not change the serum concentration of ribavirin. There have been only a few very small studies on combination therapy with peg-IFN and ribavirin (203–206). Despite high SVR rates (>50%) and effective management of ribavirin-induced anemia in these small cohorts, more experience with combination therapy and larger prospective controlled clinical trials are necessary.

HCV infection is associated with decreased survival after renal transplantation (RT) (207, 208). Furthermore, there is no safe and effective HCV treatment post RT. A meta-analysis of clinical trials of IFN-
based therapy in RT recipients showed a summary estimate for SVR and dropout rate of 18.0% (95% CI, 7.0–29%) and 35% (95% CI, 20–50%), respectively (209). The most frequent adverse event was graft dysfunction from acute rejection refractory to corticosteroid therapy. Consequently, there is a directive toward HCV treatment before kidney transplant. Pre-transplant IFN may reduce the occurrence of de novo or recurrent glomerulonephritis post-transplant (21) as well as the incidence of post-transplant diabetes mellitus (210, 211).

HCV is associated with increased morbidity and mortality in CKD patients and affects outcomes after RT. Some studies have reported impressive SVR rates with IFN-based therapy despite significant adverse events and high dropout rates. Treatment prior to RT decreases post-transplantation complications. More prospective, controlled clinical trials are necessary to optimize HCV therapy for this population.

7. CONCLUSIONS

Hepatitis C treatment is a challenge for both the patient and the caregiver. Every patient needs to be viewed with an open mind, as an individual case and not only as a member of a particular risk group. Treatment should not be denied to a patient based on the poor statistical response rate of their risk group. Prognostic factors for populations do not apply to individuals. In summary, when evaluating a member of a special population, efforts should be made to customize treatment both to the patient and to the virologic response in order to maximize chances of success.

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Extrahepatic Manifestations of Chronic Hepatitis C Infection

Douglas Meyer, MD and Henry C. Bodenheimer Jr., MD

**Contents**

- Introduction
- Spectrum of Extrahepatic Manifestations
- Conclusions
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**Key Principles**

- Extrahepatic manifestations (EHM) of chronic hepatitis C (HCV) affect a variety of organ systems and may present with significant mortality and morbidity.
- These EHM are not the direct result of viral infection but are secondary to the host immune response.
- Vascular manifestations are due to the presence of cryoglobulinemia, which causes deposition of immune complexes in the small vessels of the skin, presenting with palpable purpura.
- An association is noted between HCV and lymphoproliferative disorders, most commonly B-cell non-Hodgkin lymphoma.
- Renal disorders, most commonly membranoproliferative glomerulonephritis (MPGN) has the strongest association with HCV associated cryoglobulinemia.

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• Dermatologic manifestations include pruritus, porphyria cutanea tarda, and lichen planus.
• Thyroid diseases, in particular, autoimmune thyroid disease, are commonly associated with HCV and may be exacerbated by interferon therapy.
• Rheumatologic and neurologic diseases associated with HCV include arthritis, myositis, and peripheral neuropathy.
• Many of the EHM of HCV are modified or improved by antiviral therapy. The most durable effects are noted in those who achieve a sustained virological response (SVR).

1. INTRODUCTION

The association between chronic hepatitis C viral (HCV) infection and extrahepatic manifestations of disease was first reported in the early 1990s (1). The signs and symptoms of chronic HCV include vascular, renal, dermatologic, endocrine, rheumatologic, neurologic, and ophthalmologic presentations (Table 1). The prevalence of chronic HCV in the United States is approximately 2% of the adult population (2). The prevalence of at least one clinical extrahepatic manifestation in chronically infected HCV patients is approximately 40–75% (3, 4) (Table 2). About 10% of patients with extrahepatic manifestations of HCV infection present with significant morbidity and mortality (5).

HCV not only infects hepatocytes but can replicate in other cells, in particular, circulating peripheral monocytes (6–9). In order to accurately assess whether extrahepatic replication is actually occurring in cell types and tissue besides the hepatocyte and liver, more sensitive assays than are currently available will be required (10). This assay must be able to accurately differentiate negative-strand HCV RNA from positive strand RNA since the negative-strand RNA is the intermediate replicative molecule signifying true extrahepatic replication (11). However, most extrahepatic manifestations associated with HCV are not the direct result of the viral infection and replication but instead are secondary to the host immune response. HCV results in upregulation of the host humoral immune system with the subsequent generation of monoclonal and polyclonal autoantibodies. These antibodies form immune complexes that deposit in small- and medium-sized blood vessels of various organs where complement activation results in extrahepatic injury (12, 13). Some extrahepatic manifestations can improve with HCV treatment and even resolve after successful eradication of the virus. However, there must be caution when administering
**Table 1**
Extrahepatic Manifestations of Chronic Hepatitis C Infection

<table>
<thead>
<tr>
<th>Category</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular:</td>
<td>Type II cryoglobulinemia</td>
</tr>
<tr>
<td>Lymphoproliferative disorders:</td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Renal:</td>
<td>Membranoproliferative glomerulonephritis</td>
</tr>
<tr>
<td>Dermatological:</td>
<td>Leukocytoclastic vasculitis</td>
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<tr>
<td></td>
<td>Porphyria cutanea tarda</td>
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<td></td>
<td>Lichen planus</td>
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<td></td>
<td>Necrolytic acral erythema</td>
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<tr>
<td>Endocrine:</td>
<td>Thyroid abnormalities</td>
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<tr>
<td></td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Rheumatological:</td>
<td>Polyarteritis nodosa</td>
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<td></td>
<td>Non-deforming polyarthritis</td>
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<td></td>
<td>Sicca syndrome</td>
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<td></td>
<td>Systemic lupus erythematosus</td>
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<td></td>
<td>Anti-phospholipid syndrome</td>
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<td></td>
<td>Autoantibodies</td>
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<td></td>
<td>Osteosclerosis</td>
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<tr>
<td>Ophthalmological:</td>
<td>Mooren ulcer</td>
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<tr>
<td>Neurological:</td>
<td>Peripheral neuropathy</td>
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<tr>
<td></td>
<td>Cerebral vasculitis</td>
</tr>
<tr>
<td></td>
<td>Neurocognitive dysfunction</td>
</tr>
<tr>
<td>Pulmonary:</td>
<td>Idiopathic pulmonary fibrosis</td>
</tr>
<tr>
<td>Cardiac:</td>
<td>Cardiomyopathy</td>
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</table>

HCV therapy in patients with extrahepatic manifestations because some manifestations may be exacerbated during interferon therapy.

2. SPECTRUM OF EXTRAHEPATIC MANIFESTATIONS

2.1. Vascular

Cryoglobulins are immunoglobulins which reversibly precipitate in serum at low temperatures. There are three types of cryoglobulins: I, II, and III (14). Type I cryoglobulins are either monoclonal IgG or IgM, type II consists of two or more classes of immunoglobulin of which one is monoclonal IgM with rheumatoid factor activity; type III also consists of two or more classes of immunoglobulin with polyclonal IgM, demonstrating rheumatoid factor activity or anti-IgG activity. Type II is also called mixed essential cryoglobulinemia and is found in more than half of patients chronically infected with HCV (15–17), of whom only 10% are symptomatic (5, 17, 18). Cryoglobulins are more commonly found in HCV patients with cirrhosis, high viral load, and longer duration of disease (19–21). HCV genotype is not associated with the prevalence of cryoglobulinemia (22–24). The production of cryoglobulins appears to be driven by chronic antigen stimulation of the host immune system by HCV (12).

Patients with symptomatic cryoglobulinemia tend to be older, have longer duration of disease, higher levels of rheumatoid factor and cryoglobulins as compared to patients with asymptomatic cryoglobulinemia (25). The classical triad of symptoms in patients with HCV associated cryoglobulinemia is palpable purpura, weakness, and arthralgia. The most common skin manifestation of HCV-associated cryoglobulinemia is recurrent palpable purpura. Leg ulcers and nodules are less common

| Table 2 |
| Prevalence of Leading Clinical Signs and Symptoms of Extrahepatic Manifestations of Chronic Hepatitis C Infection |
| Arthralgia | 19–23% |
| Sensory neuropathy | 9–17% |
| Myalgia | 10–15% |
| Pruritus | 6–15% |
| Xerostomia | 12% |
| Xerophthalmia | 10% |
Palpable purpura is due to cryoglobulin-associated vasculitis with deposition of immune complexes in the small vessels of the skin (26). Symptomatic patients can also present with renal manifestations usually membranoproliferative glomerulonephritis due to deposition of immune complexes in the glomeruli of the kidney (15, 27–29). The next most commonly affected organ system is the nervous system. This commonly presents as symmetrical distal polyneuropathy. A less common presentation is mononeuritis multiplex (30). HCV RNA has been isolated from small- or medium-sized blood vessels of the affected nerves (30, 31). Diagnosis is usually made by identification of type II cryoglobulins in the serum. The diagnosis also can be made by detecting the immune complex of cryoglobulins and HCV antigen or RNA in the affected tissue. There can be false-negative results due to low serum concentration, errors in sample collection, or failure of the laboratory to detect the cryoglobulin (32).

The goal of treatment of cryoglobulinemia is to eradicate the virus, not just to ameliorate the symptoms of cryoglobulinemia (33–35). Sustained loss of HCV is usually associated with persistent loss of cryoglobulinemia and long-term resolution of symptoms while relapse after HCV therapy results in the reappearance of cryoglobulins and symptoms (32, 33). Several randomized controlled trials have evaluated the addition of corticosteroids to antiviral therapy in symptomatic HCV-related cryoglobulinemia. Corticosteroids result in more rapid and longer-lasting virological response but no improvement in sustained virological response (SVR) (36). Plasmapheresis, high dose steroids, and/or immunosuppressive agents have been used in patients with severe HCV-related cryoglobulin vasculitis (37). Rituximab, a monoclonal antibody to CD20, has been shown to be effective in open-label studies in the treatment of cryoglobulin-related vasculitis refractory to interferon therapy (38–40).

### 2.2. Lymphoproliferative Disorders

There is an association between chronic HCV particularly in patients with type II cryoglobulinemia and lymphoproliferative disorders (41). Up to 13% of patients with B-cell non-Hodgkin lymphoma (NHL) have HCV antibody (42). Furthermore, 10% of patients with mixed cryoglobulinemia, the vast majority of whom have chronic HCV, developed NHL over a 10-year follow-up period (27). The mechanism for this association is not yet established but is believed to be due to the binding of HCV E2 antigen to host CD81 receptor leading to B-cell proliferation (43, 44). Mutations, in certain genetically predisposed patients, develop in response to this chronic antigen stimulation and lead to
monoclonal proliferation of B cells resulting in lymphoma (42). One possible genetic mutation is the t(18;14) translocation, which leads to overexpression of bcl-2 oncogene, resulting in inhibition of apoptosis of B cells (45). The monoclonal B cells proliferate in the liver and bone marrow. The association of NHL with HCV and mixed cryoglobulinemia is most commonly found in patients with follicular lymphoma and lymphoplasmacytoid/immunocytoma subtypes, which are low-grade lymphomas (46–48). In addition, there is a strong association of HCV and mucosa-associated lymphoid tissue (MALT) lymphoma (49, 50). The majority of HCV-related lymphomas are extranodal located mostly in the liver and salivary glands (51, 52).

There are studies showing HCV therapy results in the regression of low-grade lymphoma particularly if there is eradication of the virus (53). Furthermore, Zuckerman and co-workers showed there is a loss of the t(18;14) mutation with interferon and ribavirin therapy, which correlated with eradication of the virus (54). High-grade lymphomas associated with HCV usually require chemotherapy.

2.3. Renal

Membranoproliferative glomerulonephritis (MPGN) has the strongest association with HCV and near complete association with type II cryoglobulinemia (15). HCV RNA, in patients with MPGN, is found in antigen–antibody complexes in the bloodstream and glomeruli. HCV has also been reported to be associated to a lesser degree with membranous and proliferative glomerulonephritis, focal segmental glomerular sclerosis, fibrillary and immunotactoid glomerulopathy (27, 55, 56).

MPGN usually is a late manifestation of HCV-associated cryoglobulinemia and typically presents with hypertension, nephrotic-range proteinuria, microscopic hematuria, rapid decline in renal function, or chronic renal insufficiency (57, 58). Renal manifestations are a leading contributor to morbidity and mortality in HCV-related cryoglobulinemia (58).

Laboratory findings include circulating type II cryoglobulins and decreased C3 and C4 complement levels (15). HCV RNA is detected in the serum and there is 1,000-fold increase in HCV RNA level in cryoprecipitate as compared to the serum (59). There are subendothelial depositions in the glomeruli consisting of IgM with rheumatoid factor, IgG, and complement (30). Electron microscopy reveals cryoprecipitates containing HCV RNA and antibody to nucleocapsid core antigen (28).
HCV interferon monotherapy alone may ameliorate proteinuria and elevated serum creatinine levels found in these patients, however, relapse off therapy with the recurrence of viremia and MPGN commonly occurs (30, 32). The addition of short-term prednisone to interferon therapy has been shown to help control severe MPGN (60). Long-term usage of prednisone is associated with significant side effects and enhanced viral replication.

There were concerns regarding the use of ribavirin in HCV in those with underlying renal disease resulting in severe hemolytic anemia. Sabry and coworkers treated 16 patients with interferon and ribavirin for 12 months after 3 months of interferon monotherapy (61). Seven patients had the dosage of ribavirin reduced mostly as a result of anemia. Only one patient cleared HCV while on therapy. There was no follow-up data but renal disease, viremia, albumin, and complement levels all significantly improved on therapy. Pegylated interferon is used in the treatment of patients with HCV-associated renal disease. In one report 4 patients were treated with pegylated interferon and ribavirin in cryoglobulin-related MPGN and 14 patients received standard interferon and ribavirin. Three of the four patients treated with pegylated interferon had response to treatment compared to 9 of 14 receiving standard interferon. Albumin levels improved in responders as compared to nonresponders; however, serum creatinine was stable in both groups (62).

There are no established guidelines for the treatment of HCV-associated MPGN. All patients should be treated with pegylated interferon and ribavirin if renal function allows, since this combination of therapy has the highest sustained viral response rate. Plasmapheresis and steroid bolus can be used as treatment during acute or severe MPGN. Case reports have detailed long-term histological improvement in HCV-related renal disease if the virus is successfully eradicated. Recent case reports have treated HCV-related MPGN unresponsive to interferon therapy and immunosuppression with rituximab resulting in improvement in proteinuria accompanied by a decreased or stable HCV viral load (38, 63). Rituximab is theorized to be of benefit since it works on the CD20 antigen of B cells. B cells are the cells that sustain the type II mixed cryoglobulinemia (38–40).

2.4. Dermatological

The prevalence rate of pruritus was 15% in the large prospective study performed by the MULTIVIRC group evaluating extrahepatic manifestations in patients with chronic HCV (4). The authors theorized the mechanism of the association of chronic HCV and pruritus
is the formation of antibody–antigen complexes, which deposit in the skin. Treatments have included PUVA, bile salt-binding agents, ursodiol, and interferon therapy (64, 65). Leukocytoclastic vasculitis is vasculitis affecting small blood vessels in many organ systems including the skin and is characterized by palpable purpura and petechiae usually on the lower extremities. Vasculitis may also present with skin ulcers or livedo reticularis on the lower extremities. This syndrome may be due to the deposition of immune complexes in walls of small blood vessels. There is an association with chronic HCV via type II cryoglobulinemia. HCV antigens and RNA are detected in blood vessel walls seen on the skin biopsy of these lesions in approximately 40% of affected patients (26).

During interferon therapy, there is improvement in the skin lesions and symptoms, which can completely resolve with sustained loss of the virus (65–67). Clinical response is associated with decrease in the serum cryoglobulin levels. Sustained viral response does not always guarantee clinical improvement. HCV therapy is more effective in treating the dermatological manifestations of type II cryoglobulinemia than the renal manifestations (60, 68). Interferon monotherapy often results in clinical improvement in the skin lesions; however, recurrence in skin lesions off therapy is common due to the low sustained viral response with interferon monotherapy (67, 69). Patients who achieve SVR with pegylated interferon and ribavirin enjoy long-term resolution of symptoms (35, 70). Combination of pegylated interferon with ribavirin presently is the preferred therapy of HCV-related type II cryoglobulinemia presenting with dermal manifestations.

Porphyria cutanea tarda (PCT) is characterized by photosensitivity, skin fragility, vesicles, and bullae. In addition, there may be hyper- or hypo-pigmentation and skin thickening. PCT typically is seen in conjunction with hepatic iron overload. There are two forms of porphyria cutanea tarda type 1 and 2. Type 1 is the more common sporadic form characterized by decreased uroporphyrinogen decarboxylase activity found only in the hepatocytes. Type 2 is the rare familial autosomal dominant form characterized by reduced uroporphyrinogen decarboxylase activity found in other cell types besides hepatocytes.

There is a strong association between PCT and chronic HCV, in particular in Mediterranean countries (71, 72). The prevalence of HCV in type 1 PCT patients ranges from 57 to 91% (71–74). The mechanism by which HCV triggers type 1 PCT is not known since the virus does not directly affect uroporphyrinogen decarboxylase activity. Pathogenesis may be related to the iron overload in the liver commonly associated with HCV (75). Studies have shown improvement in dermatologic
manifestations with interferon therapy in some patients with PCT but not all, and patients with PCT may have a decreased response to interferon therapy (76).

Lichen planus is a skin lesion characterized by flat-topped, violaceous papules that can present anywhere on the epidermis and may also involve the mucous membrane. Most cases of lichen planus are self-limited; however, oral lesions are more likely to be chronic and can even be pre-malignant. The prevalence of HCV in patients with lichen planus is 10–38% (77). This association between lichen planus and chronic HCV usually occurs in the setting of advanced liver disease (78). The pathogenesis of lichen planus is unknown but is thought to be T-cell immune mediated, and HCV-specific CD4 and CD8 T cells have been identified in the lesions of lichen planus (79).

First line therapy for the treatment of cutaneous and oral lichen planus usually is topical or intralesional steroids and antihistamines for the cutaneous form and topical anesthetics for the oral form. Interferon therapy has a variable effect on lichen planus (80, 81).

Other dermatological manifestations associated with chronic HCV include pyoderma gangrenosum, erythema nodosum, erythema multiforme, and urticaria (82–84). The pathogenic association of these syndromes with HCV is not well established. Necrolytic acral erythema is a rare condition associated with HCV that is characterized by sloughing skin lesions. It is reported to show improvement with a combination of interferon, ribavirin, and oral zinc (85, 86).

2.5. Endocrine

Thyroid diseases, in particular autoimmune thyroid disease, are commonly associated with HCV. Hypothyroidism is seen in 3.5–13% of untreated chronic HCV patients (87, 88). The prevalence of thyroid antibodies is seen in up to 12.5% of HCV patients regardless of the stage of disease and is more prevalent in older women (88, 89). In addition, the prevalence of thyroid disease and thyroid antibodies is significantly higher in HCV patients with type II mixed cryoglobulinemia than the general population (90).

Interferon therapy may induce anti-thyroid antibodies or unmask underlying thyroid disease including Hashimoto’s thyroiditis or Graves’ disease, which may not resolve after discontinuation of the interferon therapy (88, 89). Interferon-associated hypothyroidism is usually treated with hormone replacement. The development of hyperthyroidism on interferon therapy may require discontinuation of therapy (91). HCV patients, especially older women, are most at risk for the development of thyroid disease during interferon therapy particularly if
they have elevated pre-treatment titers of anti-thyroid antibodies especially anti-thyroid peroxidase antibodies (92, 93).

2.6. Rheumatologic

Arthralgia and myalgia are associated with chronic HCV. One large prospective cohort study of 1,614 chronic HCV patients reported a prevalence of arthralgia of 23% and myalgia of 15% (4). Arthralgia occurs more often in HCV patients with type II cryoglobulinemia than in those patients without cryoglobulinemia (4, 94). The prevalence of HCV-associated arthritis ranges from 4 to 11% (95, 96).

The most common presentation of HCV-associated arthritis is a symmetrical non-deforming polyarthritis most commonly affecting metacarpal-phalangeal joints, proximal interphalangeal joints, wrists, and ankles (96). The prevalence of positive rheumatoid factor in HCV patients is reported to be as high as 75% but generally most of these patients do not have clinical features of rheumatoid arthritis (3, 19, 96). One diagnostic test to help differentiate HCV-associated arthritis with positive rheumatoid factor from true rheumatoid arthritis is serum antibodies to cyclic citrullinated peptides (anti-CCP), which will be positive in true rheumatoid arthritis (97–99). HCV-associated arthritis as compared to rheumatoid arthritis usually is less intense and does not result in joint deformities.

The treatment of HCV-associated arthritis includes NSAIDs, steroids, hydroxychloroquine, and methotrexate (100, 101). Interferon therapy has been used to treat HCV-associated arthritis in patients who did not respond to other treatments (95). Clinical studies differ in the response rate to interferon therapy ranging from 17 to 76% (95).

There are case reports of HCV associated with dermatomyositis and polymyositis (102, 103). The exact mechanism for this association of HCV and myositis is not clear; however, there is a report detailing the detection of HCV RNA in muscle fibers of two symptomatic patients suggesting that the myositis is a direct result of the HCV (104).

There is a strong association between HCV and sicca syndrome, which is characterized by xerophthalmia and xerostomia due to lymphocytic sialoadenitis (105–107). This association is seen more in middle-aged women with long-standing hepatitis C (19). Approximately 10–19% of HCV patients have symptoms of sicca syndrome (4, 105–107). The prevalence of HCV in patients with sicca syndrome was 19% in one study (163). HCV-associated sicca syndrome differs from the classic Sjögren syndrome by the lack of anti-SSA and anti-SSB antibodies and glandular tubule involvement (106, 108–111). These patients tend to have milder histological findings of
lymphocytic sialoadenitis (grade I–II) than seen in the classical Sjögren syndrome (106).

The mechanism by which HCV results in sicca syndrome is not clearly established. The virus has not conclusively been shown to replicate in or directly infect salivary gland tissue (37, 105, 112). There may be molecular mimicry between the envelope E2 protein of HCV and salivary glands resulting in the stimulation of the host immune system against the salivary glands (113). Patients with HCV-related sicca syndrome frequently have cryoglobulins in the sera and lower complement levels than patients with classical or primary Sjögren syndrome suggesting a host immune-mediated mechanism rather than direct effect of the virus (107, 109). HCV-associated sicca syndrome is reported to improve with combination IFN and RBV therapy, if SVR is achieved (114).

Polyarteritis nodosa (PAN) is a necrotizing vasculitis of medium-sized arteries affecting multiple organs. The prevalence of HCV in patients with PAN ranges from 5 to 12% (115, 116). HCV-related PAN shares many clinical features with small-sized blood vessel vasculitis associated with type II cryoglobulinemia including palpable purpura, arthralgia, myalgia, and peripheral neuropathy. However, PAN has a more significant association with life-threatening systemic vasculitis than does type II cryoglobulinemia, in the form of cerebral angiitis and ischemic abdominal pain (117). PAN patients with chronic HCV as compared that those without HCV have more cutaneous involvement, lower complement levels, and more advanced liver disease (115). HCV has been found in 6–13% of patients with systemic lupus erythematosus (SLE) (118–120). HCV-associated SLE had lower levels of complement, anti-dsDNA antibodies, and cutaneous involvement than SLE patients without chronic hepatitis C (119). Antiphospholipid syndrome is characterized by the triad of positive antiphospholipid antibodies, thrombocytopenia, and a thrombotic event. Whether there is an association between hepatitis C and antiphospholipid syndrome is controversial (121, 122). Several reports have shown an association between hepatitis C infection and the presence of anti-cardiolipin antibodies, resulting in an increased prevalence of thrombocytopenia, portal hypertension, and thrombotic events (3, 121). The prevalence of anti-β2-glycoprotein-I antibodies and clinical thrombosis; however, is much less in HCV than in patients with classical antiphospholipid syndrome (3, 122, 123).

Osteosclerosis is a rare disorder characterized by diffuse bone pain with x-rays revealing sclerosis and thickening of long bone cortices. There are case reports associating chronic hepatitis C infection and osteosclerosis (124, 125).
There is a high prevalence of autoantibodies ranging from 40 to 76% in patients with chronic hepatitis C infection (3, 126, 127). The most common autoantibodies in chronic hepatitis C infection are rheumatoid factor and cryoglobulins. Other autoantibodies with high prevalence include non-organ-specific autoantibodies such as antinuclear antibody, anti-smooth muscle antibody and anti-liver kidney microsomal type I antibody, and the organ-specific anti-thyroid antibodies. The association of autoantibodies and chronic HCV has not been shown to lead to a higher prevalence of autoimmune disease except in the setting of interferon therapy (128, 129). HCV patients with autoantibodies, in particular anti-thyroid antibodies, are at increased risk for precipitation or exacerbation of autoimmune disease during interferon therapy as opposed to those without autoantibodies (130, 131). There are reports of autoimmune cytopenias being associated with HCV in interferon treatment naïve patients (132). The prevalence of HCV in patients with idiopathic thrombocytopenia (ITP) ranges from 10 to 19% (132, 133).

2.7. Neurological

Peripheral neuropathy found in patients with chronic HCV is usually associated with type II cryoglobulinemia and has manifestations ranging from symmetrical distal polyneuropathy to mononeuritis multiplex (134). Peripheral neuropathy can be sensory, sensory-motor or rarely pure motor, symmetrical or asymmetrical.

CNS involvement is very infrequently seen with type II cryoglobulinemia (135). White matter lesions are seen on magnetic resonance imaging in these patients, who suffer a higher rate of neurocognitive dysfunction than controls. The level of serum cryoglobulins correlate with the extent of impaired cognitive function.

The mechanism of peripheral neuropathy is believed to be related to vasculitis from cryoglobulin deposition in small blood vessels supplying the nerves (32, 135). These patients present with serum cryoglobulins and decreased complement levels (135). Combination anti-HCV consisting of pegylated or standard interferon with ribavirin has a high clinical response rate of up to 75% in treatment of cryoglobulin-related vasculitis (34, 60, 65). Resolution of clinical symptoms including peripheral neuropathy and CNS abnormalities depends on whether SVR is achieved. Patients who relapse or do not respond to HCV therapy usually have neurological symptoms recur within months of stopping therapy. High dose intravenous steroids or plasma exchange may be used to treat life threatening organ involvement due to type II mixed cryoglobulinemia (34).
Hilsabeck and coworkers assessed neurocognitive dysfunction in HCV patients and found impairments in attention and psychomotor speed as compared to normative means but not to patients with alcoholic liver disease (136, 137). The authors showed greater cognitive dysfunction in patients with higher stages of fibrosis.

Studies have evaluated brain metabolism in HCV patients with magnetic resonance spectroscopy (138, 139). In HCV patients with minimal hepatic fibrosis there was abnormal metabolism seen in the white matter and basal ganglia not seen in patients with chronic hepatitis B infection. A possible mechanism for abnormal brain metabolism seen in HCV patients is extrahepatic replication of HCV in the CNS. There is evidence showing replication of HCV in peripheral blood mononuclear cells (9, 140). These infected circulating monocytes may replace microglia that have turned over in the CNS (141). Negative-strand HCV RNA, a marker of extrahepatic replication of HCV has been found in various brain structures during autopsy (142). Genomic sequencing of the HCV found in brain tissue has been shown to contain mutations in the 5′ end of the genome resulting in reduced translation and replication of the virus (143). The presence of HCV in the CNS may be an important mechanism for viral relapse after antiviral therapy.

Another possible mechanism for CNS manifestations is the effect of cytokines derived from the host immune system in response to HCV. There has only been one study to date evaluating this theory, which showed no correlation between fatigue and circulating IL-1 and TNF levels (144).

Fatigue is considered an extrahepatic manifestation of HCV (145). The prevalence of fatigue in untreated HCV patients ranged from 49 to 66% and is significantly more prevalent than in healthy controls (146, 147). The mechanism for HCV resulting in fatigue has not been established. Authors have suggested social and psychiatric factors may contribute to fatigue in HCV patients (148, 149). In a multi-center trial evaluating oral ribavirin monotherapy in the treatment of chronic hepatitis C, there was improvement in fatigue without decrease in the level of viremia (150).

2.8. Pulmonary

Chronic HCV has been variably associated with idiopathic pulmonary fibrosis (IPF) (151, 152). The prevalence of anti-HCV antibodies in Italian patients with IPF was 13.3% (152).
2.9. Cardiac

Reports have shown an association between chronic HCV and hypertrophic and dilated cardiomyopathy (153). Prevalence of HCV ranged from 6.3 to 10.6% in dilated and hypertrophic cardiomyopathy patients, respectively, as compared to healthy volunteer blood donors. HCV RNA was detected in the heart on autopsy in 11.5 and 26% of patients with dilated and hypertrophic cardiomyopathy, respectively (154). The mechanism by which HCV results in cardiomyopathy has not been established.

3. CONCLUSIONS

The main target of chronic HCV is the hepatocyte. However, there are many diverse extrahepatic manifestations of chronic HCV which are due to the activation of the host immune system in response to the infection. The extrahepatic manifestations that have clearly been shown to be associated with HCV are induced by formation of type II cryoglobulinins due to chronic viral antigen stimulation of the host immune system. These extrahepatic manifestations are potentially curable with successful eradication of the virus after HCV treatment. The less established extrahepatic manifestations of HCV are of uncertain pathogenesis and usually have only epidemiologic data supporting the association.

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Anti-HCV Agents in Development

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Key Principles

- Almost half of all patients with chronic hepatitis C infection will not clear the virus with currently available therapy.
- Advances in the understanding of HCV biology are allowing investigators to develop novel therapies designed to specifically target the hepatitis C virus.

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- Specifically Targeted Antiviral Therapy for HCV (STAT-C) includes orally available small molecules, such as protease and polymerase inhibitors.
- Early data have demonstrated the high antiviral activity of several STAT-C agents, although the emergence of resistance to such agents has also been identified.
- The use of protease or polymerase inhibitors in combination with pegylated interferon (PEG-IFN) and ribavirin increases antiviral activity while also suppressing the selection of resistant variants. Therefore PEG-IFN, and probably ribavirin, will continue to remain a mainstay of HCV treatment for the foreseeable future.
- The antiviral potency of agents in development will not only improve the rate of sustained virologic response but will likely also allow for a shorter duration of therapy.
- Other emerging antiviral therapies include novel interferons, alternatives to ribavirin, nonspecific immunomodulators, and therapeutic vaccines for HCV.
- Additional approaches for HCV therapy being investigated include compounds targeting intracellular host factors that play a role in viral replication or virion production.
- The ultimate goal in the long term is to use combinations of new drugs that can completely eradicate HCV with minimal toxicity and possibly avoid the need for interferon.

1. INTRODUCTION

Chronic hepatitis C virus (HCV) is a major public health problem and the leading cause of death from liver disease in the United States, affecting at least three million Americans (1). HCV is typically transmitted through contact with blood or blood products. Since the advent of serologic testing for HCV, recipients of blood products have been at very low risk of HCV infection, and even the rate of new hepatitis C infections among intravenous drug users has dramatically decreased (2). However, given the large number of people infected many years ago and the significant number of new infections that still occur, a large burden of chronic hepatitis C infection, increasingly culminating in advanced liver disease and hepatocellular carcinoma, will continue to exist.

Acute infection with HCV is usually subclinical. Chronic infection develops in about 75% of individuals infected (3). Six genotypes of HCV have been identified, with genotype 1 accounting for a majority
of cases in the United States. Cirrhosis develops in 20–30% of patients with chronic HCV infection, typically occurring after several decades of infection (3). Among patients with cirrhosis, hepatocellular carcinoma can occur in as many as 4% of patients per year (4).

The treatment of chronic hepatitis C has improved dramatically since interferon alpha was first approved in 1991. However, a significant percentage of patients with chronic HCV infection are still not able to clear the virus and achieve a sustained virologic response with the currently available treatment, pegylated interferon (PEG-IFN) alfa-2a or alfa-2b combined with ribavirin, especially those with HCV genotype 1 infection (4). Studies focusing on the refinement of existing drugs, through the development of novel interferons or alternatives for ribavirin, are in progress. As we have gained greater insight into the life cycle of the hepatitis C virus, elucidated the crystal structures of critical viral enzymes such as the serine protease and polymerase, and refined in vitro systems, treatments capable of direct HCV inhibition have been developed. Specifically targeted antiviral therapies for HCV (STAT-C), consisting of small molecules that target the viral replication cycle, represent the most promising advance in antiviral treatment. Over the next decade, the options for antiviral therapy aimed at HCV infection are expected to greatly increase, entailing improved efficacy of treatment and possibly reduced duration of therapy. It is hoped that, in the long term, the development of novel antiviral therapy will allow for the potential replacement of interferon and/or ribavirin with their attendant toxicities.

2. LIFE CYCLE AND STRUCTURE OF HCV

HCV is a small, single-stranded RNA virus belonging to the Hepaciviridae genus of the Flaviviridae family (5). Similar to most RNA viruses, the hepatitis C virus within an infected individual exists as a population of quasispecies, which are diverse but closely related variants of the hepatitis C virus. Diversity within the genome of hepatitis C quasispecies is mainly the result of mutations produced by the error-prone RNA-dependent RNA polymerase (6). The presence of quasispecies provides HCV with a survival advantage, allowing for the selection of variants to adapt to changing conditions, including the immune response or, in the therapeutic setting, specifically targeted antiviral drugs.

The evaluation of the HCV life cycle has been hindered by the lack of effective cell culture systems and animal models to study the hepatitis C virus. An efficient cell culture system depends upon the availability of a host cell that allows viral entry and subsequent production and release
of virions capable of infecting other susceptible cells (7). The development of HCV replicon systems, which consist of genomic viral RNA that replicates from a single origin of replication, was a significant step forward in HCV research. Transfection of HCV RNA into a human hepatoma cell line, Huh-7, allowed investigators to study HCV replication first-hand (8). The development of HCV replicon systems has resulted in the creation of new cell culture lines that provide researchers the opportunity to better understand the HCV life cycle. More recently, a major advance occurred with the development of cell culture systems that can support the production of infectious virions derived from a patient with fulminant hepatitis caused by genotype 2 infection (9, 10). This is expected to greatly facilitate the study of all aspects of the viral life cycle, including entry, uncoating, replication, assembly, and release. As a corollary, the production of a robust system supporting HCV genotype 1 infection would be a highly desirable goal.

The HCV genome is composed of a 9.6 kb single-strand of positive-sense RNA flanked by untranslated regions on either end (5, 7). Translation of the RNA genome yields a polyprotein precursor that is processed by cellular and viral proteases to form ten mature proteins. The structural proteins include the core protein and envelope glycoproteins E1 and E2 that form the virus particle. The HCV core protein forms the viral nucleocapsid and is thought to interact with host cellular proteins to affect their function (5). The manner in which the core protein modulates cell signaling and gene transcription is yet to be elucidated. The envelope glycoproteins are transmembrane proteins that are instrumental for viral entry into the host cell by inducing fusion between the viral envelope and the host cell membrane. Several candidate cell surface receptors have been identified that appear to act as cofactors in viral entry, including claudin-1, CD81, and the LDL receptor (11–13).

A variety of nonstructural proteins coordinate viral replication with two viral proteases (NS2-3 and NS3-4A) being responsible for cleavage and maturation of the nonstructural proteins (5–7). HCV NS3 is a multifunctional protein, which contains a helicase domain that unwinds duplex RNA structures and a serine protease activity requiring NS4A as a noncovalent cofactor (14). As mentioned above, the NS3 serine protease is important for polyprotein processing. Analogous to most RNA viruses, HCV forms a membrane-associated complex to mediate replication. This complex consists of viral proteins, the RNA to be replicated, and a variety of altered host cell factors. Critical to the formation of the membrane-associated replication complex is NS4B, which is capable of inducing the creation of membranous webs that act as sites for complex assembly (14). Synthesis of the viral RNA is accomplished by the NS5B RNA-dependent RNA polymerase, a viral enzyme
not possessed by normal human cells and thus making it an attractive target for investigators (5–7, 14).

3. CURRENT THERAPY OF CHRONIC HCV INFECTION

The goal of therapy for chronic hepatitis C infection is to eradicate the virus and prevent progression of liver disease associated with HCV infection. Successful treatment of chronic HCV infection is best demonstrated by the presence of undetectable virus in the blood 6 months after completion of treatment, termed a sustained virologic response (SVR). Swain and colleagues demonstrated that over 99% of patients maintained their response after SVR over a 4-year period (15). Prior studies have also shown that successful treatment of HCV infection leads to a reduction in the incidence of HCC and a delay in progression of fibrosis and inflammation, thus providing a strong rationale for pursuing the most effective treatment possible (16, 17).

The first approved therapy for HCV infection was recombinant interferon alfa-2b, a cytokine that can upregulate the innate antiviral immune response by activating a variety of transcription factors and signal transducers, leading to the induction of genes encoding RNAses and inhibitors of viral protein translation. IFN-α is also important in inducing the immune system via activation of natural killer cells and memory T cells (4). However, administration of standard IFN-α alone was largely ineffective, resulting in a sustained virologic response (SVR) in only 15% of patients following 12 months of therapy while also resulting in significant adverse effects (18). The addition of ribavirin to interferon was a significant step forward, improving the rate of SVR to 40% (19). Further advances were made with the advent of pegylated interferon alpha (PEG-IFN), achieving an SVR in 54–56% of patients overall, and 42–46% of patients with genotype 1 HCV infection when used in combination with ribavirin (20–22). Hadziyannis et al. demonstrated that weight-based dosing of ribavirin (1000 mg of RBV for body weight less than 75 kg and 1200 mg of RBV for individuals greater than 75 kg) results in better treatment outcome for patients infected with genotype 1 HCV (21). Further affirmation of the superiority of weight-based RBV dosing for genotype 1, this time in a dosing scale of 800–1400 mg/day, over a flat 800 mg dose was provided by a large US trial (23). Both of these trials demonstrated the equivalent efficacy of 24 and 48 weeks of therapy for patients with HCV genotypes 2 and 3 (21, 23).

In order to have the best opportunity to achieve an SVR, patients need to reliably take as much of the prescribed amount of pegylated interferon and ribavirin as possible, namely, at least 80% of the dose over 80% of the duration of antiviral therapy (24). The use of hematopoietic
growth factors in response to anemia and leukopenia may also improve adherence to therapy, but a beneficial effect of the use of these agents on sustained virologic response has not been demonstrated in randomized clinical trials.

Studies with both of the pegylated interferons demonstrated that failure to achieve a 2-log reduction in viral load by 12 weeks predicted ultimate failure of therapy to attain SVR so strongly, with a negative predictive value of 97–100%, as to warrant discontinuation of treatment (22, 25). More recent studies have further evaluated the role of viral kinetics in tailoring the optimal length of therapy. Many patients with a rapid virologic response (RVR; undetectable viral load at 4 weeks of treatment) may be able to achieve an SVR with truncated therapy although early cessation of therapy may slightly impair the optimal chance of sustained response (26–28). Documentation of the rate at which a patient achieves an undetectable viral load, which correlates with the likelihood of SVR, is particularly helpful when managing the adverse effects that patients may experience during the course of therapy. Patients who experience significant adverse effects while on antiviral treatment can be offered a shorter duration of therapy with a reasonable, if not quite optimal, opportunity for SVR if a RVR had been achieved.

Conversely, data have shown that individuals with a slow virologic response may benefit from extending the duration of therapy to prevent relapse. Four studies, despite variability in definition of a slow response, ribavirin dose, and peginterferon formulation, have shown that genotype 1-infected patients with slow response have augmented rates of SVR if treatment is extended to 72 rather than 48 weeks (29–32).

4. REFINEMENT OF CURRENT THERAPY

4.1. Novel Interferons

It is clear that interferon will continue to play a major role in the treatment of HCV infection for the foreseeable future, and PEG-IFN has been the mainstay of antiviral therapy against hepatitis C. New formulations of interferon are being evaluated, however, in hopes of reducing adverse events, providing more convenient administration, and resulting in more effective outcomes.

Consensus interferon (CIFN) Three Rivers Pharmaceuticals is a recombinant type 1 interferon that contains the most common amino acid at each of the 166 positions of the naturally occurring interferon alpha subtypes. CIFN was developed with the rationale of creating an interferon that might lead to an enhanced response against HCV infec-
tion (33). CIFN has been proposed to have a potential role in the management of patients who were nonresponders to previous therapy (34). Bacon and colleagues recently presented final data from a multicenter trial which demonstrated that daily CIFN combined with RBV resulted in rates of SVR of 5 and 10% with CIFN doses of 9 and 15 mcg, respectively, among patients who were nonresponders to the combination of PEG-IFN and RBV. The highest rates of response were in noncirrhotic patients with substantial declines in viral load with their previous course of PEG-IFN and RBV treatment (35).

Albumin interferon is another novel interferon consisting of interferon alfa-2b fused to human serum albumin, thereby extending its half-life and allowing the drug to be administered every 2–4 weeks. A phase 2 trial in treatment naïve patients demonstrated SVR rates after 48 weeks of therapy of 58, 58, 55, and 51% with PEG-IFN alfa-2a 180 mcg weekly, albinterferon 900 mcg every 2 weeks (q 2 week), albinterferon 1200 mcg every 2 weeks, and albinterferon 1200 mcg every 4 weeks, respectively. Interestingly, the patients who received albinterferon every 4 weeks overall had slower response rates but did not have the high relapse rates generally expected with “slow respondents” (36). A study using various doses of albumin interferon in nonresponders to peginterferon and ribavirin yielded a pooled SVR rate of 17% (37). A recent phase 3 trial in genotype 1 patients (ACHIEVE 1) demonstrated that the SVR rate (48.2%) of albumin interferon 900 mcg given every 2 weeks was non-inferior to the SVR rate (51%) of PEG-IFN alfa 2a and ribavirin (37a). The rate of serious adverse events was similar in the two groups, although discontinuations due to adverse events were 10% for albumin interferon and 4.1% for PEG-IFN alfa-2a. In the companion study, for genotype 2 and 3 patients (ACHIEVE 2/3) SVR occurred in 79.8% of the albumin interferon 900 mcg recipients and 84.8% of those receiving PEG-IFN alfa 2a, again demonstrating statistical non-inferiority. The difference in SVR in this study was driven by a very high rate of SVR (95%) in Asian patients receiving PEG-IFN alfa-2a (37b). Albumin interferon represents a potential alternative formulation of interferon allowing for less frequent dosing.

4.2. Alternatives to Ribavirin

Ribavirin (RBV) is a guanosine analogue discovered in 1972 with broad-spectrum activity against several viruses. When administered as a monotherapy, RBV has minimal effect against HCV. However, in combination with interferon it has been shown to increase the viral suppression seen with interferon alone, and to reduce relapse rates. The mechanism by which ribavirin suppresses hepatitis C virus
when used with interferon has not been clearly established. Potential mechanisms of action that have been invoked include direct inhibition of HCV replication, competitive inhibition of the enzyme inosine 5'-monophosphatase which is involved in the synthesis of guanine nucleosides, an immunomodulatory effect, and induction of mutagenesis (38, 39). Ribavirin is associated with dose-related hemolytic anemia, skin reactions, and is teratogenic. Therefore alternatives to ribavirin have been sought that may mimic RBV in its effectiveness but minimize its adverse effects.

Taribavirin, previously called viramidine (Valeant), is a prodrug converted to ribavirin that becomes activated in the liver. Phase 3 trials of taribavirin demonstrated superior hematologic safety compared to ribavirin, although it was associated with inferior rates of SVR (40). It is possible that higher, weight-based dosing of taribavirin may result in better outcomes, with a study designed to evaluate this hypothesis under way.

5. SPECIFICALLY TARGETED ANTIVIRAL THERAPY (STAT-C)

5.1. Protease Inhibitors

The most promising potential advance in the therapy of chronic HCV infection lies in the development of orally available small molecules that specifically target the HCV replication cycle, termed specifically targeted antiviral therapy (STAT-C). As discussed above, the NS3/4A protease cleaves the polyprotein precursor into structural and nonstructural proteins and is vital to the life cycle of hepatitis C. The active site of the enzyme is long and shallow, initially leading to concerns about potential difficulty in developing molecules that will bind to that area (5–7). However, the first protease inhibitor evaluated, BILN 2061, a macrocyclic compound developed by structure-based drug design, demonstrated potent antiviral activity, resulting in a 3-log reduction in the HCV viral load by day 2 of administration. Unfortunately, cardiac toxicity in a monkey model precluded further investigation of the drug (41). Since then several other protease inhibitors have been developed with significant potential to become key agents in antiviral therapy.

Telaprevir (VX-950, Vertex Pharmaceuticals Inc.) inhibits the NS3/4A protease through the formation of a covalently bound complex at the active site of the enzyme. A phase 1b trial demonstrated an impressive 4.4-log median reduction in the hepatitis C viral load by day 14 among genotype 1-infected patients treated with telaprevir 750 mg every 8 hours (42). Resistant variants develop within 1 week of initia-
tion of therapy with telaprevir, indicating that monotherapy will not be a viable option (43). In another small trial, it was shown that additional viral suppression was conferred by the combination of peginterferon alfa-2a with telaprevir, with fewer resistant variants emerging, and that standard combination therapy after 14 days of telaprevir dosing suppressed resistant variants that emerged during the initial dosing period (44). Chu and colleagues recently presented their data evaluating the replication capacity of telaprevir-resistant variants. Compared to wild-type HCV, most resistant variants are not as replicatively fit, suggesting that resistant variants will tend to disappear after cessation of treatment and the viral population will revert back to wild-type predominance (45). However, the time required for this process to occur to the extent that all variants become undetectable, if this occurs at all, has yet to be documented. When used in combination with pegylated interferon and ribavirin in yet another phase 1 study, HCV RNA became undetectable in 12 of 12 subjects by 28 days of therapy (46).

The initial findings with telaprevir led to two phase 2 multicenter trials evaluating the efficacy and safety of telaprevir, administered orally at a dose of 750 mg every 8 hours in treatment-naive HCV genotype 1-infected individuals in the United States and Europe, respectively. In the US trial (PROVE 1), patients were treated with 48 weeks of pegylated interferon alfa-2a and ribavirin (n = 75), or with 12 weeks of telaprevir combined with pegylated alfa-2a and ribavirin followed by 36 weeks (n = 79) or 12 weeks (n = 79) of standard combination therapy, or no additional therapy (n = 17) (47). Patients were required to have an RVR to be eligible to stop therapy at 12 or 24 weeks. Of patients in the telaprevir groups, 79% had undetectable HCV RNA at week 4. The rate of SVR was 61% in the 24-week group and 67% in those patients receiving triple therapy for 12 weeks followed by 36 weeks of standard combination therapy. By comparison, 41% of patients receiving standard of care and 35% of those receiving triple therapy for 12 weeks achieved an SVR. Viral breakthrough occurred in 7% of patients receiving telaprevir. Discontinuation for adverse events occurred in 11% of the control patients and 21% of the patients in the three telaprevir-based groups, with rash the most common reason (47).

In the PROVE2 study (48), there was a 48-week control group (n = 82) as in PROVE1, but there was no 48 week telaprevir-containing arm. Instead, there was an arm in which patients received 12 weeks of triple therapy followed by 12 weeks of standard combination therapy (n = 81), a larger group than in PROVE1 receiving 12 weeks of triple therapy alone (n = 82), and a 12 week treatment group receiving telaprevir and pegylated interferon alone (n = 78). Unlike PROVE1, patients were not required to have an RVR in order to stop at the pre-specified
time points. In patients receiving triple therapy for only 12 weeks, SVR occurred in 60%, compared with 69% (representing follow-up week 12 results in the interim analysis). End of treatment response in the control group was pending at the time of writing. Although the SVR rates in the 12- and 24-week groups were numerically close, relapse occurred more frequently in the 12-week arm. In both the 12- and 24-week groups the importance of RVR in optimizing results was illustrated by the much lower rates of relapse in patients with RVR. One of the most impactful features of PROVE2 was the finding of a much lower rate of SVR (36%) in the ribavirin-free arm, in which lower early response rates, higher on-treatment breakthrough rates, and higher post-treatment relapse rates were all noted. The toxicity profile of telaprevir was similar to that in PROVE1 (48).

Intriguing preliminary results from PROVE3, an international trial of telaprevir in patients who have failed previous treatment with PEG-IFN and ribavirin, were reported recently. This study contained a total of 453 patients divided into four arms: standard of care with PEG-IFN alfa 2a and ribavirin for 48 weeks; telaprevir combined PEG-IFN and ribavirin for 12 weeks followed by PEG-IFN and ribavirin for 12 additional weeks; triple therapy for 24 weeks followed by 24 weeks of PEG-IFN and ribavirin; and telaprevir with PEG-IFN alone for 24 weeks. In patients with prior nonresponse to PEG-IFN and ribavirin, the rate of HCV RNA undetectability 12 weeks after cessation of therapy in the treatment group receiving 12 weeks of triple therapy followed by 12 weeks of standard therapy were 41% in prior nonresponders and 72% in relapers, but much lower in the ribavirin-free arm at 11% and 36% (48a).

Phase 3 trials are ongoing to further evaluate the efficacy of telaprevir-based therapy in treatment naïve patients and prior treatment failures and to determine the optimal duration of therapy. The results of the telaprevir trials thus far raise hopes for substantial improvements in rates of response for treatment naïve genotype 1 patients with the additional potential benefit of shortened therapy duration, particularly for rapid responders. Moreover, the addition of telaprevir to retreatment regimens have the potential to induce SVR in many patients with prior treatment failure, including the majority of prior relapers. Finally, the PROVE2 has affirmed the importance of ribavirin in treatment regimens containing STAT-C drugs.

Boceprevir (SCH503034, Schering-Plough) is another protease inhibitor currently in phase 2 trials that has demonstrated potent suppression of HCV. In a double-blind, placebo-controlled trial, boceprevir resulted in a mean maximum 2-log reduction in viral load after 2 weeks of therapy among genotype 1-infected patients who had
previously failed PEG-IFN treatment (50). Data presented by Zeuzem and colleagues demonstrated dose-related antiviral activity with patients tolerating all doses tested. An open-label, crossover phase 1 study to investigate the combination of PEG-IFN and boceprevir demonstrated a mean maximal reduction in HCV RNA of 2.9-log at a dose of 400 mg of boceprevir every 8 hours (51). When used in combination with PEG-IFN, 4 of 10 nonresponders to prior therapy became HCV-RNA negative during treatment.

Phase 2 trials were subsequently initiated in prior nonresponders, followed by a trial in treatment-naïve patients. Sustained response rates from the nonresponder study were 2% of control patients retreated with PEG-IFN alfa-2b and RBV versus 7–14% of in various arms given boceprevir 800 mg tid (lower initial doses were increased on the basis of interim analyses), PEG-IFN alfa-2b and RBV (52). The final results from SPRINT-1, a large international study evaluating boceprevir in combination with PEG-IFN alfa-2b and ribavirin, have shown substantial increases in rates of SVR relative to standard therapy (52). The study was designed to evaluate two important concepts: first, whether a lead-in phase with standard therapy alone for four weeks followed by the addition of boceprevir would results in higher rates of response and less viral breakthrough by decreasing viral levels prior to addition of the protease inhibitor; second, whether shortened therapy would be equally effective as the standard duration. Patients in the two lead-in arms received PEG-IFN alfa-2b and ribavirin for four weeks followed by triple therapy for either 24 or 44 additional weeks. Patients in the non-lead-in arms received either 24 or 48 weeks of triple therapy. Patients in a low dose ribavirin (400–1000 mg) received 48 weeks of triple therapy, and control (RBV 800–1400 mg) patients received treatment for 48 weeks.

The SVR rates in SPRINT-1 were 38% in the control group, 36% in the low dose ribavirin group (further affirming the importance not only of ribavirin but the maintenance of the standard dose in STAT-C based regimens); 55% and 56% in the shorter duration arms without and with lead-in dosing, and 67% and 75% in the longer duration arms without and with lead-in dosing (52). Rates of viral breakthrough were higher without lead-in dosing. Greater reductions in hemoglobin occurred with boceprevir, and the use of erythropoietin was associated with higher rates of SVR, opening up a potential role for further exploration of growth factors as adjuncts to treatment. Lead-in dosing is an important feature of the ongoing phase 3 boceprevir development program. Concerns about the low SVR rates in the boceprevir phase 2 nonresponder study are mitigated by the fact that this was a boceprevir dose ranging study in which some arms were also ribavirin-free, and most of
the patients were null responders. Thus, the phase 3 program focuses on both treatment naïve patients and patients with prior treatment failure.

For all HCV drugs it is important to establish safety of administration in patients with hepatic impairment, given that cirrhotic patients have the greatest need in the short term for effective treatment. Preston et al. demonstrated through pharmacokinetic studies that there is no significant difference in the plasma concentration of boceprevir among patients with increasing degree of hepatic dysfunction, laying a foundation for further exploration of this protease inhibitor in the treatment of cirrhotic patients (53).

5.2. Polymerase Inhibitors

In addition to protease inhibitors, polymerase inhibitors have great promise as a potential therapy that is specific for the hepatitis C virus. The NS5B protein contains the RNA polymerase and has been a focus of antiviral therapy development. RNA polymerase inhibitors consist of two classes of molecules, nucleoside analogues and non-nucleoside inhibitors (54). Nucleoside analogues are converted to nucleoside triphosphates after entry into the cell and their incorporation by the RNA polymerase results in premature termination of RNA replication. Conversely, non-nucleoside inhibitors bind to one of several alternate sites that change the conformation of the polymerase and confer allosteric inhibition.

The most extensively investigated polymerase inhibitor to date has been valopicitabine. Valopicitabine (NM283, Idenix Pharmaceuticals, Inc.) is a nucleoside analogue of cytidine. The development program for the drug initially centered on the concept of replacing ribavirin. Treatment-naïve patients demonstrated an early virologic response in 68% of patients when valopicitabine was used in combination with PEG-IFN starting at week 2 of treatment (55). However, significant gastrointestinal toxicity occurred, particularly with higher doses. More recent data documented a 53% end of treatment response among treatment-naïve patients administered PEG-IFN and valopicitabine (56). When compared to combination therapy with PEG-IFN and RBV, valopicitabine and PEG-IFN resulted in greater HCV RNA suppression in individuals who had previously failed standard therapy. However, no sustained responses occurred, and based on the efficacy and safety profile of the drug the US Food and Drug Administration halted its development in the summer of 2007.

The non-nucleoside inhibitor HCV-796 has also progressed to phase 2 trials with encouraging results. An early phase 1 study among treatment-naïve patients of all genotypes demonstrated a peak reduction
in HCV RNA at day 4, with viral rebound occurring in some patients (57). Further analysis of these patients documented the emergence of viral variants. Combination therapy with PEG-IFN and HCV-796 also demonstrated potent viral suppression in a 14-day randomized, placebo-controlled phase 1b study (58). Preliminary results from the phase 2 study showed that 73% of treatment naïve and 23% of non-responders achieved an undetectable HCV viral load after 12 weeks of combination therapy with HCV-796, pegylated interferon, and ribavirin, compared to 45% of treatment-naïve patients that underwent standard of care. However, 8% of patients had significant elevations in transaminases and two patients developed jaundice. Therefore, dosing of HCV-796 was suspended pending continued observation to assess safety of the medication (59).

Two additional nucleoside polymerase inhibitors under development are R1626 (Roche Laboratories) and R7128 (Pharmasset, Inc). Results from a phase 2a study demonstrated that 81% of patients receiving the combination of R1626 1500 mg bid with peg-interferon alfa-2a 180 µg/wk and RBV 1000–1200 mg/day were HCV RNA negative by week 4, with a mean viral load reduction of 5.2-log 10 IU/mL (60). Lesser but still impressive viral load reductions were seen without RBV. Furthermore, there has been no evidence for resistance after 2 weeks of monotherapy or 4 weeks of combination treatment, thus suggesting that R1626 may have a high barrier to the development of resistant viral mutants (61). Although most adverse events were mild, grade 4 neutropenia occurred in 39% of those receiving triple combination therapy including R1626 1500 mg bid, and 78% of those receiving PEG-IFN alfa-2a with R1626 3000 mg bid, compared to 10% in the standard of care group (60). Further studies with modified doses of R1626 and/or PEG-IFN alfa-2a are in progress to determine the optimal dose of both R1626 and PEG-IFN alfa-2a that will maximize response while minimizing side effects. A salient feature of this development program thus far is the attainment of very high RVR rates rivaling those attained with protease inhibitors—a significant departure from a prevailing earlier concept that protease inhibitors are uniquely suppressive. (Note added in proof: The development of R1626 was halted after observations of hematologic and ocular complications in the phase 2 program).

A Phase 1 study of R7128 monotherapy has likewise demonstrated impressive early results, with a mean 2.7-log10 decline in viral load after 2 weeks of therapy, without viral breakthrough, among patients that had previously failed an interferon-containing regimen (62). A phase 2 trial is in progress, with an early report indicating that PEG-IFN alfa-2a, ribavirin, and R7128 1500 mg bid induced RVR in 85% of patients, reinforcing the viral suppressive potential of polymerase
inhibitors when combined with PEG-IFN and RBV (62a). Gane and colleagues subsequently presented the results of a study in which prior non-responders or relapsers to IFN-based therapy were treated with 1500 mg of R7128 BID, PEG-IFN and ribavirin. An RVR was achieved in 90% of patients receiving triple therapy compared to 60% of patients receiving standard therapy (62b). Early development of other non-nucleosides with promising antiviral activity is in progress.

The role of combining protease inhibitors with polymerase inhibitors has been evaluated by Bichko and colleagues, who found that valopicitabine has similar efficacy against wild-type virus and variants resistant to protease inhibitors (63). The utility of combining protease and polymerase inhibitors in the treatment of HIV has been clearly established and further studies are needed to better elucidate the role of combination therapy for HCV infection. However, it stands to reason that using a combination of drugs with different targets within the HCV replication cycle will help to better suppress the emergence of resistant variants and thus better maintain suppression of the virus. A number of investigators have studied in vitro combinations of protease and polymerase inhibitors and demonstrated enhanced viral suppression, prevention of resistance, and suppression of pre-existing variants to one or the other drug in the combination (64–66).

5.3. Anti-HCV Nucleic Acids

In addition to suppressing HCV by inhibiting proteins and enzymes important in the life cycle of the virus, researchers are developing new ways to target the HCV genome by silencing key genes of HCV through the use of anti-HCV nucleic acids. The most common sites of the HCV genome that are targeted by anti-HCV nucleic acids include the 5' and 3' untranslated regions of the genome, since they are highly conserved (67).

Antisense oligonucleotides are short, single-stranded nucleic acids that bind to a target area within the RNA genome and interfere with its function, thereby silencing that part of the genome. Ribozymes are catalytically active RNA molecules that can be modified to target-specific RNA molecules, thereby disrupting the HCV genome. Trials of both classes of agents were thought to show initial promise either preclinically or clinically (67, 68), but their development was halted for safety and/or efficacy concerns (69). Small interfering RNA molecules (siRNA) have been shown to have promising effects in in vitro systems. In order for the promise of anti-HCV nucleic acids, including siRNAs, to become a reality, however, the development of a safe and
efficient delivery system is required that will specifically target the tissue or organ of choice (70).

6. DRUGS TARGETING THE HOST CELL

While much of the current research into novel therapies for HCV has focused on targeting viral proteins that are necessary for replication, work is also underway to evaluate drugs that target host factors, thereby indirectly disrupting the viral life cycle. By disrupting host cellular processes, rather than virus-specific pathways, the development of resistance may not be as significant a problem. Furthermore, since these drugs would work through a different mechanism of action than virus-specific therapies, a synergistic effect with other antiviral therapies may be possible.

Nitazoxanide (Alinia, Romark Laboratories, L.C.) is a thiazolide with broad-spectrum activity against intestinal parasites and anaerobic bacteria (71). Currently approved in the US for giardiasis and cryptosporidiosis, the antiviral effect of nitazoxanide (NTZ) was first realized among patients being treated for chronic parasitic infection who had concomitant hepatitis C. Although its precise mechanism of action in viral hepatitis is not entirely clear, effects on protein folding and on activation of eukaryotic initiation factors have been invoked (71). In vitro studies have demonstrated that NTZ can inhibit HCV, as well as HBV, replication. In addition, NTZ was equally effective in suppressing polymerase and protease-resistant HCV mutants (72). Rossignol and colleagues presented interim results of the efficacy of NTZ used in combination with pegylated interferon and ribavirin among HCV genotype 4-infected patients (73). While standard therapy with pegylated interferon and RBV resulted in undetectable HCV RNA at follow-up week 12 in 43% of treatment-naïve patients, the combination of pegylated interferon, RBV, and NTZ resulted in 79% of patients achieving the same endpoint (73). Studies in the US in genotype 1-infected patients are currently in progress.

Another class of drugs that has demonstrated antiviral activity against HCV infection is that of cyclosporine analogs. Cyclosporine inhibits the interaction between cyclophilin B and the hepatitis C NS5B protein, which is important in the regulation of the HCV RNA-dependent RNA polymerase. DEBIO-025 (Debiopharm), a synthetic compound that is a more potent inhibitor of cyclophilin B without the immunosuppressive activity of cyclosporine, has demonstrated significant antiviral ability in vitro (74). In vivo studies of chimeric mice with human hepatocytes demonstrated that the combination of DEBIO-025 and pegylated interferon decreased HCV viral load by 100-fold (75). A phase 1b
trial of monotherapy DEBIO-025 among patients co-infected with HIV and HCV resulted in a 3.6-log reduction of the HCV viral load without the emergence of resistance in the short term (76). Further data on this compound have not yet appeared, nor on a compound, NIM811, with a similar mechanism of action.

Glycoprotein processing, mediated by alpha-glucosidase, is a key step prior to release of the virion from the host cell. Inhibition of alpha-glucosidase prevents proper folding of the HCV envelope and adversely affects viral maturation (6). Studies have demonstrated promising results when celgosivir (MX-3253, Migenix Inc), an alpha-glucosidase inhibitor, was administered to treatment-naïve patients (77). Further studies are underway investigating combination therapy that includes celgosivir.

7. NONSPECIFIC IMMUNOMODULATORS

Immunomodulator approaches that upregulate the innate immune system without directly affecting HCV are also being developed. Stimulation of toll-like receptors (TLR), which activates pathways of innate, cellular, and humoral immunity, can produce increased levels of interferon and cytokines that mediate an enhanced immune response against the hepatitis C virus (78). CpG oligonucleotides contain motifs resembling bacterial DNA and act as agonists to TLR-9. A phase 1b study of CPG 10101 (Actilon, Coley Pharmaceuticals) demonstrated dose-dependent increases in markers of immune activation as well as decreases in HCV RNA (79). Early results of CPG 10101 used in combination with PEG-IFN and RBV among patients who relapsed after prior treatment demonstrated improved response with triple therapy, although the 48 weekend of treatment response and SVR rates in the final intention to treat analysis were disappointing (80, 81). This drug, as well as a TLR-7 agonist, has had its development program halted because of various combinations of efficacy and safety concerns in animal models.

8. HEPATITIS C VIRUS VACCINATION

Due to the significant worldwide burden of chronic HCV infection, the development of a vaccine to prevent HCV infection has been of paramount importance. However, the creation of a vaccine has been difficult given the propensity of hepatitis C to mutate and form quasispecies. An effective vaccine for HCV will induce a strong CD8+ T-cell response as well as B cells that produce antibodies against the envelope surface proteins of HCV to prevent cell entry. The development of
a vaccine for HCV has focused on a variety of approaches, including protein-based, DNA-based, and dendritic-based vaccinations (82).

Studies have evaluated the use of glycoproteins E1 and E2 as immunogens to generate cellular and humoral immune responses. Initial studies involving vaccinated chimpanzees demonstrated either resistance to viral challenge or at least partial protection, thus supporting the feasibility of protein-based vaccination (83). However, as described previously, the ability of HCV to mutate, especially within the region of its genome that encodes the E2 glycoprotein, hinders the creation of an effective protein-based vaccination (82).

DNA vaccinations, in which plasmid DNA constructs are injected intramuscularly, have been shown to induce a cytotoxic T-cell response (84). In addition, unlike protein-based vaccines, DNA vaccines are fairly easy to manufacture, making them amenable to widespread distribution. Research has focused on DNA vaccines utilizing plasmids that encode for structural proteins, such as the core protein, and nonstructural proteins, such as NS3, NS4, and NS5. Results from the mouse model demonstrate that a cellular and humoral response to DNA vaccines can be achieved, although there is still much work to be done to arrive at a true clinical benefit (85). When tested among a small number of chimpanzees, the DNA vaccine did not protect against infection, although it did alter the natural course of the infection (86). In order to make DNA vaccines more effective, new approaches are being investigated to increase the immunogenicity of the vaccine through concomitant activation of the innate immune system (82).

Targeted molecular immunogens consisting of whole, heat-killed recombinant Saccharomyces cerevisiae yeast modified to express protein targets that stimulate the immune system have been evaluated. GI-5005 (GlobeImmune) is a tarmogen comprising yeast cells expressing NS3 and core protein. In a phase 1 trial, GI-5005 was administered at varying doses with the finding of normalization of ALT and viral load reductions in some patients and the demonstration of broad-based enhancement of T-cell responses to a variety of HCV epitopes (87).

The key to the ability of vaccines to elicit an immune response lies in the ability of antigen presenting cells, such as dendritic cells, to take up antigens and activate the innate and adaptive immune system (88). The importance of dendritic cells is demonstrated by the observation that in patients with chronic hepatitis C, impaired function of antigen-presenting cells may be necessary for the virus to survive (89). Dendritic cell-based vaccines may ultimately prove to be an efficient method to induce an immune response specific to HCV, thus providing long-lasting protection from the virus.
9. CONCLUSION

Exciting advances are being made in antiviral therapy for chronic hepatitis C infection as researchers have gained greater insight into the life cycle of the virus. For years, therapy was based solely on interferon, with its broad spectrum of antiviral and immunomodulatory properties, and ribavirin, the mechanism of action of which against HCV is still much debated. Currently, a growing number of studies are being conducted to evaluate the role of specifically targeted antiviral therapy for HCV (STAT-C). These novel agents have proven to have a high level of antiviral activity but concomitantly have been associated with the selection of resistant variants. It will be important to characterize the patterns of resistance and the kinetics of both emergence and suppression of different variants with each class of drug. Protease inhibitors appear thus far to be associated with the more rapid emergence of resistance than nucleoside polymerase inhibitors. The evolution of HCV treatment in the near future will likely consist of the addition of one or more of the new drugs currently in development to peginterferon and ribavirin. There are already preliminary signals from phase 2 studies of the potential to maximize sustained response rates with shorter durations of treatment than the 1 year traditionally required for genotype 1 infection. The ultimate hope is that clinicians will be able to use combinations of orally available small molecules designed to specifically target HCV, perhaps including compounds that target host factors intrinsic to viral replication, virion production, or the immune response. Whether an immunomodulatory component of our treatment regimens will always be required is one of the central issues of the field. In the meantime, researchers are investigating refinements of current therapy, including novel interferons and alternatives to ribavirin, in parallel with the development of new agents. We stand at the dawn of a new era that promises to bring dramatic advances in our ability to treat this major public health problem.

The rapid pace of development in the field of HCV therapy has generated a wealth of data about other novel agents not available when this manuscript was initially submitted. Although we have added very recent data from phase 3 trials as the chapter has gone to press, it has not been possible to add data on other agents, including but not limited to additional protease and polymerase inhibitors, NS5A inhibitors, and cyclophilin inhibitors. The interested reader is encouraged to review the abstracts from the 2008 meeting of the American Association for the Study of Liver Diseases (Hepatology 2008;48, number 4, Supplement) and the 2009 meeting of the European Association for the Study of the Liver (Journal of Hepatology 2009; 50(Suppl 1)). The latter meeting
featured the first presentation of an early trial combining an HCV protease and nucleoside polymerase inhibitor (R7227 and R7128) demonstrating marked antiviral activity (Gane E et al, J. Hepatology 2009; 50(Suppl 1):S380) and reinforcing hopes that combinations of antiviral agents will be a major future direction for investigation.

REFERENCES


Epidemiology, Screening, and Natural History of Chronic Hepatitis B Infection

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Key Principles

- Hepatitis B virus (HBV) infection continues to be a global public health problem despite large-scale efforts to eliminate this disease through education, screening, and vaccination programs.
- An estimated 400 million individuals are chronically infected with HBV which results in nearly 1 million deaths each year from decompensated cirrhosis or HCC.
- A wide variability exists in the prevalence rates of HBV worldwide with sub-Saharan Africa, mainland China, and many countries of Southeast Asia being hyperendemic in HBV.

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• Maternal–fetal transmission of hepatitis B virus is an important problem, particularly in endemic areas such as Southeast Asia and Africa.
• Immunization against HBV has been a successful experience in various countries. It is hoped that universal vaccination campaigns will eventually reduce or eliminate the impact of this disease in many parts of the world.

1. INTRODUCTION

Hepatitis B virus (HBV) infection continues to be a global public health problem despite large-scale efforts to eliminate this disease through education, screening, and vaccination programs. Universal vaccination can prevent most acute HBV infections, although in many developing countries the potential of universal immunization remains unfulfilled. In the Western world, as a consequence of widespread vaccination programs relatively few cases of acute HBV infection are encountered. However, in these developed countries, the significant number of individuals chronically infected with HBV will continue to present with major complications of chronic infection, including decompensated cirrhosis and hepatocellular carcinoma (HCC). Also, widespread migration from Asia, sub-Saharan Africa, the Caribbean, and other areas of high prevalence to Western countries (areas of low prevalence) has resulted in immigrant communities in which the prevalence of chronic HBV infection is similar to the rates observed in the country of origin.

2. EPIDEMIOLOGY

2.1. Global Patterns of HBV Infection

HBV infection remains a global challenge, with one-third of the world’s population having serological evidence of current or previous infection. An estimated 400 million individuals are chronically infected with HBV which results in nearly 1 million deaths each year from decompensated cirrhosis or HCC (1). The virus accounts for 30% of all cases of cirrhosis and 53% of all cases of HCC worldwide (2). The prevalence of chronic HBV infection, defined as hepatitis B surface antigen (HBsAg) positivity for ≥6 months, varies widely, with rates ranging from 0.1 to 20% in different parts of the world (3). Regions may be designated as follows: (a) “High” prevalence (HBsAg positivity rates >8%), which include Asia (except Japan), parts of the Middle East, sub-Saharan Africa, and the Amazon basin; (b) “Intermediate” prevalence (HBsAg positivity rates of 2–7%), which include Japan, the
Indian subcontinent, parts of central Asia and the Middle East, Eastern and Southern Europe, as well as parts of South America; and (c) “Low” prevalence (<2% HBsAg positive), which include the United States, Northern Europe, Australia, and the southern part of South America (Fig. 1).

![Map of HBsAg positivity across the world.](image)

**Fig. 1.** Prevalence of HBsAg positivity across the world. High-prevalence regions include the Asia (except Japan), parts of the Middle East, sub-Saharan Africa, and the Amazon Basin. Intermediate-prevalence regions include Japan, the Indian subcontinent, parts of central Asia, and the Middle East, Eastern, and Southern Europe, as well as parts of South America. Low-prevalence regions include the United States, Northern Europe, Australia, and the southern part of South America.

Overall, 45% of the world population lives in “high” prevalence regions (4). In areas with a high HBV prevalence, serologic evidence of prior HBV infection (anti-hepatitis B core antibody (anti-HBc) or anti-HBs positivity) is present in the vast majority of individuals. High endemicity is perpetuated by frequent perinatal or childhood acquisition of HBV infection. Migration patterns of individuals from high to low endemic areas have had a significant impact on the epidemiology of hepatitis B (see below).

### 2.2. Patterns of HBV Infection in Specific Areas

#### 2.2.1. Africa

Sixty-five million of approximately 400 million people in the world chronically infected with HBV reside in Africa (5). Of the 1.3 million deaths attributed to HBV-related diseases recorded annually throughout the world, approximately 250,000 occur in Africa. Wide variations exist
in the prevalence of HBs antigenemia in Africa. The virus is hyperendemic (>8%) in sub-Saharan Africa, with the exception of Kenya, Zambia, Cote d’Ivoire, Liberia, Sierra Leone, and Senegal, which are areas of intermediate endemicity (2–8%). The northern African countries of Egypt, Tunisia, Algeria, and Morocco are regions of low endemicity (<2%), although pockets of high endemicity can occur within these countries (6) (Fig. 2). For example, in Tunisia prevalence rates are up to 13.3% in the south and central western regions (7, 8).

![Fig. 2. Prevalence of HBsAg positivity in Africa. High-prevalence areas in Africa include sub-Saharan Africa, with the exception of Kenya, Zambia, Cote d’Ivoire, Liberia, Sierra Leone, and Senegal, which are areas of intermediate prevalence. The northern African countries of Egypt, Tunisia, Algeria, and Morocco are regions of low prevalence, although pockets of high prevalence can occur within these countries.](image_url)

Generally, HBsAg prevalence is much higher in rural areas, and the risk of being HBsAg positive in rural areas is twice as high as in urban areas (6). HBsAg prevalence is lowest in asymptomatic blood donors and highest in patients with various clinical consequences of HBV infection. HBsAg prevalence increases with age (9).

Exposure to HBV, as measured by the prevalence of anti-hepatitis B core (anti-HBc) ranges from 1.8 to 98% in the African population, with blood donors and children found on the lower end of the scale and patients with chronic liver disease or HCC showing a higher prevalence. Exposure rates increase with age. Exposure to HBV varies in different
regions of Africa, with western Africa showing anti-HBc prevalence greater than 85% and eastern Africa showing a prevalence ranging 65–85%. Exposure rates to HBV are higher in rural than in urban populations (10). The Baka pygmies of eastern Cameroon represent the population group in Africa with the highest exposure to HBV with an anti-HBc prevalence of 93.6% (11).

Compared with other adult chronic HBV-infected patients, those in Africa have a lower rate of HBeAg positivity. Early loss of HBeAg occurs in both Bantu and Pygmy people in Cameroon, with a mean age of 31.6 and 29.5 years, respectively (12). Prevalence of HBeAg in chronic HBV-infected patients varies widely from <1 to 24% (6).

Most primary HBV infections are transmitted either perinatally or horizontally before the age of 10 years. Horizontal transmission occurs through intrafamilial, and to a lesser extent interfamilial, routes (13).

2.2.2. United States of America

In the United States, HBV prevalence is relatively low, with HBsAg positivity ranging from 2 to 7% (3). Despite the relatively low endemicity of HBV, HBV infection remains a major public health problem and is a cause of significant morbidity and mortality in certain ethnic populations and among groups of people whose behavior puts them at high risk. Large regional differences exist in HBsAg prevalence rates within the United States (3) (Fig. 3).

The emigration of people into the United States has also given rise to rapid changes in epidemiological patterns. The number of chronically HBV-infected (HBsAg-positive) individuals in the United States is often quoted as being 1.25 million. However, a recent paper that incorporated immigration data suggested that at least 2 million residents of the United States may have chronic HBV infection (14, 15). In a study from the Mayo Clinic in Rochester, Minnesota, 86% of individuals with chronic HBV infection in one Minnesota county were born in foreign countries (16). Paradoxically, deaths because of HBV infection increased during the two decades before 1999 based on data from the US Certificates of Death Registry from the National Center for Health Statistics and the Healthcare Utilization Project despite the introduction of vaccination programs within the United States (17). A fourfold increase in the rate of age-adjusted death because of HBV was predominantly observed in men and nonwhites. Findings from Chu and colleagues (18) at major referral sites across the United States have highlighted the impact of immigration on the current demographics of HBV infection. The investigators found that patients presenting for the management of HBV infection, particularly at West Coast and Northeastern referral centers, are now predominantly Asian. Additional
Fig. 3. Prevalence of HBsAg positivity in Americas. In the United States, HBV prevalence is relatively low; however, large regional differences exist in HBsAg prevalence rates within the United States. In Latin America also, wide variations in HBsAg prevalence is seen. Prevalence is low in temperate Mexico, Colombia, Argentina, Chile, Uruguay, and some Caribbean islands; intermediate in Venezuela, Peru, Ecuador, Brazil, and parts of Central America, such as Guatemala and Honduras; and high in the Dominican Republic, Haiti, and the Amazon Basin.

findings suggest that these patients have a longer duration of HBV infection that was acquired early in life, as reflected by frequent chronic HBV infection with HBeAg negativity (19).

In urban populations of the United States, transmission occurs through percutaneous or permucosal exposure to infected blood or other secretions. In adults, the predominant means of transmission are sexual intercourse, needlestick injuries in health-care workers and injection drug users. In North America, currently vertical transmission is rare because of the implementation of routine infant and childhood immunization programs. Despite these efforts, challenges remain with respect to intrafamilial transmission of HBV infection. Intrafamilial transmission has been clearly documented among Asian families who have recently immigrated to the United States. Studies among Asian American and Pacific Islander children who immigrated to the United States
found that household transmission of HBV remains relatively high at 0.5–1% annually (20), leading to the recommendation, starting in the 1980s, that all family and household members of Asians infected with HBV undergo vaccination. A recent survey on 3,163 Asian American adult volunteers in the San Francisco found that 8.9% were chronically infected with HBV and 65.4% of the chronically infected adults were unaware that they were infected. Of those who were not chronically infected, 44.8% lacked protective antibodies against HBV and were likely susceptible to future infection. Men were twice as likely as women to be chronically infected (12.1% vs. 6.4%). Asian Americans born in East Asia, Southeast Asia, or the Pacific Islands were 19.4 times more likely to be chronically infected than those born in the United States (21).

2.2.3. Latin America

In Latin America (Mexico, Central America, and South America), the total number of individuals chronically infected with HBV may exceed 6 million. The prevalence of HBsAg is low in temperate Mexico, Colombia, Argentina, Chile, Uruguay, and some Caribbean islands; moderate in Venezuela, Peru, Ecuador, Brazil, and parts of Central America, such as Guatemala and Honduras; and high in the Dominican Republic, Haiti, and the Amazon Basin (22). Hepatitis B infection has emerged as a serious problem in almost all the Amerindian communities studied in the Amazon Basin as well as in other Amazonian ecological systems from north and central South America, where it has been a major cause of morbidity and mortality.

Of an estimated 4 million chronic HBV-infected subjects in South America alone, more than 30% are located in the Amazon Basin region (including areas of Colombia, Venezuela, Peru, and the northeastern region of Brazil) (23). In these regions, a wide prevalence of antibody to hepatitis B core antigen (anti-HBc), from 1.2 to 74%, has been reported in many small seroepidemiological studies (24, 25).

An increase in prevalence is expected in South America due to immigration. Accordingly, in the state of Sao Paulo, a significantly higher rate of HBV infection exists in the people of Asian ethnicity than in the western populations (26).

In urban populations, transmission occurs through percutaneous or permucosal exposure to infected blood or other secretions. In adults, the predominant means of transmission are sexual intercourse, needle-stick injuries in health-care workers and injection drug users. Perinatal transmission plays an important role in areas where HBV infection is more prevalent among adults. In some areas of the Amazon Basin,
where most cases of acute HBV occur during the first years of life (27), there are inadequate measures to block transmission at birth, and the disease becomes evident during childhood. However, vertical transmission cannot be regarded as the sole source of endemicity in the area; other environmental, sociocultural, and sanitary factors specific to the geographical locale may contribute to the unique characteristics of the infection observed in this region. Certain cultural practices (e.g., tattooing, ear piercing, consumption of masato, sharing instruments for ritual scarification) and other factors (e.g., bat bites) have been reported to be associated with the transmission of HBV.

2.2.4. Western Pacific and Southeast Asia

Western Pacific and Southeast Asia regions are one of the most populous regions, including more than 40 countries. The region comprises highly developed countries (such as Japan, Australia, and New Zealand), countries that are rapidly industrializing (such as Singapore, Malaysia, Indonesia, and the Republic of Korea), and countries which are less prosperous (such as the Philippines and Myanmar). Hepatitis B infection is hyperendemic in this region, with the exception of Australia, New Zealand, and Japan. The prevalence of HBV in the general population in the Asian region is variable with the highest rates in Taiwan (>10%) and Thailand (>8%) and lowest in Japan 0.8%.

Much of the information on transmission of HBV in Southeast Asia and the western Pacific Region is based on inferences from cross-sectional epidemiological studies. In hyperendemic countries, the age-specific prevalence of markers of infection increases steadily with increasing age, although some decline in the carrier rate and the prevalence of antibody is often seen in the last two decades of life. In these countries, most infections seem to occur: (a) either at or shortly after birth, when newborn babies are exposed to the infected blood or blood-stained secretions of carrier mothers; (b) before starting school, when the child is part of an extended family, some of whom may be chronic HBV-infected subjects; and (c) in early adult life, after the onset of sexual activity. Some infections may be transmitted by multiple use of nonsterile needles or instruments and transfusion of unscreened blood. While the modes of transmission of HBV are similar throughout the region, there are considerable variations in the importance of perinatal and household transmission and transmission by inoculation of blood, secretions, or sexual intercourse from country to country. In hyperendemic areas, most infections are acquired early in life, either from the child’s mother or some other person in the extended household. By contrast, in low-prevalence groups, most infections are acquired in early
adult life through intravenous drug abuse and unprotected sexual intercourse.

Immigration has also contributed to the HBV-infected patients’ pool in the Pacific region especially Australia. In Australia, HBsAg prevalence rates reflect those found in the countries in which the individual’s parents or grandparents were born, being lowest (about 0.1%) in descendants of British migrants, higher (2–5%) among migrants and the children of migrants from the Mediterranean region, and highest (5–15%) among migrants from the Pacific islands and Southeast Asia (28). A considerable proportion of Australia’s chronic HBV-infected subjects have entered the country as refugees from Vietnam and Cambodia. The notification rate of HBV fell in Australia to 1.5 per 100,000 in 1997 but has since increased to 2.2 per 100,000 in 2001. This increase in notifications is thought to be due to the increase in migrants and refugees from high-prevalence countries. A recent study in central Sydney (New South Wales) found that almost 70% of those with chronic HBV were born overseas (29).

2.2.5. China (Mainland)

China is an endemic area of HBV infection with 90% of the adult population having been infected. The national seroepidemiological survey on viral hepatitis conducted in 1992 showed that the prevalence of HBsAg positivity was 9.75% in the general population (30). It is estimated that each year 300,000 deaths in China are caused by chronic hepatitis B-related conditions. According to the 2007 Chinese Health Statistical Digest published by the Ministry of Health, the incidence of viral hepatitis is the highest among the 35 infectious diseases that require compulsory reporting since 1978 (31). In China, HBV infection is spread mainly through mother-to-infant (mostly perinatal) transmission and early childhood transmission. The Ministry of Health has recommended routine infant HBV immunization in rest of the China since 1992, but the families had to pay for the vaccination. Finally, in 2005, the Chinese government adopted a completely free HBV vaccination program for all neonates (32). According to a national survey, in 1999, the HBV vaccine coverage in infants under 12 months of age was 88.5 and 62.7% for urban and rural areas, respectively, with four economically privileged provinces having a coverage rate of more than 90% (33). Comparison of the two national surveys of vaccination coverage indicated that the estimated coverage of three doses of the hepatitis B vaccine increased substantially overall: from 70.7% among children born in 1997 to 89.8% among children born in 2003 (32).

The positive impact of HBV vaccination has been demonstrated beyond doubt. In 1998, Lu et al. conducted a sampled survey on
hepatitis A, B, and C in Yunnan province, China. The prevalence of HBsAg was 2.0% in 452 serum samples collected from elementary school students in three different counties (34). Jia et al. (1999) reported hepatitis B vaccination of newborns in four capital cities and its suburbs involving 1,271 children in late 1997. The hepatitis B vaccine coverage rate in the cities (92.1%) was significantly higher than that in suburbs (77.0%). HBsAg decreased from 7.1% in nonvaccinated controls to 1.66% in vaccinees (35). In a study from Nanning, China, in 2004, during the 14 years after hepatitis B vaccination, the HBsAg-positive rates were found to be an average of 1.5%. The incidence of hepatitis B dropped from 3.27/10,000 to 0.17/10,000, a 94.8% decrease, in the group of 0–19-year-olds (36).

2.2.6. TAIWAN

In the early 1980 s, 15–20% of the population of Taiwan was estimated to be chronically infected with HBV. A program of mass vaccination against hepatitis B was launched in 1984. In the first 2 years of the program, newborns of all HBsAg-positive mothers were vaccinated. Since 1986, all newborns, and then preschool children, primary school children, adolescents, young adults, and others have also been vaccinated. Vaccination coverage was greater than 90% for newborns and 79% of pregnant women being screened for HBsAg. The proportion of babies who were born to highly infectious HBV positive mothers and became chronically HBV infected decreased from 96 to 14%, whereas the decrease was from 12 to 4% for babies of less infectious mothers. Within 10 years of introduction of the vaccine, the HBsAg prevalence declined from 9.3 to 1.3% in children younger than 12 years in Taiwan (37). Similar changes in prevalence have been reported for HCC and fulminant hepatitis B (see below).

2.2.7. INDIA

HBsAg prevalence rates among blood donors range from 1 to 6% and in pregnant women from 2.6 to 9.5% (38). Community data on HBsAg and anti-HBs positivity in the Indian population are scarce. However, among studies in healthy individuals, the HBsAg and anti-HBs positivity has been reported to be 1.6–2.1% and 17.1–19.5%, respectively (39, 40). The HBsAg positivity rate varies regionally and overall was quoted to be as high as 4.7% (Fig. 4) (40). This is the weighted mean of various studies and includes high-risk populations. Lodha et al. did a systematic review of literature and concluded that the prevalence of hepatitis B in India was 1–2% (41). A recent meta-analysis found the prevalence in nontribal populations is 2.4% (95% CI: 2.2–2.7%) and the
prevalence among tribal populations is considerably higher at 15.9% (CI: 11.4–20.4%) (42).

Higher prevalence rates have been reported in high-risk populations. The HBsAg positivity among professional blood donors has been reported to be 15–20%. Moreover, among health-care workers, HBsAg positivity has been reported to be 1.7–40% (38). Recently, studies have shown that within India hyperendemic regions for HBV infection may exist in tribal populations with HBsAg prevalence reaching up to 23% (42, 43).

Indian studies on age-stratified HBsAg and anti-HBs positivity rates indicate that a chronic HBsAg positivity rate of 2–3% is reached by the age of 5 years (44) which does not increase further with age. However, the anti-HBs frequency continues to increase with age (45). This indicates that exposure to HBV infection occurs mainly in childhood. The predominant route of transmission among children is horizontal transmission during preschool and early school age. Studies evaluating HBV infection frequencies among household contacts also indicate that horizontal spread of HBV infection is the predominant route of transmission in this situation (46). The introduction of mandatory HBsAg screening of blood donors in India during the 1990s has resulted in a marked decrease in posttransfusion HBV infection (38). Despite donor screening for HBsAg, about 25% of posttransfusion hepatitis is still due
to HBV (38). It may be possible to prevent posttransfusion hepatitis B by eliminating anti-HBc positive donors. However, due to a general shortage of donated blood in India, discarding anti-HBc positive blood remains problematic. Another possible route of transmission of HBV infection is the use of nondisposable glass syringes in rural India (47). Data on the significance in India of other routes of HBV transmission, such as tattooing, visits to barbers, body-piercing practices, and homosexual activities are limited.

2.2.8. MIDDLE EAST

Studies in the Middle East show the prevalence of HBsAg to range from 3 to 11% overall. The highest prevalence rates have been reported from Saudi Arabia and the Republic of Yemen (48).

In the Middle East, the majority of infections occur through childhood and perinatal transmissions. There is limited information on sexual transmission in these societies. Parenteral transmission in health institutions is limited due to routine screening of blood products. The number of intravenous drug users in the Middle East remains low compared to other regions, but there is little information on unsafe injection practices.

Studies on the magnitude of perinatal and childhood transmission in the Middle East have produced differing results. While some studies suggest that childhood transmission is the major mode of transmission of HBV infection with perinatal transmission being uncommon, others propose that perinatal transmission plays an important role in contributing to the pool of chronic HBV-infected patients. Considering the heterogeneity of Middle East populations and the varying prevalence of chronic HBV infection among women of childbearing age, intercountry differences in the mode of transmission probably exist (48).

2.2.9. EUROPE

The prevalence of HBsAg positivity ranges from <0.1% in north-west Europe to 1–5% in southern Europe. The highest rates are found in the south and east, particularly the central Asian republics. The highest reported rates in the countries of central and eastern Europe are in Bulgaria, Estonia, Latvia, Kazakhstan, Kyrgyzstan, Moldova, the Russian Federation, and Uzbekistan (49).

Improvements in health-care delivery, better living standards, safer sexual practices, and implementation of immunization programs have led to decreasing HBsAg prevalence in many European countries including Italy, Greece, and Spain (50).
2.3. Patterns of HBV Transmission

HBV is present in the blood, saliva, semen, vaginal secretions, menstrual blood, and, to a lesser extent, perspiration, breast milk, tears, and urine of infected individuals. The virus is resistant to breakdown, can survive outside the body, and is easily transmitted through contact with infected body fluids (3). Sexual activity and injection-drug use account for the majority of HBV transmission in low-prevalence areas (1). Perinatal (vertical) transmission is most common in Far East Asian countries and Oceania (3). Not all HBV infection in endemic areas is vertically transmitted. Early childhood (horizontal) transmission is particularly important in sub-Saharan Africa, Alaska, and Mediterranean countries where, in contrast to Asia, perinatal transmission is less common (1). The exact mode of early childhood transmission is unknown, but is thought to occur via unapparent blood or body fluid exposures from parents, siblings, or playmates that inoculate HBV into cutaneous scratches, abrasions, or onto mucosal surfaces. Person-to-person horizontal transmission occurs from intimate contact with exchange of bodily fluids or from parenteral exposure, such as illicit drug use. However, up to 40% of adults with acute HBV infection cannot or will not identify a specific route of exposure, which in part reflects the highly infectious nature of this virus even in the absence of recognized risk factors. In contrast with the acutely infected infant or child, the otherwise healthy adult with acute HBV is highly likely to successfully clear the infection. Paradoxically, the more symptomatic the acute illness, the more likely the HBV is to resolve without event and without progression to chronicity. This is because a brisk immune response is believed to produce more hepatocyte injury during viral clearance. Immunocompromised individuals, such as those infected with HIV, dialysis patients, and the elderly are more likely to have sub-clinical acute HBV and develop chronic infection. The recognition that an exacerbation of chronic HBV infection can mimic initial acute HBV infection led to a reevaluation of the rate of chronicity in healthy adults, which is now estimated to be less than 1% rather than the more frequently quoted rate of 5% (51).

2.3.1. Specific Modes of Transmission

Mother-to-Child Transmission

Maternal–fetal transmission of hepatitis B virus is an important problem, particularly in endemic areas such as Southeast Asia and Africa. The placenta forms an excellent barrier against transmission of this large virus, and intrauterine infection with HBV is rare. It does occur, however, probably as a result of transplacental leakage, as in threatened abortion and at the time of delivery.
If a mother is infected with HBV during pregnancy and develops acute hepatitis B, the infant’s infection depends on the gestational age at the time of maternal hepatitis. Acute hepatitis in the first to second trimester rarely causes HBV infection of the newborn, whereas hepatitis in the third trimester or during the postpartum period frequently leads to HBV infection of the newborn infant, which suggests that mother-to-infant transmission of HBV occur mainly in the perinatal period, rather than in utero.

Vertical transmission of HBV from mother to infant occurs peripartum or in the initial months after birth. Transmission in utero can occur, but accounts for less than 2% of perinatal transmissions (52). Breast feeding is not considered a major route of mother-to-infant infection of HBV.

HBeAg status has a putative role in facilitating vertical transmission of HBV from mother to child. Infants born to mothers who are both HBeAg and HBsAg positive have up to a 95% risk of acquiring HBV infection in the absence of immunoprophylaxis. By contrast, transmission rates range from 10 to 40% for infants born to HBeAg-negative mothers. Appropriate immunoprophylaxis interrupts transmission in more than 90% of cases. However, immunological blockade fails in approximately, 10% of neonates who later became chronically infected with HBV. It is currently thought that the major cause of unsuccessful immunological blockade is the intrauterine infection of HBV, which occurs when the placental barrier becomes infected (53).

Blood and Tissue Donations

Universal screening of blood donors with HBsAg has reduced the risk for transfusion-transmitted HBV. Many countries with low prevalence have also added tests for anti-HBc to detect chronically HBV-infected patients with low-level viremia who may not have detectable HbsAg (occult HBV). These two tests decrease infection rates to approximately 2.5–15.3 per million units of blood in low-prevalence areas (54). The United States, Canada, Japan, Australia, and some European countries perform the more sensitive nucleic acid tests in addition to these two serologic tests. The magnitude of the incremental yield and clinical benefit of nucleic acid tests over serologic tests in these low-prevalence areas, as well as the need for performing serologic tests in addition to nucleic acid tests, is unknown. Anti-HBc cannot be used as a screening test in high-prevalence areas because up to 90% of adults may have serologic evidence of either past or ongoing hepatitis B infection. Hence, combined HBsAg and anti-HBc screening would disqualify most volunteer blood donors. Therefore, in such countries, HBsAg alone is currently used for screening this population.
However, in high-prevalence areas, 3–30% of individuals who are anti-HBc-positive and HBsAg-negative are HBV DNA positive in blood. It has been estimated that HBsAg-negative, HBV DNA positive blood carries an approximately 10% risk of transmitting HBV to susceptible recipients (55). With donor screening strategies using only HBsAg, the risk for transfusion-transmitted HBV infection in Taiwan was recently estimated to be 100 per million units, that is, at least 7–40 times higher than in low-prevalence areas (56). The yield of nucleic acid tests was projected to be 20 times greater in high-prevalence areas than in low-prevalence areas, where it is currently used, making it that much more cost-effective per infection prevented in these countries (55). However, nucleic acid tests vary in sensitivity and specificity and are too expensive for routine use in high-prevalence areas (57). Another potential mode of transmission for HBV is from organ or tissue donation. It has been estimated that undetected viremia at the time of donation may be more common among tissue donors than blood donors (58). Although the simplest way to prevent posttransplant infection is to exclude all anti-HBc-positive individuals as potential donors, this approach may be impractical in high-prevalence areas where the majority of the population has been previously exposed. Therefore, the addition of nucleic acid testing to strategies in the screening of tissue donors may be the best option for reducing the risk of transmission.

**Renal Replacement Therapy**

The prevalence of HBV infection in patients on renal replacement therapy varies considerably among different areas of the world varying from 1.5 to 53% among hemodialysis patients and 2.4–31% among renal transplant recipients (59). It is usually similar in dialysis patients and renal transplant recipients, but is increased compared with the general population, indicating that infections occur mainly during the time of dialysis. Two epidemiological patterns can be observed. First is a geographical distribution, which reflects the disease burden in the general population. It is higher in the Middle East and Far East compared with Western countries. Within Europe, a north–south gradient of increased prevalence has been reported. Second, countries with a lower socioeconomic status have a higher prevalence among dialysis patients, indicating lower resources for maintenance of hemodialysis units, HBV vaccination programs, and erythropoietin treatment (60).

Repetitive blood transfusions are the single most important factor for hepatitis virus transmission, whereas infection through contaminated hemodialysis equipment occurs less frequently. Finally, patient-to-patient transmission of HBV with transplanted organs has been reported. Sharing of supplies, instruments, or medications between
hemodialysis patients and reuse of dialyzers would in theory increase the spread of HBV between patients.

**Transmission by Unsafe Medical Practices**

Transmissions through contaminated and nonsterile injection practices and through inadequately sterilized medical instruments remain a significant problem in low-income areas. Unsafe injection practices coupled with the popular and sometimes unnecessary use of injections in low-income countries is a complex public health problem. Low-income countries include most of Africa, the Indian subcontinent, some Southeast Asian countries, and Mongolia. Infection control policies, guidelines, and practices to enhance the safety of patients and health workers have been widely researched, implemented, and evaluated in high-income countries. Consequently, the risk of nosocomial HBV infection due to unsafe injection practices in developed countries is extremely small. However, in low-income developing countries, unsafe injection practices are comparatively common, placing both patients and health workers at risk of infection with hepatitis B and other bloodborne viruses (61). Patients are at risk because both single-use disposable and reusable needles and syringes are reused, and the methods employed to clean and sterilize the equipment between patients are often suboptimal, if used at all. Health workers are at risk because they are required to handle used injecting equipment in order to clean and sterilize it for reuse.

The number of HBV infections attributable to unsafe injection practices (defined as the reuse of a syringe or needle from patient to patient without sterilization) in low-income countries has been calculated as 8–16 million infections globally every year (62). The World Health Report (2002) reports that unsafe injection practices account for 30% of HBV infections worldwide.

Episodes of HBV transmission from one patient to another in healthcare settings have also been reported from developed countries (63). In most cases, these transmissions resulted from noncompliance with recommended infection control practices that were designed to prevent cross contamination of medical equipment and devices.

**Health-Care Workers and Hepatitis B**

In the prevaccination era, transmission of HBV in medical settings was a severe public health problem. A high rate of HBV infections in health-care workers (HCW) was observed. HBV transmission was especially frequent in cases of direct contacts with blood, such as surgery, hemodialysis units, or oncology wards. However, the numbers
of HBV infections of HCW have dropped significantly during the last few decades due to vaccination. Because more than 95% of vaccines developed protective antibodies, the risk of vaccinated HCW to acquire HBV during their professional activities is now minimal. However, not all HCW are vaccinated or are responders to vaccine and, therefore, are at risk to acquire HBV infection. In a recent study from India, only 55% of the 2,162 HCWs screened had received vaccination, 28% were unvaccinated, and 17% were unaware of their vaccination status. Protective (>10 IU/ml) anti-HBs titers were seen in only 61.7%. One percent HCWs were HBsAg positive, 24.7% had past exposure (IgG anti-HBc positive), and occult HBV was detected in 5% of HCWs with past exposure (64).

HCWs, who are chronically infected with HBV, represent a potential risk for their patients. Since the early 1970s, HBV-infected HCWs have been identified as the source of infection for patients who underwent surgical procedures. The risk for patients becoming infected during surgical procedures depends on several factors including serum HBV DNA level and HBeAg positivity, skill, and medical condition of the HCW. Other factors include duration of surgery, volume of blood transmitted, and route of transmission (percutaneous vs. mucosal). Although various routes of transmission of HBV have been described, most HBV infections are caused by contact with infected blood. Sharp injuries with needles or other sharp devices can occur during the treatment of patients. During operative procedures, the risk for contact between the HCW’s and patient’s blood has been estimated to be 2.02–2.21% (65, 66). A recent survey on surgeons in training at 17 medical centers across the United States found that 83% of surgeons in training had had a needlestick injury during training (67). The estimated annual frequency of pathogen-specific percutaneous injuries was 1,168 for hepatitis B in a nationwide survey in Taiwanese health-care workers (68). The amount of infected blood transmitted affects the risk of transmission. The number of HBV particles transmitted by a needlestick and during delivery depends on the viral load and the volume of blood transmitted. HBeAg/anti-HBe status is often used as a marker of infectivity. However, serum HBeAg is at best an indirect measure of hepatitis B viremia because of possible mutations. The infection risk after exposure to HBV-infected blood depends on the viral load which has only been determined in a few investigations. Sera of transmitting surgeons were found to contain HBV DNA levels between $5.0 \times 10^9$ and $6.35 \times 10^4$ g Eq./ml. The lowest measured HBV DNA level in serum from a transmitting surgeon was $4.0 \times 10^4$ g Eq./ml in a sample taken at least 3 months after transmission (69). The proportion of patients infected
with HBV after treatment by an infected HCW varies between 0.5 and 13.1% in different investigations (70).

The risk estimate for HBV in a model including four determinants (prevalence of HBV in medical staff, frequency of percutaneous injuries, frequency of a sharp objects contact with susceptible patients, and frequency of HBV transmission after exposure, e.g., by needle-stick injuries) was 2,400 HBV transmissions per 1 million surgical procedures (71). For comparison, the corresponding risks for hepatitis C is about 140 per million (72), and for HIV, about 24 per million surgical procedures (71). In this calculation the influence of surgeons’ HBV titers was not considered, although this factor may have an important impact on the rate of HBV transmission. Overall, the available estimates indicate that the risk of HBV transmission from infected medical personnel to patients is much higher than that for HCV or HIV.

2.4. Effect of Vaccination on the Epidemiology of Chronic Hepatitis B

Immunization against HBV has always been a successful experience in universal vaccination campaigns in different countries. It is well known that success is not achieved when the strategy is oriented to vaccinating only risk groups. It is estimated that 25–30% of persons with hepatitis B deny having any risk factor whatever for acquiring the infection and, therefore, are not identified as vaccination targets. In the United States, only after vaccination was recommended for all newborns in 1991, and in 1996, routine immunization of adolescents from 11 to 12 years of age, was a substantial reduction found in the incidence of hepatitis B (73).

Despite availability of vaccines, their introduction in infant immunization programs globally was limited during the 1980s; the use of these vaccines being largely confined to protecting individuals at risk. In 1990, Ghendon’s WHO strategy for global elimination of new cases of hepatitis B, Younde Meeting in 1991, and then the Fourteenth Meeting of the Global Advisory Group of the Expanded program on Immunization recommended the integration of hepatitis B vaccine into national immunization systems in all countries with a hepatitis B carrier prevalence (HBsAg) of 8% or greater. Following the endorsement of the recommendations of the Global Advisory Group by the World Health Assembly in 1992, the number of countries with hepatitis B vaccine incorporated within their national immunization program has been continuously rising. The introduction of hepatitis B vaccine into developing countries received a further boost with the formation of the Global
Alliance on Vaccines and Immunization (GAVI), which is an international coalition of immunization partners (74).

Primary indicators of a positive impact of hepatitis B vaccination may include: (1) decline in acute cases of hepatitis B; (2) reduction in the proportion of deaths attributable to complications of HBV (cirrhosis or HCC); and (3) falling seroprevalence of HBsAg in the vaccinated population. Unlike the situation with other vaccine-preventable diseases, the efficacy of hepatitis B prevention programs is not based solely on surveillance of acute disease. In particular, because most infections in children are asymptomatic, acute disease surveillance will not reliably measure the initial impact of routine infant vaccination. However, trends in acute hepatitis B disease incidence can be used to evaluate the effectiveness of programs directed at adolescents and adults who are more likely to have symptomatic infections after HBV exposure. Because most of the HBV-associated morbidity and mortality is related to chronic infection, demonstrating a reduction in the prevalence of chronic infection in children is the major early indicator of success. The serious adverse outcomes due to chronic HBV infection occur several decades after exposure; thus, much of the benefit of infant immunization on prevention of cirrhosis and HCC will not be realized for decades after a program is started.

The positive impact of HBV vaccination has been demonstrated beyond doubt. In July 1984, Taiwan was one of the first countries in the world to mandate infant immunization against HBV. Within 10 years of introduction of universal immunization, HBsAg prevalence declined from 9.3 to 1.3% in children younger than 12 years (37). The incidence of HCC in children aged 6–14 years declined from 0.7 per 100,000 between 1981 and 1986, to 0.57 per 100,000 between 1986 and 1990, and to 0.36 per 100,000 between 1990 and 1996 (75). The HBsAg prevalence among persons younger than 15 years of age has declined from 9.8% in 1984 to 0.7% in 1999, thereby converting a hyperendemic into low endemic country within 15 years of the introduction of the hepatitis B immunization program (76). In Formosa, the mortality rate from fulminant hepatitis B in nursing infants, between the years 1974 and 1984, before the universal vaccination era, was 5.36/100,000. This rate fell to 1.71/100,000 between 1985 and 1998, after the vaccination program was launched. Universal vaccination against hepatitis B in these high endemicity countries effectively reduced both perinatal and horizontal transmissions, thus diminishing the rates of chronic hepatitis B (76, 77). In Formosa, the incidence of chronic HBV-infected subjects diminished from 10 to 1% in children under the age of 15 years (77). In Thailand, in less than 10 years after initiation of a vaccination program, the overall HBsAg prevalence declined from 3.4
to 0.7% among 1–18-year-olds (78). Vaccination has impacted the incidence and prevalence of hepatitis B infection in other Asian countries, converting high-prevalence to intermediate-prevalence areas, with the possible exception of China (79). The persistence of higher prevalence in China has been attributed to vaccine coverage of only 25–30% of the population in this country (80).

Similar effects have been reported from African countries as well. In the Gambian Hepatitis Intervention Study (81), the protective efficacy of the vaccine in preventing chronic infection in the first 3 years of life was estimated to be 95%. Despite a relatively high prevalence of HBV infection in the general population and women of childbearing age, integrating infant immunization within the current schedule of 6, 10, and 14 weeks had a significant impact on reducing the prevalence of chronic infection, as shown in South Africa where the HBsAg prevalence rate declined from 12.8% in 3-year-olds to 3.0% within 5 years of initiation of the vaccination program (82).

Similarly, in Saudi Arabia, following the introduction of the vaccine, the overall HBsAg prevalence dropped from 6.7% in 1989 to 0.3% in 1997 in children aged 1–12 years (83).

The effect of vaccination has also been marked in European countries. Italy was one of the few countries in Europe to introduce hepatitis B vaccination, initially based on a high-risk approach, but expanded to cover both infant and adolescents in 1991. Between 1985 and 1996, the prevalence of chronic HBV infection rate has fallen from 3.4 to 0.9% with the greatest decline in the age group 15–24 years (84, 85). In Bulgaria, following the introduction of HBV immunization in 1991, a coverage level of 71.3% was reached by 1992 and a dramatic decline in the reported annual incidence of acute hepatitis B in infants was seen, from 22 to 30 per 100,000 in the early 1980s to 5.6 per 100,000 (86).

In the United States, following the adoption of universal infant vaccination in 1991, the incidence of acute hepatitis B in children and adolescents has decreased by 89%, and the racial disparities in the prevalence of chronic HBV infection have narrowed (87). There has been a marked decrease in new infections following vaccination in Alaska, where HBV is endemic (88). This dramatic decline can be expected to result in a reduced incidence of cirrhosis and HCC in the coming decades.

Therefore, effectiveness of routine infant hepatitis B immunization in significantly reducing or eliminating the prevalence of chronic HBV infection has been demonstrated in a variety of countries and settings. In general, the greatest impact has been achieved in high HBV endemic areas that have achieved high vaccine coverage among infants, and where the vaccine was administered at birth. However, HBV
vaccination programs have produced remarkable results even when the policy is not universal or consistent throughout a country. For example, a reduction in the HBsAg prevalence among children from 10.0 to 0.6% and from 18.7 to 2.2%, has been reported in Gambia and Senegal, respectively, the two African countries that have been able to implement vaccination in only a portion of their population (89).

Many long-term hepatitis B immunization programs have demonstrated a greater than expected benefit in terms of disease reduction when compared with levels of vaccination coverage. A possible reason for this finding is that the pool of individuals that is highly infectious to others is greatly reduced by preventing chronic infections in children. Not only are new infections in children more likely to become chronic, but chronically infected children are likely to be HBeAg positive and highly infectious to others. Thus, by preventing infections in children, the number of HBeAg-positive persons declines rapidly over time because of the inherent clearance of HBeAg in older persons (90).

At present, the development of mutants as a result of vaccination programs is of concern in high endemicity countries. The emergence of surface gene mutants, with mutations mainly occurring within the “a” determinant, has been observed in some vaccinees in several areas of the world. The mutants may go undetectable by current diagnostic assays and potentially cause breakthrough infections in a vaccinated population (91), thus posing a potential threat to the long-term success of vaccination programs.

The prevalence of surface antigen gene mutants increases gradually 5–10 years after the programs are instituted. In Taiwan, the prevalence of “a” determinant mutants in HBV DNA positive children was 7.8% in 1984, significantly increased to 19.6% in 1989, peaked at 28.1% in 1994, and remained at 23.1% in 1999; it was higher in those fully vaccinated compared with those not vaccinated. However, the number of mutant infected children in each survey was stable in the first 5–10-year period but decreased 10–15 years post vaccination. More HBsAg positive “a” determinant mutants emerged in children fully vaccinated with plasma-derived vaccine than those given recombinant vaccine. Thus, although “a” determinant variants have an advantage in infecting immunized children, they do not threaten current HBV vaccination strategies in Taiwan (92).

2.5. Epidemiology of Special Situations

2.5.1. HBV HCV COINFECTION

The exact number of patients infected with both HCV and HBV is unknown. One Eastern European study found a rate of dual infection
in 0.68% of a randomly selected healthy population of over 2,200 individuals (93). In patients with chronic hepatitis B, estimates of the rates of HCV coinfection vary from 9 to 30%, depending on the geographic region. One Italian study found that rates of dual infection increased with age, and was more common in patients over 50 years of age (94). These numbers may underestimate the true number of patients with both viral infections because no large-scale studies have been performed, and occult HBV (presence of HBV DNA in the sera and/or liver of persons who are HBsAg-negative) is a problem in patients with chronic hepatitis C (see later). Occult HBV infection has been found to be very common (45%) in HCV-infected injection-drug users in the United States (95).

2.5.2. HBV-HIV COINFECTION

There is evidence of prior HBV infection in approximately 90% of HIV-infected persons whereas chronic HBV infection (indicated by reactive HBsAg) is detected in 5–15% of HIV-infected persons globally (96). HIV infection is associated with the failure to seroconvert following acute infection and a greater risk of developing chronic hepatitis B infection compared to HIV seronegative person. Detection of HBV DNA in the serum in the absence of HBsAg has been reported in persons with HIV infection, particularly those with anti-HBc IgG alone. However, estimates of the prevalence of occult HBV infection vary considerably from 0 to 10% (97, 98). Further, in several studies, occult HBV infection has not been associated with elevation in serum ALT and the clinical significance in HIV-infected persons is uncertain.

2.5.3. EPIDEMIOLOGY OF OCCULT HBV

Occult hepatitis B virus infection (HBV) can be defined as the long-lasting persistence of viral genomes in the liver tissue (and in some cases also in the serum) of individuals negative for the HBsAg. The gold standard to test for occult HBV is the analysis of DNA extracts, from liver as well as from blood samples, performed by a “nested” PCR technique and the use of oligonucleotide primers specific for at least three different HBV genomic regions. With this approach, only the cases in which HBV DNA is detected using at least two different sets of primers may be considered positive for the occult infection (99). Occult HBV infection is a worldwide entity, although its distribution may reflect the general prevalence of the HBV in various geographic areas and in various populations. HCV-infected patients are the category of individuals with the highest prevalence of occult HBV. In particular, HBV DNA is detectable in about one-third of HBsAg-negative HCV carriers in the Mediterranean Basin and this prevalence is even higher in Far East
Asian countries. In a recent Lebanese study, the prevalence of occult HBV infection ranged from 11.9 to 44.4% in HCV-infected patients and it increased with increasing severity of the liver disease. Although Lebanon is an area of low endemicity for both HBV and HCV, occult HBV infection is common in HCV-infected patients (100).

A variable prevalence (2–24%) of occult HBV infection has been reported in subjects with cryptogenic liver disease (101, 102). Besides patients with liver disease, individuals at high risk of parenterally transmitted infections have also been widely investigated for occult HBV. In general, a high prevalence has been reported, with 45% intravenous drug addicts in Baltimore (95) and 51% hemophiliacs in Japan (103) having occult HBV infection. The studies performed on hemodialysis patients provide widely divergent results, reporting a prevalence of occult HBV ranging from 0 to 36% (104). These discrepancies appear to be mainly dependent on the different sensitivity and specificity of the assays utilized in the various studies.

Widely divergent prevalence of occult HBV, ranging from 0 to 89% (105, 106), has been reported in HIV positive individuals. This difference relates to the great difference in sensitivity and specificity of the assays used in these various studies. The study finding the highest prevalence of occult HBV both used a very sensitive HBV DNA amplification procedure and examined serial serum samples from each individual (105, 106). Since serum HBV DNA levels fluctuate even in occult HBV-infected patients, repeating the HBV DNA test over time is a useful tool in identifying the occult HBV cases.

Among the populations of apparently healthy individuals, occult HBV has been extensively explored in blood donors and much less in the general population. Among blood donors, occult HBV infection is of rare occurrence in the western world, whereas it is frequently detected in the developing world. As far as the general population is concerned, a recent study evaluating occult HBV prevalence in HBsAg-negative residents of a Canadian Inuit community detected HBV DNA in 18% of anti-HBe-positive subjects and in 8% of HBV seronegative individuals, respectively (107), whereas occult HBV infection was detected in 3.4% among voluntary blood donors from India (102).

### 2.6. Molecular Epidemiology

HBV, the hepadnavirus infecting humans, is classified into eight genotypes (A, B, C, D, E, F, G, and H) by phylogenetic analyses using alignments of whole genomes. HBV genotypes differ by at least 8%. Due to the genetic diversity of HBV, numerous subgenotypes of HBV have been described (108, 109) (Table 1; Fig. 5).
<table>
<thead>
<tr>
<th>Subgenotype</th>
<th>Synonyms</th>
<th>Geographic Origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A1</td>
<td>Aa, A'</td>
<td>(112, 118, 121)</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>Ae, A–A'</td>
<td>Europe</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>Ac</td>
<td>Gabon, Cameroon</td>
</tr>
<tr>
<td></td>
<td>A4</td>
<td></td>
<td>Mali</td>
</tr>
<tr>
<td></td>
<td>A5</td>
<td></td>
<td>Nigeria</td>
</tr>
<tr>
<td>B</td>
<td>B1</td>
<td>Bj</td>
<td>Japan</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>Ba</td>
<td>Asia (excluding Japan)</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td></td>
<td>Indonesia, Philippines</td>
</tr>
<tr>
<td></td>
<td>B4</td>
<td></td>
<td>Vietnam</td>
</tr>
<tr>
<td></td>
<td>B5</td>
<td></td>
<td>Philippines</td>
</tr>
<tr>
<td></td>
<td>B6</td>
<td></td>
<td>Alaska</td>
</tr>
<tr>
<td>C</td>
<td>C1</td>
<td>Cs</td>
<td>South East Asia (Vietnam, Myanmar, Thailand, Southern China)</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>Ce</td>
<td>Far East (Korea, Japan, Northern China)</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td></td>
<td>Micronesia</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td></td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td>C5</td>
<td></td>
<td>Philippines, Vietnam</td>
</tr>
<tr>
<td>D</td>
<td>D1</td>
<td></td>
<td>Mongolia, Belarus, Europe</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td></td>
<td>India</td>
</tr>
<tr>
<td></td>
<td>D3</td>
<td></td>
<td>South Africa, East India, Serbia</td>
</tr>
<tr>
<td></td>
<td>D4</td>
<td></td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td>D5</td>
<td></td>
<td>East India</td>
</tr>
<tr>
<td>F</td>
<td>F1</td>
<td></td>
<td>South and Central America</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td></td>
<td>South America</td>
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<tr>
<td></td>
<td>F3</td>
<td></td>
<td>Bolivia, Colombia, and Venezuela</td>
</tr>
<tr>
<td></td>
<td>F4</td>
<td></td>
<td>Argentina</td>
</tr>
</tbody>
</table>
HBV subgenotypes differ by at least 4%. In genotypes A, B, and C, epidemiological data show that the respective subgenotype pairs A1/A2 (formerly termed Aa/Ae) (110), B1,6/B2–5 (formerly Bj/Ba) (110), and C1/C2 (formerly Cs/Ce) differ substantially in many virological and probably some clinical parameters. Subgenotypes also show distinct geographic distribution. However, this is not true for genotype D with subgenotypes D1, D2, and D3 being described as widespread in the world; e.g., D3 was found in Asia (East India) (111), South Africa (112), and Europe (Serbia). HBV genotype B (HBV/B) can be divided into two major geographically distinct groups, provisionally designated “Bj” (“j” for “Japan”) and “Ba” (“a” for “Asia”) (113, 114). Molecular evolutionary analyses have shown that HBV/Ba includes four subgenotypes B2–B5 (113–115). Distinguishing these from the HBV/Bj/B1 subgenotype, the HBV/Ba/B2–B5 subgenotypes demonstrate evidence of intergenotypic recombination with HBV/C, in a genome part corresponding to the core promoter/precore/core (Cp/preC/C) genomic region (115). Clinically, HBV/Ba infection has been found in many Asian countries such as Taiwan to be associated with a higher risk of development of HCC in chronic HBV-infected subjects (116). By
contrast, in Japan, HCC has less commonly been associated with subgenotype Bj (117). In addition, the prevalence of HBeAg in persons infected with HBV/Bj has been found to be lower than that in persons infected with HBV/C or HBV/Ba (117). Although genotypes B and C are more prevalent in Asian countries, in India, genotypes D and A are more prevalent (118). A novel subgenotype, “B6,” of HBV/B in indigenous Arctic populations with chronic liver disease has been described (119). Thus, it has been suggested that HBV/B be considered to be a genotype present in two major forms: a nonrecombinant type (Bj/B1 and B6) and a recombinant type (Ba/B2–B5) (119).

Except for genotype E and G, all HBV genotypes can be divided into subgenotypes. The absence of subgenotypes in HBV genotype E has been assumed to be the consequence of a recent genesis for genotype E. The case for HBV genotype G appears to be less clear. Genotype G was originally found in the United States, France, and Germany. Later, partial sequencing of HBV genes pointed to a high prevalence of HBV genotype G in Mexico. Nevertheless, the geographic origin of HBV genotype G remains unknown (120).

2.6.1. DOUBLE INFECTIONS AND RECOMBINANTS

Double infections with two different HBV genotypes have been known since typing was done serologically. Subsequently, evidence of super infection with HBV isolates of the same or different genotype was described in chronic HBV patients. Additional observations came from patients treated with interferon, where after treatment and relapse, a switch of the genotype has been described (135). Using different methods for genotyping, several reports described high rates of double infection with two different HBV genotypes in all parts of the world. Using these methods double infections have been found in 4.4–17.5% (136). Even triple infections with HBV of genotypes A, B, and C have been described in 0.9% of HBV-infected intravenous drug users (137). Infection with HBV of genotype G seems to be associated very often with an infection of HBV genotype A (138). Japanese strain of HBV having a recombination between genotypes C and D have been reported (139). Recently, recombinant genotypes A and D have been identified from India (140).

3. SCREENING HIGH-RISK POPULATIONS TO IDENTIFY HBV-INFECTED PERSONS

In developed countries such as the United States where most of the infants, children, and adolescents have been vaccinated against HBV, the risk of transmitting HBV in daycare centers or schools is
extremely low and HBsAg-positive children should not be isolated or prevented from participating in activities including sports. Table 2 displays AASLD recommendations for the population and high-risk groups that should be screened for HBV infection and immunized if seronegative (1). The tests used to screen persons for HBV should

<table>
<thead>
<tr>
<th>Individuals born in areas of high (&gt;8%) and intermediate-prevalence rates (2–7%) for HBV including immigrants and adopted children#</th>
</tr>
</thead>
<tbody>
<tr>
<td>— South Asia (except Sri Lanka)</td>
</tr>
<tr>
<td>— Africa</td>
</tr>
<tr>
<td>— South Pacific Islands</td>
</tr>
<tr>
<td>— Middle East (except Cyprus)</td>
</tr>
<tr>
<td>— European Mediterranean: Greece, Italy, Malta, Portugal, and Spain</td>
</tr>
<tr>
<td>— The Arctic (indigenous populations)</td>
</tr>
<tr>
<td>— South America: Argentina, Bolivia, Brazil, Ecuador, Guyana, Suriname, Venezuela, and Amazon region of Colombia and Peru</td>
</tr>
<tr>
<td>— Independent states of former Soviet Union</td>
</tr>
<tr>
<td>— Eastern Europe, including Russia, except Hungary</td>
</tr>
<tr>
<td>— Caribbean: Antigua and Barbuda, Dominica, Dominican Republic, Granada, Haiti, Jamaica, Puerto Rico, St Kitts and Nevis, St Lucia, St Vincent and Grenadines, Trinidad and Tobago, and Turks and Caicos</td>
</tr>
</tbody>
</table>

**Other high-risk groups recommended for screening**

— Household and sexual contacts of HBsAg-positive persons

— Persons who have ever injected drugs

— Persons with multiple sexual partners or history of sexually transmitted disease

— Men who have sex with men

— Inmates of correctional facilities

— Individuals with chronically elevated ALT or AST

— Individuals infected with HCV or HIV

— Patients undergoing renal dialysis

— All pregnant women

# If HBsAg-positive persons are found in the first generation, subsequent generations should be tested.

*Those who are seronegative should receive hepatitis B vaccine.
include HBsAg and hepatitis B surface antibody (anti-HBs). Alternatively, hepatitis B core antibody (anti-HBc) can be utilized as long as those who test positive are further tested for both HBsAg and anti-HBs to differentiate infection from exposure. Some persons may test positive for anti-HBc but not HBsAg or anti-HBs. The finding of isolated anti-HBc can occur for a variety of reasons:

(1). Anti-HBc may be an indicator of chronic HBV infection; in these persons, HBsAg had decreased to undetectable levels but HBV DNA often remains detectable, more so in the liver than in serum (Occult HBV infection).

(2). Anti-HBc may be a marker of immunity after recovery from a prior infection. In these persons, anti-HBs had decreased to undetectable levels but anamnestic response can be observed after one dose of HBV vaccine (141).

(3). Anti-HBc may be a false positive test result particularly in persons from low-prevalence areas with no risk factors for HBV infection. These individuals respond to hepatitis B vaccination similar to persons without any HBV seromarkers (142).

4. NATURAL HISTORY OF CHRONIC HBV INFECTION

A number of phases of chronic HBV infection are recognized, reflecting the dynamic interaction between the virus and the human host immune system (143).

4.1. Phases of Chronic HBV Infection Following Vertical Transmission

4.1.1. IMMUNE-TOLERANT PHASE

In patients with perinatally acquired HBV infection, the first phase (immune tolerance) is characterized by the absence of elevated transaminase levels despite evidence of active HBV replication (presence of HBeAg and HBV DNA in serum). During this phase, which may last 1–4 decades, spontaneous and treatment-induced HBeAg seroconversion is infrequent (<5% per year). Liver biopsy during immune tolerance often reveals an absence of inflammation and scarring. HBCAg is abundant in hepatocyte nuclei during this phase. A study from Taiwan followed 240 patients (54% men, mean age 27.6 years) who presented in this phase and found that only 5% progressed to cirrhosis and none to HCC during a follow-up period of 10.5 years (144). These findings indicate that prognosis is generally favorable for patients who are in the immune tolerant phase. However, recent data have shown that more than one-third of HBeAg-positive HBV-infected patients
with persistently normal ALT have significant fibrosis on liver biopsy (145). In patients with childhood- or adult-acquired HBV infection, the “immune tolerant” phase is short-lived or absent.

4.1.2. IMMUNE-REACTIVE PHASE

During the immune-reactive phase (immune clearance/HBeAg-positive chronic hepatitis), symptoms of liver disease may appear for the first time. Increased immune pressure on the virus during this phase leads to suppression of serum HBV DNA levels and accelerated clearance of HBeAg with seroconversion to anti-HBe. This phase is characterized by the presence of HBeAg, high or fluctuating serum HBV DNA levels, persistent or intermittent elevation in serum aminotransferases, and active inflammation on liver biopsy. The “flares” of aminotransferases are believed to be manifestations of immune-mediated lysis of infected hepatocytes secondary to increased T-cell responses to hepatitis B core antigen (HBCAg) and HBeAg. These flares may precede HBeAg seroconversion, but many flares only result in transient decreases in serum HBV DNA levels without loss of HBeAg and some flares may lead to hepatic decompensation. Generally, the flare subsides after a variable period of time, although the associated liver injury may not regress and fibrosis can result. Lobular hepatitis now becomes evident on histology and the histological changes may mimic acute hepatitis. However, the portal tracts tend to enlarge with fibrosis, and HBsAg can be demonstrated by specific stainings in hepatocytes, which is not seen in acute hepatitis B. HBCAg, which in the previous phase resided in the hepatocytes nucleus, is now frequently seen also in the cytoplasm. Histological changes can vary from mild hepatitis to cirrhosis. The duration of the “immune clearance” phase, and the frequency and severity of the flares, correlate with the risk of cirrhosis and HCC. Recurrent “flares” are more common in men and may explain why HBV-related cirrhosis and HCC are more common in men than in women. An important outcome of the “immune clearance” phase is HBeAg to anti-HBe seroconversion. Factors associated with higher rates of spontaneous HBeAg seroconversion include older age, higher aminotransferase levels, and HBV genotypes. High aminotransferase level is a surrogate marker for vigorous host immune response, accounting for its strong correlation with spontaneous as well as treatment-related HBeAg seroconversion. Studies from Asian countries, where genotypes B and C predominate, showed that genotype B is associated with a lower prevalence of HBeAg, HBeAg seroconversion at an earlier age, and more sustained virological and biochemical remission after HBeAg seroconversion (146, 147). Flares in aminotransferases were more typical in genotype C patients, suggesting that greater liver injury
at the time of seroconversion is also a feature of infection with this genotype (148). In a prospective cohort study, 1,158 Alaska Native persons were tested serially for HBeAg for a median of 20.5 years and were genotyped. Genotypes A, B, C, D, and F were identified. Genotype C persons initially HBeAg-positive were more likely than those with other genotypes to be positive on initial and final specimens and time to HBeAg clearance was longer \((P < 0.001)\). Age at which 50% of persons cleared HBeAg was <20 years for those infected with genotypes A, B, D, and F and 47.8 years in genotype C \((P < 0.001)\). After losing HBeAg, those with genotypes C and F were more likely to revert to the HBeAg-positive state \((P < 0.001)\) (149).

4.1.3. **Low-Replicative Phase**

After destruction of the hepatocytes producing HBV, active replication of HBV decreases. In the serum, HBeAg disappears and anti-HBe starts to appear, ALT normalizes, and HBV DNA decreases. However, HBsAg is continuously produced by the liver cells containing integrated HBV genome. When the HBV genome starts to integrate within the hosts’ chromosomal DNA is unclear, but integration can be demonstrated readily at this stage. Because of the relatively lower HBV replication in the liver, hepatocytes are spared from attacks of the immune system, and the host once again becomes tolerant to HBV. The residual underlying pathology already present at the time of cessation of the HBV replication determines the outcome of the illness. If there is no cirrhosis caused by events during clearance phase, the previous liver injury may regress now. However, if cirrhotic changes are already present at the time of HBeAg seroconversion the cirrhosis remains and is likely to progress. Liver biopsy during this phase usually shows mild hepatitis and minimal fibrosis, but more severe liver damage including cirrhosis may be observed in patients who had accrued severe liver injury during the preceding “immune clearance” phase. HBeAg is generally not seen in the liver. This phase was referred to as the “healthy carrier” state in the past, but this is an erroneous label given that a significant proportion of patients may have high HBV DNA levels, hepatic fibrosis and the potential for further disease flares, and other complications such as HCC. Indeed, for patients with infection acquired at an early age, the majority of complications occur after HBeAg seroconversion. The so-called “inactive carriers” are rarely inactive and hence this term should preferably be abandoned. A better term would be “chronic HBV infection, with or without liver disease”; based on defined clinical, biochemical, and histological criteria (145).

This relatively low-replicative phase may persist indefinitely, in which case the prognosis is generally favorable, especially if this state
is reached early. This is supported by a long-term follow-up study of HBsAg-positive healthy blood donors in northern Italy (150). No difference in survival was found between 296 HBsAg-positive blood donors and 157 uninfected controls over a 30-year period, and no episodes of hepatic decompensation were reported. Unfortunately, some patients in this phase have reactivation of HBV replication. Reactivation may occur spontaneously or as a result of immunosuppression. In one study of 283 Taiwanese patients followed for a median of 8.6 years after spontaneous HBeAg seroconversion, 67% had sustained remission, 4% had HBeAg reversion, and 24% had HBeAg-negative chronic hepatitis B. Cirrhosis developed in 8% and HCC in 2%, the risk being higher in those who had active hepatitis after HBeAg seroconversion (151). Recent data from India have shown that 13.8% of HBeAg-negative CHBV-infected patients with persistently normal ALT have significant fibrosis on liver biopsy (145).

4.1.4. REACTIVATION PHASE

The fourth phase (reactivation of HBV replication/HBeAg-negative chronic hepatitis B) is characterized by negative HBeAg, positive anti-HBe, detectable HBV DNA, elevated aminotransferases, and continued necroinflammation. Whereas most patients reach this phase after a variable duration of nonreplicative phase, some progress directly from HBeAg-positive chronic hepatitis to HBeAg-negative chronic hepatitis. Patients in this phase are usually older and have more advanced liver disease because this represents a later phase in the course of chronic HBV infection. Serum HBV DNA levels are lower than in HBeAg-positive patients but may be high. The higher levels of serum HBV DNA result from a spontaneous mutation in the core or core promoter region of the viral genome. The precore mutation produces a stop codon in a region of the HBV genome that prevents the formation of HBeAg, whereas the basal core promoter mutation affects HBeAg transcription. These mutations either singly or in combination permit HBV replication in the absence of HBeAg. The hallmark of this phase is its fluctuating course. In a study of 164 anti-HBe-positive patients who were monitored at monthly intervals for a median period of 21 months, 64% had fluctuating alanine aminotransferase (ALT) levels, including 44% whose ALT levels were intermittently normal (152). Several investigators have attempted to define cutoff HBV DNA levels that would differentiate patients with HBeAg-negative chronic hepatitis from inactive carriers, but in view of the fluctuating course, serial testing is more reliable than a single test. A recent study found that reactivation of hepatitis B following HBeAg seroconversion correlated significantly with genotype C (P = 0.003), male sex (P = 0.03), alanine aminotransferase
levels >5 x upper normal limit during the HBeAg-positive phase \( P = 0.02 \), and age at HBeAg seroconversion >40 years \( P = 0.002 \) (153).

HBeAg-negative chronic hepatitis B was originally reported in Mediterranean countries. It has now been reported in all parts of the world. The geographic variations in prevalence of HBeAg-negative chronic hepatitis B are related to the predominant HBV genotype in that region. Recent studies in Europe, Asia, and the United States have all reported an increased prevalence of HBeAg-negative chronic hepatitis and a decreased prevalence of HBeAg-positive chronic hepatitis (154); this may be related to increased awareness, decrease in new HBV infections, and aging of existing chronic HBV-infected subjects.

Spontaneous HBsAg seroclearance has been reported to occur at the rate of 0.5–1% per year in patients with chronic HBV infection. HBsAg seroclearance is generally accompanied by undetectable serum HBV DNA, normalization of liver biochemistries, and improved liver histology. However, HCC has been reported in a small percent of patients, the risk being higher in those with cirrhosis, HCV coinfection, or older age at the time of HBsAg seroclearance (155).

### 4.2. Phases of Chronic HBV Infection Following Horizontal Transmission

Horizontally acquired disease also evolves through a number of phases with active replication and hepatic necroinflammatory activity in the early months and years of chronic HBV infection. With time, replication often diminishes and host immune pressure results in HBeAg/anti-HBe seroconversion. This is followed by a quiescent phase of infection with lessened liver injury and evolution into a non-replicative phase. Certain patients appear to suffer little morbidity after HBeAg seroconversion. For instance, studies of HBsAg-positive Italian patients in the inactive carrier state initially identified when they were rejected as blood donors showed that these individuals experienced no appreciable increase in liver-related morbidity over many years (150).

### 4.3. Predictors of Disease Progression in Chronic HBV Infection

#### 4.3.1. Chronic HBV Infection and Cirrhosis

The annual incidence of cirrhosis has been estimated to be 2–6% for HBeAg-positive and 8–10% for HBeAg-negative patients. The higher rate of cirrhosis among HBeAg-negative patients is related to older age and more advanced liver disease at presentation. Among HBeAg-positive patients, the rate of cirrhosis development is higher in those who remained HBeAg positive during follow-up. Other factors that
have been identified to be associated with progression to cirrhosis include significant alcohol intake, concurrent infection with hepatitis C virus (HCV) or human immunodeficiency virus (HIV), high levels of HBV replication, and HBV genotype (C>B). In a recent study from Taiwan, the 10-year cumulative probability of cirrhosis among chronic hepatitis B patients with HCV superinfection, HDV superinfection, and no superinfection was 48, 21, and 9%, respectively (156). Coinfection of HIV and HBV has also been shown to increase the risk of cirrhosis and liver-related mortality compared with HBV monoinfection. Patients who had HBeAg reversion had increased risk of cirrhosis compared with those who had sustained HBeAg seroconversion. In one study of 3,774 chronic HBsAg-positive patients aged 30–65 years, the adjusted relative risk of cirrhosis for patients with baseline serum HBV DNA >10^4 and >10^6 copies/mL was 2.3 (95% CI, 1.6–3.5) and 9.3 (95% CI, 6.5–13.1), respectively (157). Thus, persistent high levels of HBV replication (with accompanying hepatitis) increase the risk of cirrhosis, but the prognostic significance of a high serum HBV DNA level at a single time point in a young carrier (<30 years old) is unclear. Studies in Asia showed that genotype C is associated with HBeAg seroconversion at a later age and more active hepatitis than genotype B; these studies also found that genotype C is associated with a more rapid rate of progression to cirrhosis than genotype B (158, 159).

4.3.2. CHRONIC HBV INFECTION AND HCC

The incidence of HCC is higher in those with cirrhosis, as compared to those without cirrhosis. The annual incidence of HCC has been estimated to be <1% for HBV-infected subjects without cirrhosis, compared to 2–3% in those with cirrhosis (160). Other factors associated with development of HCC include coinfection with HCV (161), a family history of HCC (162, 163), significant alcohol intake (153), high levels of HBV replication (164), HBV genotype (C>B, D>A) (165–167), and core promoter mutations (168). Obesity, diabetes, and smoking may also contribute to the risk of HCC (169).

Several lines of evidence support an association between HBV replication and the risk of HCC. In a prospective study of 11,893 Taiwanese men aged 30–65 years, followed for a mean of 8.5 years, the adjusted relative risk of HCC was six- to sevenfold higher among HBsAg-positive men who were HBeAg positive at entry than those who were HBeAg negative (170). Another study from Taiwan found that the risk of HCC increased with increasing baseline serum HBV DNA level. The adjusted odds ratio for patients with the highest quartile of HBV DNA level vs. those with the lowest was 7.26 (95% CI, 3.54–14.89) (171). Studies in Senegal and mainland China also confirmed an increased
risk of HCC among HBV-infected patients with high baseline serum HBV DNA levels (164). Unfortunately, none of these studies monitored serum HBV DNA and aminotransferase levels over time. The duration of high levels of HBV replication as well as the intensity and frequency of hepatitis activity may be more important than a high HBV DNA level on a random occasion in predicting the risk of HCC in individual patients.

Several studies from Asia demonstrated that genotype C is associated with increased risk of HCC compared with genotype B (165, 166). This may be related to a longer duration of high levels of HBV replication and active hepatitis and a higher frequency of core promoter mutations. Core promoter mutations have been shown in many studies to be associated with increased risk of HCC and to precede HCC diagnosis (168). Core promoter mutations also have been found to be associated with more active hepatitis and are more frequently associated with genotype C than B (168). In addition, the most common core promoter mutations (A1762T, G1764A) result in corresponding changes in the overlapping X gene. The HBx protein is a potent transactivator and may activate host genes including oncogenes. HBV genotype D has been suggested to be predictive of the occurrence of HCC in young patients. In a study from India, genotype D was more prevalent in HCC patients < 40 years of age, as compared to incidentally detected asymptomatic HBsAg-positive subjects (63 vs. 44%, P = 0.06) (167).

4.3.3. HBV DNA Levels and Disease Progression

During the past few years, the key relationship between elevated and persistent viremia and disease outcomes (cirrhosis, HCC, and liver disease-related deaths) has been recognized. These data have shown that relatively low-level persistent viremia can increase an individual’s risk for these HBV-related outcomes. A study from Taiwan has clearly established a relationship between persistent HBV replication and the subsequent development of cirrhosis and HCC in patients with presumed early acquisition of HBV infection (172). The investigators in this prospective cohort study enrolled 3,653 chronically HBV-infected individuals, the majority of whom were HBeAg negative. Over a mean follow-up of 11.4 years without therapeutic intervention, the cumulative incidence of HCC was significantly related to baseline HBV DNA levels: 14.89% of those with baseline HBV DNA levels >1 million copies/mL (~200,000 IU/mL) at entry developed HCC compared with only 1.30% of those with baseline HBV DNA levels < 300 copies/mL (~60 IU/mL). The relationship between baseline HBV DNA levels and the development of HCC was constant in patients with an absence of cirrhosis and normal alanine aminotransferase (ALT) levels at baseline,
and this relationship persisted after adjusting for age, sex, alcohol use, and smoking status. Follow-up serum HBV DNA levels were available from some of the study participants. These values revealed that although a spontaneous reduction in HBV DNA levels during the course of the study diminished the risk for HCC, it did not eliminate it. The same group of investigators also correlated the development of cirrhosis with baseline HBV DNA levels (157). The researchers prospectively evaluated 3,582 individuals without clinical evidence of cirrhosis at entry and found that the incidence of cirrhosis began to increase when the baseline HBV DNA level was >10,000 copies/mL (~2,000 IU/mL). The relative risk of cirrhosis development correlated with HBV DNA and was independent of HBeAg status and the ALT level. An unresolved issue is whether there is a threshold level of HBV DNA below which complications such as cirrhosis do not occur. Yuen and colleagues (173) prospectively assessed the complications associated with chronic HBV infection in Chinese patients over a 4-year period and correlated the occurrence of these complications with serum HBV DNA levels. However 28.9% of patients with complications had HBV DNA levels <200 copies/mL.

4.3.4. ALT LEVELS AND DISEASE PROGRESSION

ALT level is commonly used as a biochemical marker of liver disease and has been important in defining which patients are candidates for therapy. The extent of liver cell necrosis and degree of elevated ALT level do not always correlate, and ALT measurements may fail to identify patients with necroinflammatory activity or fibrosis. In addition, the ALT activity may vary with body mass index, sex, abnormal lipid, and carbohydrate metabolism as well as the time of the day.

According to a Korean study of 94,533 men and 47,522 women in the age range of 35–59 years, the relative risk for liver-related mortality was significantly higher in patients with ALT levels between 0.5 and 1x ULN compared to those with <0.5 x ULN (174), although this study did not test HBsAg and anti-HCV. Currently recommended and revised upper limit of normal ALT values are 30 IU/L for men and 19 IU/L for women. These values are almost half of the values traditionally accepted values of the upper limit of normal ALT. Elevated ALT has been considered to be associated with active liver disease on histology while normal ALT with inactive histology. Many initial studies had shown that among patients with HBV infection with normal ALT, about 50–90% of patients had either minimal changes or chronic persistent hepatitis on biopsy. Recently, however studies have described contrary findings also (145).
4.3.5. HBV DNA Levels in Patients with Normal ALT

Patients who are HBeAg positive with normal ALT (immunotolerance phase) have high levels of serum HBV DNA. Among patients who are HBeAg negative with normal ALT, it has traditionally been believed that they have low HBV DNA levels. However, in a recent study that investigated the correlation between serum ALT level and hepatitis B viral factors in HBeAg-negative patients with persistently normal serum ALT level (PNALT), it was found that 47.5% of patients with low-normal ALT (levels of less than 0.5x upper limit of normal) and 63.4% with high-normal ALT had serum HBV DNA level >10^4 copies/ml. Also compared with chronic HBV-infected subjects with low-normal ALT, those with high-normal ALT were older (41 vs. 37 years, P<0.001) (175). Studies from China have demonstrated that a significant proportion of patients with persistently normal ALT had elevated HBV DNA levels (145,176) (Table 3).

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>HBeAg (+) PNALT  ( N=73 )</th>
<th>HBeAg (−) PNALT  ( N=116 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAI, median (range)</td>
<td>5.0(1.0–11.0)</td>
<td>3(1–10)</td>
</tr>
<tr>
<td>&gt;3, N (%)</td>
<td>46(63)</td>
<td>23(39.7)</td>
</tr>
<tr>
<td>F score, median (range)</td>
<td>1.0(0.0–4.0)</td>
<td>1(0–3)</td>
</tr>
<tr>
<td>Fibrosis stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[0/1/2/3/4] [%]</td>
<td>17.8/42.5/26.0/11.0/2.7</td>
<td>39.7/46.6/8.6/5.2/0.0</td>
</tr>
<tr>
<td>Baseline HBV DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(log cp/ml), N (%)</td>
<td>5.23(2.78–9.27)</td>
<td>4.29(2.78–9.20)</td>
</tr>
<tr>
<td>&lt;3</td>
<td>5(7.0%)</td>
<td>29(25.0%)</td>
</tr>
<tr>
<td>3–3.999</td>
<td>10(14.0%)</td>
<td>23(20.0%)</td>
</tr>
<tr>
<td>4–4.999</td>
<td>14(19.0%)</td>
<td>23(20.0%)</td>
</tr>
<tr>
<td>≥5</td>
<td>44(60.0%)</td>
<td>41(35.0%)</td>
</tr>
</tbody>
</table>

4.3.6. Histology in Patients with Normal ALT

Elevated ALT has been considered to be associated with active liver disease on histology. Many initial studies had shown that among patients with chronic HBV infection with normal ALT, about 50–90%
of patients had either minimal changes or chronic persistent hepatitis on biopsy. Recent studies have, however, described higher prevalence of liver injury in such patients. In a recent analysis of chronic HBV patients, increasing age, higher ALT, higher grade of inflammation on biopsy, and HBeAg positivity predicted fibrosis: 18% of patients with PNALT had stage 2+ fibrosis and 34% had grade 2 or 3 inflammation. Overall, 37% of patients with PNALT had significant fibrosis or inflammation (177). A recent study from India found that 40.2 and 13.8% of HBeAg-positive and HBeAg-negative patients, respectively, with persistently normal ALT had significant fibrosis on histology (Tables 3 and 4) (145).

Table 4
Histological Findings According to Baseline HBV DNA Levels Among HBeAg-Negative Patients with PNALT (Adapted from Reference 145)

<table>
<thead>
<tr>
<th>Group</th>
<th>HBVDNA &lt; 10^5 copies/ml 75</th>
<th>HBV DNA &lt; 3x10^4 copies/ml 65</th>
<th>HBV DNA &lt; 10^5 copies/ml 52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline biopsy N (%)</td>
<td>29(38.7)</td>
<td>22(33.8)</td>
<td>9(17.3)</td>
</tr>
<tr>
<td>HAI [median (range)]</td>
<td>2.0(1–5)</td>
<td>2.0(1–5)</td>
<td>2.0(1–5)</td>
</tr>
<tr>
<td>HAI &gt;3, N (%)</td>
<td>6(20.7)</td>
<td>6(27.3)</td>
<td>3(33.3%)</td>
</tr>
<tr>
<td>F score [median (range)]</td>
<td>1.0(0.0–1.0)</td>
<td>1.0(0.0–1.0)</td>
<td>1.0(0.0–1.0)</td>
</tr>
<tr>
<td>Any fibrosis, N (%)</td>
<td>15(51.7)</td>
<td>14(63.3)</td>
<td>6(66.7%)</td>
</tr>
<tr>
<td>Inactive liver disease [HAI &lt;3, F ≤ 1], N (%)</td>
<td>23(79.3)</td>
<td>16(72.7)</td>
<td>7(77.8%)</td>
</tr>
<tr>
<td>Active liver disease [HAI ≥3 and/or F ≥2], N (%)</td>
<td>6(20.7)</td>
<td>6(27.2)</td>
<td>2(22.2)</td>
</tr>
</tbody>
</table>

Therefore, a considerable proportion of patients with normal or minimally elevated ALT are likely to have significant hepatic fibrosis.

According to a large-scale study, there are differences in the cumulative risks of development of cirrhosis-related complications and HCC at 10 years of follow-up for patients with different ALT levels on presentation. Patients with ALT values near the upper range (0.5–1 x ULN)
have the same risk of development of complications as those with ALT 2–6 x ULN (173).

4.3.7. Interventions to Modify the Natural History of Chronic HBV Infection

Accumulating evidence in the past 25 years showed that the risk of cirrhosis and HCC is higher in chronic HBV-infected subjects who undergo HBeAg seroconversion later in life, have persistent high levels of HBV replication, and long durations of active hepatitis. Thus, treatment that is effective in inducing sustained suppression of HBV replication may reduce the risk of cirrhosis and HCC. Long-term follow-up studies found that interferon therapy has a minimal effect on reducing the risk of cirrhosis, HCC, and liver-related mortality (178). However, a significant benefit has been observed among responders (178). The overall lack of benefit may be related to the low rate of sustained response after a single course of interferon therapy. The availability of oral antiviral therapy with minimal side effects has changed the paradigm of hepatitis B treatment. A study of patients who have received 3 years of lamivudine therapy found that necroinflammation as well as fibrosis was decreased, although maintenance of histologic benefit was mainly seen in patients who did not have evidence of lamivudine resistance (179). Lamivudine treatment has been associated with decreased risk of cirrhosis and major complications and improved survival. In a prospective, double-blind, randomized, controlled trial of lamivudine reported in 2004 (180), 651 Asian patients with compensated liver disease, who were positive for HBeAg or had serum HBV DNA >700,000 genome Eq./mL, with bridging fibrosis or cirrhosis on liver biopsy were randomized to receive lamivudine or placebo. After a median follow-up of 32 months, a significant difference in disease progression as well as HCC was observed between the two groups despite a very high rate (49%) of lamivudine resistance. These data indicate that antiviral therapy can modify the natural history of chronic HBV infection. The results are likely to be better with newer antiviral agents such as adefovir and entecavir that have lower rates of drug resistance.

4.4. Natural History of HBV- and HCV-Coinfected Patients

HBV and HCV interact with each other and affect immune responses. HCV infection can suppress HBV replication; patients with chronic hepatitis B who are coinfected with HCV have lower HBV DNA levels, decreased activity of HBV DNA polymerase, and decreased expression of HBsAg and hepatitis B core antigen in the liver. Furthermore,
patients with chronic HBV infection who become superinfected with HCV can undergo seroconversion of HBeAg and HBsAg to respective antibodies. The annual incidence of HBsAg seroconversion was found to be 2.08% in HCV-coinfected patients compared to 0.43% in patients with HBV monoinfection (181).

HBV can reciprocally inhibit HCV replication as well. In one Italian study, coinfection patients had a rate of HCV RNA clearance of 71% compared to 14% with HCV monoinfection (182). HBV replication in coinfection individuals may result in more liver inflammation.

Furthermore, coinfection patients have been demonstrated to have lower levels of both HBV DNA and HCV RNA than corresponding monoinfected controls, indicating that concurrent suppression of both viruses by the other virus can also occur (183).

Overall, both viruses can inhibit each other simultaneously; either virus can play a dominant role; both viruses have the ability to induce seroconversion of the other; the chronology of infection has a role in determining the dominant virus; and HBV and HCV can alternate their dominance. However, the dominant effect appears to be HCV suppression of HBV (184).

There are various immune profiles of dually infected patients with chronic hepatitis. One possibility is dually active HBV and HCV, in which patients have detectable serum HBV DNA and HCV RNA. These patients are at the highest risk of progression to cirrhosis and decompensated liver disease. Another possibility is active HCV infection (positive HCV RNA) in the setting of an inactive HBsAg carrier. Such patients behave similar to patients with HCV monoinfection and likely exhibit HCV viral suppression of HBV activity. Another possibility is active HBV infection in patients with inactive or prior HCV infection (HBV DNA positive/HBeAg positive/HCV RNA negative/anti-HCV positive). This immune profile is less common and indicates HBV suppression of HCV.

Coinfected patients have higher rates of cirrhosis with decompensation. One cross-sectional study found higher rates of cirrhosis (44% vs. 21%) and decompensated liver disease (24% vs. 6%) in coinfection patients compared to patients with chronic HBV monoinfection (185). HBV replication in coinfection patients (detectable serum HBV DNA) has been correlated with higher rates of cirrhosis, Knodell score, piecemeal necrosis, and fibrosis (186).

Coinfection with HBV and HCV has been shown in many case-control studies to correlate with an increased risk of developing HCC (187). A longitudinal study reported a rate of incidence of HCC (per100 person years) of 6.4 in dually infected patients, compared to 2.0 in HBV- and 3.7 in HCV-monoinfected patients, and a 45% cumulative
4.5. Natural History of Chronic Hepatitis B in HIV-Coinfected Patients

The outcome of HBV infection depends on the strength and quality of the innate and adaptive humoral and cellular immune responses. HIV-infected individuals show a quantitative depletion of CD4 T cells but also an overall immune dysfunction including dysregulation of the cytokine network, a decrease of the functional capability of CD8 T cells and, at later stages, a significant reduction in their number.

Even in most cases with an absent host defense, HBV replication in the liver is noncytopathic. In HIV-infected patients, host deficiencies are not “absent” even in the most advanced stages and are often aberrantly activated. In such cases, the quality of HIV-induced deregulation of a still active anti-HBV immune response may be crucial in determining the necroinflammation and fibrosis.

Coinfection with HIV significantly modifies the natural history of HBV infection. In patients with HBV infection, HIV coinfection is associated with higher chronicity rate of acute hepatitis B, higher levels of HBV replication, a lower rate of spontaneous loss of HBeAg and/or HBsAg, and seroconversion to anti-HBe and anti-HBs, and a high rate of seroreversion after CD4 T-cell depletion in those with spontaneous- or interferon-induced anti-HBe seroconversion (189).

There are contradictory data on the activity of inflammatory liver disease in coinfected patients. Cohort studies from northern Europe and United States of homosexual men showed a significantly less severe necroinflammatory activity in HIV-coinfected patients (190, 191). In contrast, some studies performed in Californian and French cohorts that included injection drug users showed increased necroinflammatory activity in HIV seropositive subjects (192). There are also contradictory data on the impact of HIV on hepatitis B progression toward cirrhosis: some studies from northern Europe and United States performed in the pre-HAART era did not reveal an unfavorable impact of HIV coinfection on hepatitis B evolution (193), while three French studies (194–196) identified a more rapid progression toward cirrhosis in HIV-coinfected individuals. These discrepancies could be related to differences in the prevalence of infecting HBV genotypes and of mutant HBeAg-defective HBV strains or the degree of immune suppression or to the different prevalence of cofactors associated with liver injury (alcohol, HCV, HDV) in the various cohorts. A higher rate of decompensation has been observed in HIV/HBV-coinfected individuals with
cirrhosis (197). There is no data supporting an accelerated progression toward HCC in HIV/HBV-coinfected people, although there is a more severe presentation of hepatocellular carcinoma and a lower survival in HBsAg-positive patients with HIV infection (198). Individuals coinfected with HIV and HBV are at a greater risk of liver-related death than those infected by HIV or HBV alone (or not infected with either virus) (199).

Natural history of HBV-related disease in HIV-infected patients can be modified due to the highly active antiretroviral therapy (HAART). HAART may either prevent or treat opportunistic infections and some opportunistic tumors, thus decreasing the incidence of AIDS and increasing life expectancy of HIV-infected patients. The prolonged survival of HIV-infected individuals allows a longer time for cirrhosis to develop and, therefore, since the introduction of HAART, the relative proportion of deaths attributable to liver disease has been rising. This increase is due partly to an absolute decrease of classical opportunistic infections and tumors, but possibly also to an absolute increase in liver-related deaths (200). HAART may have a major impact on HBV coinfection because of restoration of immune responses and decrease of aberrant activation and deregulation of the immune system. In addition, at least three antiretrovirals (lamivudine, tenofovir, and emtricitabine) are potent inhibitors of HBV replication. HAART may therefore influence the natural history of chronic hepatitis B as follows:

(a) By preventing acute infection in patients using antiretrovirals with dual activity. However, use of lamivudine as an antiretroviral was not associated with a decreased incidence of acute hepatitis B in a large cohort of HIV-infected subjects (201).

(b) By inhibiting HBV replication after the introduction of antiretrovirals with dual activity. Tenofovir, lamivudine, and emtricitabine are able to suppress HBV replication depending on immune status, despite the frequent persistence of residual replication. The prolonged suppression of HBV replication leads to histological improvement and induces a significant decrease or normalization of aminotransferase levels and prevents disease progression to cirrhosis and end-stage liver disease (202). However, anti-HBe and anti-HBs seroconversion have been observed in a minority of patients starting HAART regimens.

(c) By reactivating HBV replication after withdrawal of antiretrovirals with dual activity or emergence of HBV-resistant strains. Withdrawal of antivirals with dual activity has been associated with reactivation of HBV infection and with flares of liver enzyme elevations and hepatic decompensation in patients with advanced liver disease (203). Similar phenomenon has been observed after the occurrence of HBV genome mutations that are associated with resistance to lamivudine (204).
(d) By restoring adaptive HBV-specific immune response and innate non-specific immune response, and reducing aberrant activation of the immune system. Independent of the use of antivirals with dual activity, HAART may induce reconstitution of the adaptive immune response and this is a “double-edged sword” in patients infected with HBV: on one side, HBV seroconversion can occur leading to a better control of HBV replication with or without flares of necroinflammatory activity; on the other hand, flares of necroinflammatory activity have been reported without modification of HBV markers and of HBV replication levels (205). Additionally, the rapid phase inhibition of HIV replication that occurs when HAART is started has been associated with a transient increase of HBV replication. This association may be related to cessation of HIV-induced secretion of cytokines with antiviral activity and may trigger flares of necroinflammatory activity in the very early phases of HAART. Given the complex interactions between HAART and HBV coinfection, it is not so easy to establish if HAART has a beneficial or a negative effect on chronic hepatitis B. Thio and coworkers observed an increase of liver-related mortality in HBsAg-positive people living with HIV in the HAART era (96). They failed to observe a significant association between lamivudine use and a lower liver-related mortality because of lack of information in most patients. However, several direct and indirect data support a positive effect of HAART on the natural history of hepatitis B. In a recent analysis of data from the Italian ICONA cohort, the use of lamivudine as a part of the first HAART regimen in naïve patients was independently associated with lower liver decompensation-related morbidity (206). Low CD4 T-cell count nadir value has been associated with higher liver-related mortality in HBsAg-positive patients and severe cases of reactivation of hepatitis B related to immune restoration (206). Therefore, an earlier use of HAART for prevention of severe immune dysfunction and the inclusion of a drug with dual anti-HBV activity might slow down progression of chronic hepatitis B in HIV-infected patients.

Occult HBV in HIV-coinfected patients pose different kinds of problems. Reactivation of HBV infection following severe immune depletion and/or lamivudine withdrawal can occur in HIV-coinfected patients without HBsAg and with markers of past HBV infection. However, HBsAg seroreversion occurred in only one out of 98 subjects followed prospectively, at a rate of 0.23/100 patient-year (207).

4.6. Natural History of Occult HBV Infection

The virological and clinical reactivation of occult HBV infection may occur in certain clinical situations. In fact, an occult HBV-infected patient who becomes immunocompromised may show a reactivation
of viral replication because of faulty immunological control. Once recovery is achieved and immune surveillance reconstituted, T-lymphocyte-mediated hepatocyte injury may lead to the development of hepatitis. The virological and clinical reactivation of occult HBV infection has been observed in several different clinical conditions including hematological malignancies, HIV infection, hematopoietic stem cell transplantation, and organ transplantation. Occult HBV-infected patients undergoing OLT may present reinfection of the new liver and occasionally this event may be followed by virological and clinical reactivation. The availability of new, potent immunosuppressive drugs (like anti-CD20, anti-CD52, and anti-TNF monoclonal antibodies) used in various clinical settings, have further increased the risk of HBV reactivation in occult HBV-infected individuals (189).

The frequency of occult HBV reactivation is still unclear. In a recently reported study performed on 14 anti-HBs/anti-HBc-positive patients who underwent allogenic hematopoietic stem cell transplantation (190), 12 individuals experienced progressive and persistent disappearance of anti-HBs and 7 patients had HBsAg seroconversion. Of these last seven cases, only one developed symptomatic acute hepatitis and needed hospitalization. This suggests that occult HBV reactivation is quite a frequent event in immunocompromised patients, but since it is usually investigated only in the case of development of acute hepatitis, its recognition might be missed in most of the cases. Thus it seems prudent to monitor all patients undergoing immunosuppressive therapy very carefully for HBV serology and/or viremia and to continue this monitoring for months (or even years) after stopping treatment. In fact, early identification of a virological reactivation makes it possible to begin specific antiviral therapy early, which may prevent the occurrence of reactivation.

Individuals who have recovered from self-limited acute hepatitis may persistently carry HBV genomes for decades without showing any clinical or biochemical sign of liver damage. However, when the liver tissue of these subjects examined, histological patterns of a mild necroinflammation have been revealed up to 30 years after the resolution of acute hepatitis (208–210).

Occult HBV infection might accelerate the progression of chronic liver disease in HCV-infected individuals (211, 212). Several studies have suggested that occult HBV infection may also exert its negative influence on chronic hepatitis C in terms of a reduced response to IFN therapy (213). The mechanisms by which occult HBV may help HCV to resist IFN are unknown. Most cited reports have used conventional IFN therapy, whereas there are no reliable studies evaluating whether occult HBV infection may interfere with the response to PEG-IFN plus
Ribavirin (the present gold-standard therapy for the chronic hepatitis C treatment). Several reports indicate that occult HBV infection is associated with the progression of liver fibrosis and cirrhosis development in patients with cryptogenic liver disease (214, 215). Occult HBV infection has also been cited as a risk factor for HCC development (216).

5. SUMMARY AND FUTURE DIRECTIVES

In recent years, there have been great advances in the understanding of the epidemiology and natural history of chronic hepatitis B virus infection. Key relationships have been discovered regarding HBV DNA and ALT and disease outcomes. The concept of the so-called “Inactive carrier” has not been found to be convincing and is gradually fading. However, many issues still remain unanswered. One key issue is the importance and natural history of various genotypes in varying geographical regions. Another crucial issue is whether earlier treatment of chronically infected individuals with active viral replication who do not have the current conventional indications for antiviral therapy (i.e., elevated aminotransferases and necroinflammatory damage on liver biopsy) will alter their natural history. Only long-term prospective studies can answer these questions, but as the benefits of effective viral suppression have become more apparent, it will be more difficult to perform placebo-controlled trials of HBV therapy.

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Current Treatment of Chronic Hepatitis B

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and Emmet B. Keffe, MD

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Key Principles

• The natural history of HBV infection can be divided into four phases: immune tolerance, immune clearance (HBeAg-positive chronic hepatitis B), nonreplicative (inactive HBsAg carrier), and reactivation (HBeAg-negative chronic hepatitis B).
• Patients with chronic hepatitis B should receive treatment when alanine aminotransferase (ALT) levels are elevated (>19 U/L for

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women and >30 U/L for men) and serum HBV DNA levels are ≥20,000 IU/mL (HBeAg-positive disease) or ≥2,000 IU/mL (HBeAg-negative disease).

- The preferred treatment of chronic hepatitis B in 2008 includes adefovir, entecavir, peginterferon alfa-2a, telbivudine, and tenofovir. Standard interferon alfa-2b has been replaced by peginterferon alfa-2a, and lamivudine is not a preferred first-line drug due to high rates of resistance.

- With use of the oral agents, serum HBV DNA should be less than 2000 IU/mL or undetectable 24 weeks after the initiation of therapy for maximal efficacy and the lowest rate of antiviral drug resistance (“roadmap” concept).

- The rates of genotypic resistance with long-term therapy are high with lamivudine (65–70% at 4–5 years), intermediate with telbivudine (22% in HBeAg-positive and 9% in HBeAg-negative patients at year 2), lower with adefovir (29% at 5 years of therapy), and lowest with entecavir in nucleoside-naïve patients (~1% at year 4) but higher in lamivudine-resistant patients (~40% at year 4). Interferon and peginterferon therapy have not been associated with the development of antiviral drug resistance.

- Potential future therapies for chronic hepatitis B include clevudine and peginterferon alfa-2b. There is an evolving role for combination therapy primarily to reduce the rate of resistance with long-term therapy and manage resistance when it occurs.

1. INTRODUCTION

Despite the advances in the diagnosis and treatment of viral hepatitis, chronic infection with hepatitis B virus (HBV) continues to have a major worldwide impact. As many as 2 billion persons have been infected with HBV worldwide, and 350 million people are chronically infected, including at least 1.25 million individuals living in the United States (1, 2). In addition, the prevalence of chronic hepatitis B (CHB) in the United States has been underestimated, considering the lack of inclusion of the prison population with their high infection rate in epidemiological studies and the continuous influx of immigrants from endemic areas with high rates of HBV infection. Individuals with chronic HBV infection are at an increased risk of dying prematurely from the development of hepatocellular carcinoma (HCC) or cirrhosis (3). Therefore, diagnosis and treatment of CHB in its early stages is imperative to potentially prevent such complications. In addition, the degree of HBV replication as assessed by serum HBV DNA levels is associated with the risk of development of HCC (Fig. 1) (4).
Fig. 1. HBV viral load level correlates with the risk of development of hepatocellular carcinoma (adapted from Chen CJ et al. (4)).

Compared to only two available therapies for the management of CHB in the 1990s, i.e., interferon alfa-2b and lamivudine, there are now five additional agents that have been approved by the United States Food and Drug Administration (FDA) over the last decade: adefovir dipivoxil, entecavir, peginterferon alfa-2a, telbivudine, and tenofovir. The future management of CHB appears to be even more promising based on a number of additional drugs in development and newer treatment strategies.

2. NATURAL HISTORY

An individual’s age and time of infection have a strong impact on the course of acute HBV infection. Infection at birth or in early childhood results in the development of chronic HBV infection in more than 90% of individuals. In contrast, infection in adulthood is most often self-limited, with more than 95% clearing the virus (5). The hallmark of acute HBV infection is the presence of detectable hepatitis B surface antigen (HBsAg), IgM antibody to hepatitis B core antigen (anti-HBc), and hepatitis B e antigen (HBeAg).

Once chronicity is established, patients infected at birth or early in life typically enter into an initial immune tolerance phase, which is characterized by high levels of viral replication (elevated HBV DNA levels and presence of HBeAg) but normal alanine aminotransferase (ALT) levels (Table 1). This phase is usually short-lived or absent when infection occurs in adults and long-lived when infection occurs in infancy or early childhood. Although a liver biopsy is not usually
Table 1
Phases of Chronic HBV Infection

<table>
<thead>
<tr>
<th>Definitions</th>
<th>Diagnostic Criteria</th>
<th>Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune tolerance phase</td>
<td>HBsAg positive &gt; 6 months</td>
<td>Normal or minor nonspecific changes</td>
</tr>
<tr>
<td></td>
<td>HBeAg positive, anti-HBe negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBV DNA &gt; 20,000 IU/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal ALT</td>
<td></td>
</tr>
<tr>
<td>Immune clearance phase (HBeAg-positive CHB)</td>
<td>HBsAg positive &gt; 6 months</td>
<td>Active necroinflammation, with or without fibrosis</td>
</tr>
<tr>
<td></td>
<td>HBeAg positive, anti-HBe negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBV DNA &gt; 20,000 IU/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevated or fluctuating ALT levels</td>
<td></td>
</tr>
<tr>
<td>Inactive HBsAg carrier state</td>
<td>HBsAg positive &gt; 6 months</td>
<td>Absence of significant hepatitis</td>
</tr>
<tr>
<td></td>
<td>HBeAg negative, anti-HBe positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBV DNA undetectable or less than 2,000 IU/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal ALT levels</td>
<td></td>
</tr>
<tr>
<td>Reactivation phase (HBeAg-negative CHB)</td>
<td>HBsAg positive &gt; 6 months</td>
<td>Active necroinflammation, with or without fibrosis</td>
</tr>
<tr>
<td></td>
<td>HBeAg negative, anti-HBe positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBV &gt; 2,000 IU/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluctuating ALT levels</td>
<td></td>
</tr>
</tbody>
</table>

pursued in this stage in practice, studies have shown the presence of minimal or absent necroinflammation and fibrosis on histological examination (5–7).

The immune clearance phase follows the immune tolerance phase and is characterized by clearance of infected hepatocytes, resulting in elevated or fluctuating ALT levels and persistently high HBV DNA levels. Necroinflammation with various degrees of fibrosis is the hallmark of this stage on liver biopsy. Repetitive hepatitis flare and prolonged duration of this phase can lead to advanced fibrosis or cirrhosis. During this phase, patients seroconvert and develop antibodies to HBeAg (anti-HBe) at an annual rate of 5–15% without therapy but can be accelerated by initiation of antiviral therapy (6, 7).

Seroconversion from HBeAg to anti-HBe signals the transition to the nonreplicative phase of infection, and patients in this stage are referred to as inactive HBsAg carriers (8). Normal ALT levels, undetectable or low levels of serum HBV DNA, and the presence of anti-HBe are the hallmarks of this phase. In addition, liver biopsy reveals either resolution or minimal lingering necroinflammation, with vari-
able amounts of fibrosis that may have developed during the immune clearance phase. Inactive carriers, particularly older subjects, may spontaneously lose HBsAg and seroconvert to hepatitis B surface antibody (anti-HBs). However, small amounts of serum HBV DNA can often be detected using sensitive, real-time polymerase chain reaction techniques (9).

 Reactivation of HBV infection may occur in a subgroup of patients despite HBeAg seroconversion. Reactivation usually occurs close to the time of HBeAg loss or can occur many years later, with the finding of high levels of serum HBV DNA and significant disease on liver biopsy (8, 10, 11). Emergence of the precore or double-core promoter mutants of HBV is responsible for such reactivation (9, 11). This phenomenon was first recognized in the Mediterranean area (9); however, it is becoming more commonly recognized in the United States. Chu and colleagues (12) reported a prevalence rate of 27% and 44% for the precore and the core promoter variants, respectively, in a survey of patients in the United States. Patients with HBeAg-negative CHB tend to have more severe liver disease than patients with HBeAg-positive CHB. Serum ALT levels may remain normal and serum HBV DNA undetectable for many years before later detection. Many patients with HBeAg-negative CHB are erroneously labeled as inactive carriers due to intermittently low or normal ALT levels and undetectable HBV DNA. However, frequent monitoring of HBV DNA and ALT can easily detect fluctuation in viremia level in such a group of patients, leading to early detection and intervention prior to the development of progressive liver disease (13). A serum HBV DNA level greater than 2000 IU/mL should raise the suspicion for HBeAg-negative CHB rather than an inactive carrier state (14). Therefore, it is not surprising that significant necroinflammatory changes and fibrosis are present in more than 50% of patients. The majority of the patients with HBeAg-negative CHB have a poor long-term prognosis since the diagnosis of CHB is made late in the course of the disease, with 25–50% having cirrhosis at the time of diagnosis (8, 9, 15).

3. INDICATIONS FOR TREATMENT

The goals of antiviral treatment are to achieve maximal viral suppression, reduce progression to cirrhosis, and decrease the risk of developing HCC. The presence of elevated ALT and serum HBV DNA levels is an indication for treatment of patients in both the immune clearance phase and the reactivation phase. The target goals of treatment of patients with HBeAg-positive CHB are loss of HBeAg and/or seroconversion to anti-HBe, normalization of ALT levels, and maximal
suppression of HBV DNA to undetectable or low levels. If these goals can be achieved, improvement of liver histology, reduction in disease progression, and decrease in incidence of HCC are likely (17). Recent data from a Korean-based population revealed the presence of higher risk of liver-related mortality with ALT levels greater than 20 U/L for females and 30 U/L for males (18). Those findings have led to the proposal that a lower threshold for an elevated serum ALT be used when considering who should be treated (8, 19).

Standard practice guidelines (8, 19) recommend initiation of treatment in patients with HBeAg-positive CHB when serum HBV DNA is at least 20,000 IU/mL (~100,000 copies/mL) and ALT levels are either elevated above normal (8) or greater than two times the upper limits of normal (ULN) (19). A liver biopsy should be considered in patients with ALT less than two times the ULN, as approximately 20% of these individuals if over age 35–40 years will have significant hepatic fibrosis (8). Active necroinflammatory activity on a liver biopsy is an indication for treatment regardless of the level of serum HBV DNA. Patients with HBV DNA levels over the threshold for treatment and high ALT levels can be monitored for 3–6 months to assess for the development of HBeAg seroconversion, or started right away on therapy. However, if such patients have high serum bilirubin levels or signs of liver decompensation, prompt initiation of therapy is warranted.

The goals of treatment patients with HBeAg-negative CHB are to achieve maximal viral suppression and normalization of ALT levels. Initiation of treatment is indicated at a lower HBV DNA threshold compared to patients with HBeAg-positive CHB (8). Approximately 50% of patients with HBeAg-negative CHB and HBV DNA levels less than 100,000 copies/ml have significant inflammation on liver biopsy (14). In addition, the course of HBeAg-negative CHB is marked by fluctuation of ALT levels. Therefore, any ALT elevation in the setting of HBV DNA greater than 2,000 IU/ml (~10,000 copies/ml) is an indication to initiate therapy. A liver biopsy is recommended in patients with normal ALT levels to assess for the presence of necroinflammation. If active hepatitis is found on liver biopsy, treatment is indicated. While antiviral therapy can be discontinued after treatment for an additional 6–12 months after seroconversion in patients with HBeAg-positive CHB, long-term therapy is recommended for HBeAg-negative CHB, as relapse is common if therapy is stopped (8, 19).

Patients with a positive or negative HBeAg, low HBV DNA levels, and a normal ALT level do not warrant treatment. Such patients need to be monitored every 3 months for 1 year after initial diagnosis and then every 6–12 months thereafter.
4. APPROVED DRUGS FOR TREATMENT OF CHRONIC HEPATITIS B

Patients with CHB can be treated with two classes of drugs: immunomodulatory agents (interferon and peginterferon) and antiviral agents (nucleoside and nucleotide analogues). Each class of drugs has its own advantages and limitations. Interferons may not be tolerated based on significant side effects and poor tolerability, while the efficacy of antiviral agents is limited by the emergence of resistance over time. There are seven drugs currently approved by the FDA for the treatment of CHB: interferon alfa-2b, peginterferon alfa-2a, lamivudine, adefovir, entecavir, telbivudine, and tenofovir.

While all of the approved agents can be used as first-line therapy for CHB, lamivudine is not recommended as a first-line agent secondary to a high rate of antiviral drug resistance of 65–70% after 5 years of therapy (8, 19). In addition, telbivudine is recommended only in patients who achieved undetectable viral load by week 24 of therapy, based on an overall rate of resistance of 22% in patients with HBeAg-positive and 9% in patients with HBeAg-negative disease. However, studies have shown that achieving an undetectable level of HBV viremia by week 24 is associated with a very low rate of resistance and continued efficacy at week 96 (20).

4.1. Peginterferon Alfa-2a

Standard interferon alfa-2b has been replaced in routine practice with peginterferon alfa-2a for the treatment of chronic hepatitis B. Rather than the daily or thrice weekly injection schedule of interferon alfa-2b, peginterferon alfa-2a is given weekly due to its longer half-life. It also has comparable, if not better, efficacy than interferon alfa-2b (8, 19). Standard interferon alfa-2b therapy is associated with 37% loss of serum HBV DNA, 18% HBeAg seroconversion rate, and 80–90% durability of response in HBeAg-positive individuals (8). Standard interferon therapy in patients with HBeAg-negative CHB results in 60–70% loss of serum HBV DNA, 48% histological improvement, and 20–30% durability of response (21). Treatment with peginterferon alfa-2a for 48 weeks results in 25% loss of HBV DNA, 27% HBeAg seroconversion, and a higher seroconversion rate of 32% at week 72. No added benefit has been reported in HBeAg-positive and HBeAg-negative CHB with the addition of lamivudine to peginterferon monotherapy (22, 23). Unlike oral antiviral therapy where genotype does not predict the response to therapy, genotype has been noted to be associated with the response to therapy with peginterferon: genotype A responds more favorably than genotype D and genotype B better than genotype C in
some but not all studies (8, 22). Patients with CHB genotype A or B are optimal candidates for peginterferon therapy if they are young, lack comorbidities, have HBV DNA levels less than $10^9$ copies/mL, and ALT levels at least 2–3 times the upper limit of normal (24).

Fixed duration of therapy and lack of antiviral resistance are advantages of peginterferon therapy compared with oral antiviral therapy. However, the need for injections and presence of significant side effects has limited the use of peginterferon therapy in the United States.

### 4.2. Lamivudine

Lamivudine was the first licensed oral agent for the treatment of CHB. It has similar efficacy to interferon-based therapy. Like other oral antiviral agents, it has a lower durability of response in HBeAg-positive patients (50–80%) and even lower durability in HBeAg-negative patients (20–25%) (8, 21). Treatment is typically for 1 year but is extended until HBeAg seroconversion occurs. It is further continued for an additional 6 months to enhance the durability of response in HBeAg-positive patients. In addition, prolonged treatment has been associated with decreased rate of disease progression and lowering the incidence of HCC in individuals with advanced fibrosis or cirrhosis (17). However, prolonged use of lamivudine is associated with the development of the YMDD mutation at a rate of ~20% per year, increasing to approximately 70% by year 4 of therapy. Therefore, lamivudine is no longer recommended as a first-line agent for the treatment of CHB (8, 19).

Patients who develop lamivudine resistance were historically switched to adefovir or entecavir (8, 19). However, the risk of subsequent adefovir resistance is increased after switching from lamivudine to adefovir therapy, and thus adefovir should be added to continue lamivudine therapy, particularly in cirrhotic patients to also avoid a flare of hepatitis B (8, 19, 25). In addition, lamivudine can be successfully used to treat patients who developed adefovir mutation (26).

### 4.3. Adefovir Dipivoxil

Adefovir dipivoxil is a nucleotide analogue approved for the treatment of CHB at a dose of 10 mg daily. Higher doses have been associated with significant nephrotoxicity. One year of therapy results in a 12% HBeAg seroconversion rate in patients with HBeAg-positive CHB. Adefovir results in a 53% and 63% rate of histological improvement in HBeAg-positive and HBeAg-negative patients, respectively, when compared with placebo (27, 28). HBeAg seroconversion marks the end of therapy in HBeAg-positive patients, while patients with
HBeAg-negative CHB are treated long-term. Once HBeAg seroconversion occurs with adefovir therapy, it is sustained in 91% of patients. Recent studies have demonstrated the continuous beneficial effect of adefovir, i.e., improvement in fibrosis, ALT normalization, and viral suppression with prolonged duration of therapy up to 4–5 years (29, 30). Resistance to adefovir develops at a slower rate than resistance to lamivudine. Rates of resistance are 0%, 3%, 18%, and 29% after 1, 2, 4, and 5 years of therapy (29). Elevated serum HBV DNA levels after 48 weeks of adefovir therapy correlate with the emergence of resistance (31).

### 4.4. Entecavir

Entecavir is the most potent licensed nucleoside analogue, which results in a 6.98 log_{10} copies/mL decrease of HBV DNA levels in HBeAg-positive patients at a dose of 0.5 mg daily. It is more potent when compared to lamivudine in achieving better rates of histological improvement (72% vs. 62%), normalization of ALT (78% vs. 70%), and viral suppression of HBV DNA to less than 400 copies/mL (69% vs. 38%). However, the rate of HBeAg seroconversion was comparable to lamivudine after 1 year of therapy (32, 33). Entecavir was also found to be superior to lamivudine in HBeAg-negative CHB patients in regard to histological improvement (70% vs. 61%) and viral suppression (91% vs. 73%) (33). Maintenance of the virologic response has been documented in long-term follow-up studies extending up to 96 weeks (34). Low resistance rate (<1%) has been observed in treatment-naïve patients after 4 years of therapy. However, patients with lamivudine resistance have a higher rate of development of resistance. They also require a higher dose of entecavir (1 mg daily). Resistance in this population has been reported as 1%, 10%, 27%, and 39% at 1, 2, 3, and 4 years, respectively (35, 36).

### 4.5. Telbivudine

Telbivudine is a nucleoside analogue of thymidine. It is a potent inhibitor of HBV DNA polymerase. Telbivudine was found to be superior to lamivudine in a phase III trial (GLOBE trial) in HBeAg-positive CHB patients, with a HBeAg seroconversion rate of 22% and HBV DNA undetectability (<300 copies/mL) of 60% after 1 year of therapy (37). The GLOBE trial, a randomized phase III study, provided 2 years follow-up data on patients with HBeAg-negative CHB and HBeAg-positive CHB, confirming superiority of telbivudine over lamivudine (38). Genotypic resistance to telbivudine at 1 year and 2 years is 4.4% and 21.6%, respectively, in HBeAg-positive patients and 2.7% and
8.6%, respectively, in HBeAg-negative patients (38). Secondary analysis of the GLOBE data demonstrated the pivotal role of week 24 level of serum HBV DNA. Patients with undetectable HBV DNA at week 24 achieved better outcomes after 1 and 2 years of therapy in terms of HBeAg seroconversion, ALT normalization, and a lower rate of antiviral drug resistance (20).

Telbivudine also achieved superiority to adeovir in terms of early viral suppression at week 24 in HBeAg-positive patients with CHB who were initially treated with telbivudine or switched from adeovir to telbivudine (39). The early virologic suppression correlated with better rates of HBeAg seroconversion, ALT normalization, and undetectable HBV DNA.

4.6. Tenofovir

Tenofovir is a nucleotide analogue structurally related to adeovir. It has been in widespread use for the treatment of human immunodeficiency virus (HIV) infection and just gained FDA approval for the treatment of CHB. Results from a phase III study in HBeAg-positive patients comparing tenofovir to adeovir showed superiority of tenofovir in achieving combined viral suppression (<400 copies/mL) and histological improvement (67% vs. 12%) at 48 weeks. In addition, tenofovir therapy was associated with higher rates of HBsAg loss (3.2% vs. 0%) and HBV DNA <400 copies/mL (76% vs. 13%) when compared to adeovir therapy (40, 41). Similar results were found in another phase III study of patients with HBeAg-negative CHB who were treated with tenofovir vs. adeovir, with regard to better combined viral suppression, improved inflammatory score (71% vs. 49%), and better viral suppression to <400 copies/ml (93% vs. 63%) (41).

5. DRUGS IN DEVELOPMENT FOR TREATMENT OF CHRONIC HEPATITIS B

Many drugs are currently in different stages of development for the treatment of CHB, including emtricitabine and clevudine.

6. NEW TREATMENT STRATEGIES

6.1. Combination Therapy

Combination therapy with peginterferon and lamivudine was not superior to peginterferon alone in both HBeAg-positive and HBeAg-negative CHB (22, 23). The combination of peginterferon with other nucleoside/nucleotide analogues might be more beneficial. The addition
of adefovir to peginterferon results in better early virologic suppression at week 24 than peginterferon alone (71% vs. 41%) (42). In addition, combination therapy of adefovir and peginterferon for 48 weeks results in a marked decrease from baseline of serum HBV DNA and intrahepatic covalently closed circular DNA, which are closely related with reduced HBsAg (43).

Switching from lamivudine to adefovir in lamivudine-resistant cases is associated with higher rate of emergence of adefovir resistance (44), while combination therapy resulted in lower rate of development of adefovir resistance (45). The timing of addition of adefovir to lamivudine in the case of the presence of YMDD mutation is also paramount. Patients who have clinical evidence of lamivudine resistance (HBV DNA >10^6 copies/mL and elevated ALT) at the time of addition of adefovir achieved worse control of viral replication than patients who had only genotypic expression of lamivudine resistance (46). Therefore, the addition of adefovir to lamivudine therapy should be pursued as soon as the genotypic resistance is detected.

The cross-resistance for lamivudine and telbivudine at codon 204 makes the combination or switching therapy between lamivudine and telbivudine not desirable, even though a multicenter, randomized, phase III trial of HBeAg-negative and HBeAg-positive patients with persistent HBV viremia who were treated with lamivudine vs. switching to telbivudine showed improvement of HBV DNA suppression at week 24 (80% vs. 56%, p<0.001) (47). It will be interesting to see in the follow-up results of this study whether cross-resistance became a major limiting factor.

The combination of nucleosides and nucleotides appears to be beneficial. The in vitro addition of tenofovir to lamivudine, emtricitabine, telbivudine, or entecavir appears to be beneficial (48). However, more issues need to be addressed such as resistance profile of the agents, prior therapies, and drug–drug interactions before clinically embarking on such an approach (49).

### 7. ROADMAP CONCEPT FOR ON-TREATMENT MANAGEMENT

Current treatment guidelines do not offer recommendations regarding on-treatment monitoring. A panel of international experts recently recommended a “roadmap” approach with on-treatment monitoring of patients with CHB (50). The roadmap approach recommends making management decisions based on stratification of the patients according to week 12 and 24 serum HBV DNA levels (Fig. 2). Patients with <1 log_{10} IU/mL reduction of serum HBV DNA from baseline at week
Fig. 2. The roadmap concept for on-treatment management of CHB (51).

12 are classified as primary treatment failures. It is also important to assess for patient compliance with therapy prior to labeling the patients as primary treatment failures. The addition of a more potent drug is recommended in the primary treatment failure. Patients are then further stratified at week 24 based on their virologic response: complete (HBV DNA <60 IU/mL), partial (HBV DNA 60 to less than 2000 IU/mL), or inadequate (HBV DNA >2000 IU/mL). The expert panel also recommended continuous monitoring for virologic breakthrough on therapy every 3–6 months. While the proposed roadmap applies generally to HBeAg-positive and HBeAg-negative patients, relapse is common in HBeAg-negative patients once therapy is discontinued, regardless of the HBV DNA negativity period while on therapy (51).

8. CONCLUSION

CHB is a global health concern with a significant economic burden from acute and chronic infection. However, HBV infection is a vaccine-preventable disease, and broader vaccine recommendations have the potential to markedly reduce infection with this virus. The primary goal of treatment of CHB is to prevent the progression to cirrhosis, liver failure, and HCC. Treatment strategies of CHB continue to evolve with the introduction of new drugs and lower threshold for initiation of therapy. This more aggressive approach to treatment has the potential to interrupt the inevitable progression of CHB to cirrhosis or HCC. The proposed active on-treatment monitoring strategy will help guide clinicians
in assessing response to therapy and make adjustments when needed to optimize the outcome of therapy with the lowest rate of resistance.

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Management of Patients Co-Infected with Chronic Hepatitis B (CHB) and the Human Immunodeficiency Virus (HIV)

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**CONTENTS**

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*Key Principles*

- Certain populations with CHB present additional clinical complexities. These include populations co-infected with the human immunodeficiency virus (HIV) chronic hepatitis B (CHB).
- CHB does not appear to affect the progression of HIV infection; conversely, HIV infection does adversely influence several aspects of the natural history of CHB.
- Current guidelines recommend that all HIV positive patients should be tested for the hepatitis B surface antigen (HBsAg), and

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that all HIV positive individuals who are seronegative for markers of HBV infection undergo vaccination.

- The decision to treat CHB infection in HIV-infected individuals must be based on careful consideration of the need for antiretroviral therapy, the severity of liver disease, and potential adverse effects.
- Liver transplantation is a feasible option for those with co-infection and advanced liver disease.

1. INTRODUCTION

Despite recent improvements in therapy, the management of chronic hepatitis B virus (HBV) infection remains challenging. Certain populations with chronic HBV infection present additional clinical complexities. One such population comprises those co-infected with human immunodeficiency virus 1 (HIV-1). This chapter will address the major issues relevant to the management of the HBV/HIV co-infected individuals. Important differences in the natural history and therapy will be discussed and suggested approaches to management based on the available data and guidelines are offered.

2. EPIDEMIOLOGY

Approximately 2 billion people worldwide have been exposed to HBV with an estimated 370 million people with chronic infection (1). Forty million people are estimated to be infected with HIV worldwide (2). Reports have suggested that 5–10% of persons with HIV infection are co-infected with HBV (3, 4). However, the prevalence varies depending on the route of transmission, with the highest rates of co-infection occurring in men who have sex with men (9–17%), intermediate in injection drug users (7–10%), and lowest in heterosexuals (4–6%) (1).

Co-infection with HBV/HIV is common because of the similar modes and routes of transmission of the two viruses. Both are blood-borne viruses that are transmitted through sexual contact, the sharing of needles through illicit drug use, or acquired at birth from mother to child.

Eight hepatitis B viral genotypes have been identified that have a variable geographical distribution (5). Individual genotypes differ by a genome nucleotide sequence divergence of greater than 8%. Among co-infected patients, the distribution of HBV genotypes mirrors the local genotype distribution (6–8). Though not routinely determined in
the management of chronic HBV infection, recent studies have suggested a role for HBV genotypes in the natural history of liver disease and outcome of interferon therapy. Genotype A was shown to have a better response to nucleoside or nucleotide (nucleos(t)ide) agents in HBV/HIV co-infection (6), although in the HBV mono-infected population response to nucleos(t)ide analogues was shown to be independent of genotype (9). HBV genotype G has been associated with more rapid liver fibrosis progression in co-infected patients (10). Further studies in the mono- and co-infected population will be needed in order to better clarify the role of HBV genotypes in clinical practice.

3. NATURAL HISTORY

3.1. Influence of HBV on Outcome of HIV Disease

The effect of HBV infection on the natural history of HIV infection has been evaluated in several large cohorts (3, 4). Analysis of 9,802 HIV positive patients (9% of whom were HBsAg positive) from 72 European centers found a negligible influence of HBV on the progression of HIV disease (4). The presence of HBV did not appear to affect the HIV virological or immunologic response to highly active anti-retroviral therapy (HAART), the frequency of improvements in CD4+ counts, or time to achieve an undetectable HIV RNA in serum. These studies also reported no effect of HBV on the progression of HIV infection to clinical AIDS (3, 4).

3.2. Influence of HIV on Outcome of HBV Disease

In contrast, HIV infection does appear to influence several aspects of the natural history of HBV infection. Markers of HBV replication are significantly higher in co-infected patients including higher HBV DNA levels and a higher prevalence of hepatitis B e antigen (HBeAg) positivity (11, 12). Paradoxically, serum ALT levels are lower, perhaps due to the HIV-induced immunosuppression (12). Immune reconstitution associated with Highly Active Anti-Retroviral Therapy (HAART) may be associated with hepatitis reactivation and ALT flares. The risk of liver-related death is higher among HBV/HIV co-infected persons than among those infected with either virus alone (4, 13–15). Finally, spontaneous resolution of HBV infection indicated by loss of HBsAg occurs far less commonly in co-infected patients as opposed to HBV mono-infected patients (16).

Acute HBV infection resolves in the majority of HIV negative adults with less than 10% developing chronic infection. However clearance rates of HBV are significantly reduced in persons with HIV
co-infection, with 21% developing chronic infection in one study (11). The higher chronicity rate is felt to be due to the impaired cellular immunity associated with HIV infection.

Studies during the pre-HAART era suggested that HIV infection did not influence the rate of fibrosis progression in patients with CHB perhaps due to a variety of confounding factors, such as alcohol use, hepatitis delta co-infection, and degree of immunosuppression (3, 13, 16, 17). However, more recent studies have suggested that progression to cirrhosis is more rapid in HBV/HIV co-infected persons particularly those with a CD4+ count below 200 mm$^3$ (12, 18). Further corroborating the untoward effect of HIV on HBV related liver disease, several prospective cohort studies have reported an increased rate of liver-related complications and death in HBV/HIV co-infected persons (4, 14, 19). The advent of HAART for HIV infection has greatly diminished mortality from HIV infection and consequently, liver disease as a cause of morbidity and mortality has become more prominent. In the Multi Center AIDS Cohort Study, death in HBV/HIV co-infected persons was 14-fold higher compared to patients with either HBV or HIV infection alone (14). Similar findings have been reported from other HIV cohorts (4, 19). Notably, liver-related mortality was significantly increased in those with lower CD4+ counts (14). Interestingly, despite the accelerated progression of liver fibrosis in co-infected patients, an increased prevalence of hepatocellular carcinoma has not been observed (20).

HBV reactivation in patients with resolved infection (HBsAg negative, anti-HBc positive with or without anti-HBs) is uncommon in the absence of immunosuppression or chemotherapy; however, it has been reported in HBV/HIV co-infection (18). This is most likely due to the persistence of covalently closed circular HBV DNA (cccDNA), which serves as the template for viral transcription and resides in the nucleus of hepatocytes.

3.3. Elevated Serum ALT Levels During HAART Therapy in HBV/HIV Co-infection

The development of serum ALT flares on HAART therapy in HBV/HIV–co-infected patients may be related to a number of diagnostic possibilities. Initiation of HAART therapy can be associated with immune reconstitution with resulting ALT flares due to enhanced cytotoxic T-cell recognition of HBV infected hepatocytes. Serum ALT flares while on a stable HAART regimen can be associated with the development of HBV viral resistance to the anti-HBV components of the HAART: or could represent immunoclearance of HBV with the
concomitant loss of HBeAg, superinfection with hepatitis delta or hepatotoxicity due to HAART therapy. Serum ALT flares in the setting of discontinuation of HAART therapy may represent reactivation of a previously suppressed HBV infection by the anti-HBV component. Significant ALT flares on HAART should warrant further evaluation.

4. MANAGEMENT

4.1. Prevention

Given the increased morbidity and mortality from liver disease in HIV infection, several guidelines recommend that all HIV positive patients should be tested for HBsAg particularly since effective vaccines for the prevention of HBV exist (21). Since 1991, universal vaccination against hepatitis B for children was implemented in the United States with coverage rates equivalent to other standard childhood vaccines. The Advisory Committee on Immunization Practices, the Department of Health and Human Services (DHHS), and American Association for the Study of Liver Diseases (AASLD) all recommended vaccination of HIV positive individuals who are seronegative for markers of HBV infection (22).

Primary vaccine response exceeded 90% in HIV uninfected individuals who completed three or more doses of hepatitis B vaccine but was reduced in HIV positive individuals (23). In HIV patients without an initial hepatitis B surface antibody response to vaccination, additional cycles of HBV vaccine appeared to improve acquisition of immunity (24). The use of a double dose regimen has been shown to improve response rates to HBV vaccination (25). In one study, the use of a double dose regimen was associated with a 58% rate of anti-HBs positivity in previous vaccine failures (25). Postvaccination testing is recommended in HBV- and HIV-susceptible individuals to confirm a primary vaccine response.

4.2. Therapy

4.2.1. Goals

The goals of therapy are to stop progression of liver disease and to prevent the development of cirrhosis and hepatocellular carcinoma. Many unanswered questions remain regarding who to treat, when to treat, how long to treat, and when to stop.

4.2.2. Who and When to Treat HBV

The decision to treat CHB in HIV-infected individuals must be based on careful consideration of the need for antiretroviral therapy for HIV
infection, the severity of liver disease, and the likelihood of response to anti-HBV agents and potential adverse events. In general, the indications for treatment of HBV infection in the setting of HIV infection are the same as those for HBV mono-infected persons as recommended by the AASLD, the European AIDS Clinical Society (EACS) on the Treatment of HBV and HCV in HIV co-infected patients and the DHHS guidelines on the management of adults and adolescents with HIV infection (21, 26, 27).

Hepatitis B e antigen positive patients with a serum HBV DNA level >20,000 IU/ml and elevated serum ALT levels greater than 2 x ULN are candidates for treatment. HBeAg positive patients with normal ALT levels should be monitored without HBV therapy if HAART therapy is not indicated. The HBV DNA level for treatment in HBeAg negative patients is lower with a threshold of >2,000 IU/ml. Whether a liver biopsy is necessary before initiating therapy in HBV HIV co-infected patients is an unsettled issue. Given the association between HBV DNA level and outcome of CHB coupled with the rapidity by which liver histology can progress in chronic HBV infection, there has been a notable shift in clinical practice toward performing fewer liver biopsies in patients with chronic HBV infection and instead basing therapy on HBV DNA and serum ALT levels. Performing a liver biopsy remains a reasonable consideration in individuals with mildly elevated (<1–2 x ULN) or fluctuating ALT levels in the process of gathering data to determine initiation of treatment. Decompensated patients are best managed at liver transplant centers.

4.2.3. TREATMENT MEDICATIONS

Currently, six drugs are licensed for the treatment of chronic HBV. Two are formulations of interferon that require injection as the route of delivery. The other agents are all oral nucleos(t)ide analogues that target the viral reverse transcriptase and include lamivudine, adefovir, entecavir, and telbivudine. Additionally, two nucleos(t)ide analogues licensed for HIV therapy, tenofovir and emtricitabine, have known activity against HBV. Potency, side effects, and resistance profiles vary among the different agents and are essential to consider when selecting agent(s).

4.3. Interferon

Two interferon preparations have been approved for the treatment of chronic HBV infection, standard interferon alfa-2b and pegylated interferon alfa-2a. Very few studies have been conducted in the co-infected population and most reported a lower response rate as determined by the loss of HBeAg and a higher rate of HBV reactivation as assessed
by the reappearance of HBeAg when compared to mono-HBV infected patients (18).

4.4. Lamivudine

Lamivudine was the first nucleoside analogue approved for chronic HBV and also has activity against HIV. Therapy with lamivudine in HBV/HIV co-infected persons resulted in a mean reduction in HBV viral levels of 2.7–3 log_{10} copies/ml and undetectable HBV DNA in 40% by PCR assay (lower limit of detection 400 copies/ml) after 1 year of therapy (28). HBeAg seroconversion rates ranged from 11 to 17% which were comparable to that reported for HBV mono-infected subjects at 16–21% (29, 30). Resistance to lamivudine occurred at a rate of 14–24% after 1 year of treatment and >70% after 5 years of therapy in HBV mono-infected persons (29–31). Unfortunately, lamivudine resistance developed more rapidly in HBV/HIV co-infected persons. Emtricitabine and telbivudine have similar antiviral effects as lamivudine and develop similar patterns of HBV polymerase genotypic resistance (32).

4.5. Adefovir

Adefovir is an acyclic phosphonate nucleotide analogue with activity against HBV but not against HIV at the approved dose of 10 mg. Adefovir is effective at suppressing HBV DNA and is associated with a median reduction in HBV DNA level of 3.5–3.7 log_{10} copies/ml after 48 weeks of therapy (33, 34). However, a high rate of primary non-response (20–50%) has been reported indicating inadequate viral suppression (35). Rates of resistance to adefovir were 2% at 2 years and 29% after 5 years of therapy in HBV mono-infected persons (36).

Adefovir is active against lamivudine-resistant mutants. In one study, 35 HBV/HIV co-infected persons with lamivudine resistance received adefovir 10 mg daily (37). The median reduction in HBV DNA levels reported at 48, 96, and 144 weeks were 4.7, 5.5, and 5.9 log_{10} copies/ml (37). For the management of lamivudine resistance, adding adefovir to lamivudine is preferred to switching to adefovir because of a lower rate of developing adefovir resistance (38–40).

4.6. Entecavir

Entecavir is a carbocyclic analogue of 2-deoxyguanosine with potent activity against HBV and a high genetic barrier to the development of resistance in HBV mono-infected individuals (41, 42). In treatment of naïve HBV mono-infected patients, entecavir 0.5 mg daily for 48 weeks was associated with a 6.9 log_{10} copies/ml reduction in HBV DNA level and 67% tested HBV DNA negative using an assay with a lower limit of
detection of 300 copies/ml (42). No genotypic resistance was reported at 48 weeks. In extended use, entecavir continued to demonstrate a low rate of resistance, 1.2% at 5 years (43).

For management of lamivudine resistance, the recommended dose of entecavir is 1.0 mg daily. However, even at twice the naïve patient dose, the efficacy is reduced in mono-infected patients with lamivudine-resistant HBV raising the development of entecavir resistance as an issue in this population. After 5 years of therapy in lamivudine-resistant chronic HBV patients, 51% developed evidence of genotypic resistance (43). This is because pre-existence of lamivudine-resistant mutations resulted in an increase in the rate of entecavir resistance. In one randomized study of entecavir in HBV/HIV co-infected patients with lamivudine resistance, the mean reduction of HBV DNA was 3.66 log_{10} copies/ml and only 8% of patients developed undetectable HBV after 1 year of therapy (44).

Entecavir was initially believed not to have activity against HIV and was therefore an ideal therapeutic choice for HBV/HIV co-infected individuals who did not require HAART. However, recent reports demonstrated that entecavir has anti-viral activity against HIV and selects for the M184V HIV mutation (45). Thus, entecavir monotherapy for HBV in co-infected individuals should be not be used unless combined with HAART therapy.

4.7. Tenofovir

Tenofovir is an acyclic nucleotide analogue with activity against both HBV and HIV. It has been approved for use in HIV and more recently for HBV infection. Tenofovir exhibited more potent anti-HBV activity than adefovir in both HBV mono- and HBV/HIV co-infected persons (46, 47). This was demonstrated in a small randomized trial comparing tenofovir 300 mg daily to adefovir 10 mg daily, where 94% of subjects had lamivudine resistance (48). The trial was terminated early after the primary non-inferiority endpoint was met. The mean reduction in HBV DNA was 4.4 log_{10} copies/ml with tenofovir and 3.2 copies/ml with adefovir. Similar results were reported in a retrospective analysis of the efficacy of tenofovir in HBV/HIV co-infected persons (49). No resistance has been reported as yet with tenofovir.

4.8. Which Regimen to Use?

Selection of an appropriate regimen will depend on whether the patient only warrants treatment for just CHB alone, just the HIV infection alone, or both infections simultaneously, Table 1.
### Table 1
Summary Of Therapeutic Guidelines For The Management of HBV–HIV Co-Infection

<table>
<thead>
<tr>
<th>Clinical scenario</th>
<th>DHHS panel (50)</th>
<th>EACS (21)</th>
<th>AASLD guidelines (26)</th>
</tr>
</thead>
</table>
| Treat HBV         | • Pegylated interferon alfa-2a  
• Adefovir         | • Pegylated interferon alfa-2a  
• Telbivudine with adefovir add-on if HBV DNA positive at 24 weeks  
• Adefovir and telbivudine combination therapy  
• Early HAART initiation with tenofovir and lamivudine or emtricitabine | • Pegylated interferon alfa-2a  
• Adefovir |
| Treat HIV         | • HAART including tenofovir and lamivudine or emtricitabine | • HAART including tenofovir and lamivudine or emtricitabine | • Tenofovir and lamivudine or emtricitabine |
| Lamivudine resistance present | • Add on/replace with tenofovir | • Add-on tenofovir or adefovir | |

### 4.9. Patients Who Do Not Require Treatment for HIV

Agents that have dual activity against HBV and HIV should not be used as monotherapy to treat HBV in the absence or HAART because of the risk of developing HIV resistance which may limit future HIV therapeutic options. In HBeAg positive patients with low viral load and high serum ALT levels or genotype A and who have preserved CD4 counts (>500 mm$^3$) consideration can be given to using pegylated interferon alfa-2a. The limitation of pegIFN is its poor tolerability and lower efficacy in the HIV setting. Moreover, the drug is contraindicated
in decompensated cirrhosis. The only nucleos(t)ide analogue without HIV activity is adefovir. However, given its relatively low potency and increased rate of resistance over time, monotherapy with adefovir is not advised. Because of the risk for HIV drug resistance, the use of emtricitabine, lamivudine, tenofovir, or entecavir without a full combination antiretroviral regimen should be avoided. Recent DHHS guidelines recommended initiating HAART therapy if HBV treatment alone is necessary (50).

4.10. Patients Who Require Therapy for HIV and HBV

HAART therapy that includes two dual acting drugs against HBV are suitable options for treating co-infected patients. The best choice is to combine a nucleoside and a nucleotide analogue that do not share cross-resistance. Thus, tenofovir plus either emtricitabine or lamivudine would be considered as first-line options. The use of tenofovir, lamivudine, or emtricitabine as the only anti-HBV agent should be avoided because of the risk of developing HBV resistance. Entecavir could be substituted for either emtricitabine or lamivudine. If patients need to change their anti-HIV regimen because of treatment failure or side effects, the anti-HBV component should be continued even if it is not part of the new regimen. A degree of caution should be exercised when stopping HBV therapy as it has been associated with reactivation of HBV and ALT flares.

4.11. Patients Who Require Treatment for HIV but not HBV

If treatment is indicated for the HIV infection in co-infected individuals, then the ability to avoid treatment of the HBV infection becomes problematic as most antiretroviral regimens contain agents with activity against HBV. In this situation, the preferred HAART backbone should include tenofovir in combination with either lamivudine or emtricitabine in order to treat the HBV infection concomitantly (50).

4.12. When to Stop Therapy?

Durable treatment endpoints in HBV/HIV co-infected persons have not been well studied. HBV DNA levels should be monitored every 3–6 months to detect emergence of virological breakthrough heralding drug resistance. The durability of HBeAg and HBsAg loss or seroconversion has not been reported in HBV/HIV co-infected persons. Since in most circumstances therapy for HIV will be necessarily long term, there is little need to discontinue anti-HBV therapy even if these serological endpoints are achieved.
4.13. Management of End-Stage Liver Disease in Co-Infection

The development of signs and symptoms of abdominal ascites, hepatic encephalopathy, and gastrointestinal varices heralds the progression to decompensated liver disease. The treatment and management of these complications in co-infected individuals is identical to the management of end-stage liver disease caused by other chronic liver conditions (51).

4.13.1. Liver Transplantation

Liver transplantation has been evaluated as a medical option for co-infected patients who progress to end-stage liver disease. Prior to the advent of HAART therapy, the presence of HIV infection was considered a contraindication for liver transplantation. However, HAART therapy has altered the natural history of HIV infection such that liver disease is among the leading causes of mortality in co-infected patients. As such, liver transplantation has been assessed as a therapeutic option in end-stage co-infected individuals. In general, HBV/HIV co-infected patients who are highly selected candidates appeared to have comparable survival as those who are HIV mono-infected 2 years after transplantation. However, the number of co-infected patients reported in these studies was small and thus rendering it difficult to generalize their findings (52, 53). Improved management of the complexities associated with the medical management after liver transplantation has required heightened attention to drug–drug interactions, infections, and rejection episodes (51).

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Management of Antiviral Resistance in Chronic Hepatitis B

Edward Doo, MD and Marc Ghany, MD

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Key Principles

- Antiviral resistance in HBV infection is becoming an increasingly encountered clinical problem.
- Characterizing and standardizing definitions of antiviral resistance is of paramount importance in defining a framework within which efficacy may be judged.
- The different classes of antiviral agents have varying risks of resistance. The development of resistant mutations may limit future treatment options due to cross-resistance to other antiviral agents.


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- Management strategies include early detection, add-on therapy, and salvage or rescue therapy based on cross-resistant profile of the mutations present.

1. INTRODUCTION

Almost one-third of the world’s population has been exposed to hepatitis B virus (HBV) and it is estimated that there are 370 million chronic HBV carriers (1). When unrecognized or if left untreated, 40% of individuals will be at risk for developing significant liver disease including cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC). Thus, chronic hepatitis B is a considerable global public health issue. The availability of oral nucleos(t)ide analogue treatment has revolutionized the management of chronic HBV infection. The advantages of oral nucleos(t)ide analogues include their ease of administration, high antiviral efficacy, and a favorable side effect profile. These characteristics represent a major advancement over interferon therapy, which required the use of injection as the route of administration and was associated with many side effects. The development of drug resistance is a major problem associated with the administration of nucleos(t)ide agents. The current challenge is how to best use these agents, whether singly, in combination, or sequentially in order to minimize the development of viral resistance. This chapter will review antiviral resistance of chronic hepatitis B therapy in terms of nomenclature, rates of occurrence, and management.

2. WHY DOES ANTIVIRAL RESISTANCE DEVELOP?

Several viral, host, and drug factors that contribute to the development of nucleos(t)ide resistance have been identified (2, 3). The viral polymerase is the target of nucleos(t)ide analogue therapy. The absence of proofreading function in the viral polymerase results in a relatively high mutation rate of $10^{-4}$ to $10^{-5}$ mutations per base per cycle. This high mutation rate coupled with a high replication rate of $10^{12}$ to $10^{13}$ virions per day means that all possible mutations can be generated daily for every nucleotide site within the viral genome. In general, most viral mutations are not tolerated and viral variants replicate less well compared to wild-type virus. Therefore, wild-type virus is the predominant viral population in the absence of selective pressure such as nucleos(t)ide treatment.

Another important factor is the relationship between the potency of an antiviral agent and its ability to induce selection pressure on the virus. A drug with low antiviral potency does not exert significant
pressure upon the virus, and the chance of developing drug resistance is low. Similarly, a potent drug with rapid and complete suppression of viral replication allows little opportunity for the emergence of resistant virus, as mutagenesis is replication dependent. An agent exerting modest antiviral activity directed at a single target site would potentially lead to the highest probability of selecting for drug-resistant virus. Several other characteristics of the antiviral agent are important. These include the structure of the antiviral agent and the genetic barrier to resistance. The genetic barrier to resistance refers to the number of mutations that the virus must accumulate to restore replication fitness in order to circumvent the effect of the antiviral agent. The higher the genetic barrier of a therapeutic agent, the less likely is the chance of developing resistance.

Regarding the host, the immune status of the patient is one important factor determining the development of viral resistance. Immune suppression, whether acquired primarily or secondarily, indirectly affects the rate of viral replication and the level of circulating virus. Obesity is another factor that can affect drug concentrations in the setting of fixed dosing by altering volumes of distribution; and patient nonadherence can affect steady-state drug levels, all of which are important in the development of drug resistance. Since many of these agents are prodrugs or require phosphorylation for their function, the activity of host cellular enzymes is also an important determinant of antiviral drug resistance.

3. NOMENCLATURE OF ANTIVIRAL DRUG RESISTANCE

Characterizing and standardizing definitions of antiviral resistance has been a major effort of investigators in the field of viral hepatitis B. Standardized definitions acceptable by all investigators allow for more equitable comparisons among antiviral agents in different studies and provide a framework upon which efficacy can be judged. At a National Institutes of Health Workshop on the Management of Chronic HBV: 2006, a panel of experts proposed a consensus opinion of standardized definitions of response to treatment (4, 5).

3.1. Primary Antiviral Treatment Failure (or Primary Non-Response)

Primary non-response is defined as the inability of a nucleos(t)ide analogue to reduce serum HBV DNA by ≥1 log_{10} IU/ml after the initial 6 months of therapy (4, 5). This definition was chosen because it exceeded the variability of the HBV DNA assays and reflected a true virological response, but not a clinical one. Potency of the antiviral
agent is probably the most important factor contributing to primary non-response. Monitoring of primary non-response is important because a high residual viral level after 1 year of treatment is associated with a high rate of antiviral resistance (6).

3.2. Secondary Antiviral Treatment Failure (or Virological Breakthrough)

Virological breakthrough is defined as a consistent increase in serum HBV DNA by $\geq 1 \log_{10}$ IU/ml above nadir while on treatment, after achieving an initial virological response in patients who are compliant with their medication (4, 5). Virological breakthrough is usually the result of drug resistance. Virological breakthrough may occur with or without a concomitant rise in serum alanine aminotransferase (ALT) level.

3.3. Biochemical Breakthrough

Biochemical breakthrough is defined as elevation in serum ALT level during treatment in a patient who has achieved initial normalization (4, 5). Biochemical breakthrough may occur concomitantly with viral breakthrough and in some cases may be accompanied by a hepatitis flare (defined as an ALT level $> 5\times$ upper limit of normal) and clinical decompensation (7, 8).

3.4. Genotypic Resistance

Genotypic resistance is defined as the presence of mutations within the HBV polymerase that have been demonstrated to decrease susceptibility to treatment (4, 5). Primary drug-resistant mutations cause an amino acid substitution that results in reduced susceptibility to an antiviral agent. A secondary or compensatory mutation results in amino acid substitutions that restore functional defects in viral polymerase activity associated with primary drug resistance.

3.5. Phenotypic Resistance

Phenotypic resistance refers to the in vitro confirmation that the genotypic mutation decreases susceptibility to treatment and is the gold standard to confirm antiviral resistance (4, 5).

3.6. Recognition of Antiviral Resistance

Detection of antiviral resistance requires the implementation of an adequate monitoring schedule while on therapy. There are no firm recommendations on what constitutes an appropriate monitoring
schedule but a reasonable approach would be to monitor the serum ALT and HBV DNA every 3 months while on treatment. Virological breakthrough is typically the first manifestation of antiviral resistance (Fig. 1). Therefore HBV DNA levels should be monitored in all patients receiving nucleos(t)ide analogues to document an initial virological response and to monitor for virological breakthrough during treatment in those who had an initial virological response. It is important to exclude non-compliance in patients with virological breakthrough and this should be considered before ordering expensive tests to confirm the presence of drug-resistant mutants. Investigation into the specific resistant mutations should be performed if testing is available so as to confirm genotypic resistance and to help direct the selection of salvage therapy. The development of viral breakthrough is more often than not followed by biochemical breakthrough, which may occasionally rise to the level of a hepatitis flare.

4. RATES OF RESISTANCE TO APPROVED AGENTS

4.1. L-Nucleosides

Currently, lamivudine and telbivudine are the only L-nucleosides approved for treatment of chronic HBV infection in the United States. Other L-nucleoside agents with antiviral activity against HBV include emtricitabine and clevudine. These compounds have a similar

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**Fig. 1.** The rates of genotypic resistance for nucleos(t)ide analogues over time.
molecular structure and target site of action, and as a result, similar patterns of antiviral-resistant mutations. Primary mutations conferring resistance to L-nucleosides occur in the YMDD motif of the reverse transcriptase, which is located in the catalytic site for viral polymerase (9). The specific primary mutations conferring resistance to lamivudine are the rtM204V/I substitutions and for telbivudine, only the rtM204I substitution (9, 10). In vitro studies have shown that these mutations result in a >1000-fold decreased susceptibility of the viral polymerase for lamivudine and reduced functional capacity of the viral polymerase (9). As a result, compensatory secondary mutations including rtV173L, rtL180M, and rtL80I that restore the viral polymerase function to near wild-type levels are almost always associated with the primary resistance mutations (11). The primary lamivudine-resistant mutations are cross-resistant with other agents in the L-nucleoside class. Therefore, other L-nucleoside agents have significantly reduced activity against the lamivudine-resistant mutant virus and should not be used as salvage therapy (12).

In clinical trials, the rate of resistance to lamivudine is relatively high with 14%–24% developing genotypic resistance at 1 year and >70% at 5 years (7, 13, 14). Telbivudine is associated with a lower rate of resistance compared to lamivudine at 1 year, 12%, but the rate almost doubles at two years to 22%, suggesting that the development of resistance may be a significant problem with longer duration of therapy.

4.2. Acyclic Phosphonates

Adefovir and more recently tenofovir are acyclic phosphonates approved for therapy of HBV. Tenofovir has been shown to have superior antiviral activity against HBV compared to adefovir (15). Mutations that confer resistance to adefovir reside outside of the YMDD motif and result in only a modest increase (two- to ninefold) in the EC_{50}. Nevertheless, virological breakthrough occurs with this subtle change in the binding of adefovir to the mutant HBV polymerase (16). The primary adefovir-resistant mutations are the rtA181T and rtN236T substitutions in the viral polymerase (16). The adefovir-resistant mutations are partially cross-resistant with tenofovir but are sensitive to the L-nucleoside and cyclopentane class of agents. The one exception being the rtA181T mutation, which has been shown to result in primary lamivudine resistance in the absence of the rtM204V/I mutations. Accordingly, lamivudine should not be used as salvage therapy for adefovir resistance if this mutation is present (17).

Long-term follow-up results of registration studies indicated the rate of genotypic resistance to adefovir was 2% at 2 years but increased to
29% at 5 years in HBeAg-negative and 20% at a median of 235 weeks in HBeAg-positive subjects (18–20). No resistance to tenofovir has been reported at 1 year of use.

### 4.3. Cyclopentane Group

Entecavir is a carbocyclic analogue of 2-deoxyguanosine with potent activity against HBV. Entecavir acts at three stages in the viral life cycle by inhibiting priming as well as inhibiting synthesis of both negative and positive strands. Entecavir has a high genetic barrier to resistance, which means that the virus must accumulate several mutations before virological breakthrough is observed. Two patterns of mutations have been reported (21). One pattern includes rtI169T + rtL180M + rtM204V + rtM250V and the other rtL180M + rtT184G + rtS202I, and rtM204V (21). Both patterns of mutations encompass the primary lamivudine-resistant mutation, rtM204I/V. In the absence of the primary lamivudine-resistant mutation, the entecavir-associated resistant mutations results in only a modest change in EC\textsubscript{50} (<10-fold) as opposed to over a 1000-fold susceptibility in the presence of the lamivudine-resistant mutant (21).

Entecavir is associated with a low rate of antiviral resistance in nucleoside-naïve patients, 0% at 1 year and 1.2% at 5 years (22). In lamivudine-experienced patients with rtM204V/I ± rtL180M mutations, the rate of entecavir resistance is significantly increased from 1% at 1 year to 51% at 5 years (23). Therefore, careful consideration should be given to other therapeutic options before selecting entecavir as salvage therapy for lamivudine resistance. The entecavir-resistant mutations have been shown to be responsive to adefovir and tenofovir (24).

### 5. CLINICAL CONSEQUENCES OF ANTIVIRAL RESISTANCE

The development of antiviral resistance is usually associated with loss of initial virological, biochemical, and histological response. In a long-term follow-up study of patients treated with lamivudine 100 mg daily, liver biopsies were performed at baseline, 1, and 3 years (25). Histological improvement was observed more frequently in patients without lamivudine resistance, 77% versus 44%, and histological worsening was less common, 15% versus 5%, compared to patients with lamivudine resistance (25). Patients with resistance for >2 years were least likely to demonstrate histological improvement (36%). An important randomized placebo-controlled study of lamivudine to prevent disease
progression in patients with bridging fibrosis or cirrhosis showed that resistance diminished the therapeutic benefits of lamivudine (26). In this study, half of the patients developed lamivudine resistance. Although benefit was observed among treated patients who developed lamivudine resistance when compared to placebo, the benefit was clearly less relative to lamivudine-treated patients without resistance (26).

Hepatitis flares and hepatic decompensation may occur following the development of antiviral-resistant mutants. In a study of almost 1000 HBeAg-positive patients with compensated liver disease who received lamivudine for a median of 4 years, the risk of hepatitis flares increased with the duration of lamivudine resistance such that 80% patients who had lamivudine-resistant mutants for >4 years would have experienced at least one hepatitis flare (7). The risk of hepatic decompensation was extremely low in this cohort, less than 1%, except for the group who had lamivudine resistance for more than 4 years. The generally favorable short-term clinical outcome of this cohort may be related to their young age (mean 32 years old) and the fact that only 10% had cirrhosis at the onset of treatment.

Development of antiviral resistance may limit future treatment options due to mutations that confer cross-resistance to other antiviral agents. For example, development of lamivudine-resistant mutation rtM204V/I is associated with cross-resistance to other L-nucleoside analogues, such as telbivudine and emtricitabine, as well as entecavir. The presence of the rtM204V/I mutation effectively eliminates these drugs as rescue agents for these patients (12).

6. MANAGEMENT OF ANTIVIRAL RESISTANCE

Several important concepts of management of antiviral resistance have emerged in recent years. The earlier therapy is altered after detecting the emergence of virological breakthrough, the better the long-term virological and biochemical outcome. Additionally, the strategy of add-on therapy appears to be a better approach as opposed to switching therapy with regard to preventing subsequent multi-drug resistance. Salvage therapy should be implemented immediately in patients with cirrhosis to prevent the possibility of hepatitis flares and subsequent hepatic decompensation. Finally, the choice of rescue therapy for a patient with drug-resistant HBV should be based on the cross-resistant profile of the mutations present, the potency of available agents against these mutations, and the presence of co-morbid conditions such as renal insufficiency (Table 1).
Table 1
General Principles of Management

- Obtain pre-treatment HBV DNA level
- Assess on-treatment virological response profile
- Document previous antiviral therapy if any
- Know potency and resistance profile of antiviral drugs
- Know of cross-resistance profile of drug
- Assess severity of underlying liver disease
- Address Co-morbid medical conditions e.g. renal disease
- ? Perform resistance testing

In a study to assess whether the timing of administration of rescue therapy influenced outcome, adefovir was initiated either at the time of detection of genotypic resistance without biochemical breakthrough or at the time of genotypic and clinical breakthrough in patients treated with lamivudine (27). At 2 years, the virological response was 100% in patients treated at the time of genotypic resistance only but 78% in those with both genotypic and clinical breakthrough, suggesting that early implementation of rescue therapy was associated with a more favorable clinical outcome (27). Several studies have demonstrated that when adefovir was used to manage lamivudine resistance, the strategy of add-on was superior to switching in terms of preventing future adefovir resistance (28, 29). In a retrospective analysis of 588 patients with lamivudine resistance, 303 were switched to adefovir and 285 had adefovir added in combination with lamivudine (29). The rates of virological response were similar in both the switched and combination groups after 3 years of therapy (71% versus 78%, respectively). However, the rate of virological breakthrough (30% versus 6%, respectively) and the genotypic resistance to adefovir (16% versus 0%) were significantly higher in the group switched to adefovir monotherapy as opposed to adefovir add-on therapy respectively, underscoring the benefit of combination therapy in preventing the development of multi-drug resistance (29).

6.1. Options for Management of Antiviral Resistance

Data on management options for patients with lamivudine resistance are evolving and are available from clinical trials and in vitro testing. Therapeutic options include adding adefovir, switching to drugs with
high potency and genetic barriers to resistance such as entecavir or tenofovir or the combination of tenofovir plus emtricitabine (off label use) (3). Rates of undetectable HBV DNA by PCR in HBeAg-positive patients treated with adefovir 10 mg daily as add-on therapy or who were switched to entecavir 1.0 mg daily or tenofovir 300 mg daily were reported as 35%, 21%, and 91%, respectively (30–32). Although these results are not directly comparable because of different baseline characteristics, the 1-year rate of resistance to adefovir add-on or switching to entecavir or tenofovir were 0%, 1% and 0%, respectively (30–32). These data would support all of these strategies at least in the short term but longer term data are necessary before one strategy can be recommended over the other. Of concern is recently released 5-year data on rates of genotypic resistance to entecavir in lamivudine-resistant patients, indicating that 51% developed resistance (23).

Lamivudine-resistant HBeAg-negative patients showed better virological responses compared to HBeAg-positive patients as was the case in the treatment of naïve patients (28, 29). In one study, 67% and 69% of patients treated with switching or add-on adefovir therapy, respectively, achieved a virological response, but add-on therapy was associated with a lower rate of subsequent adefovir resistance (29). Currently, only case reports are available on the use of entecavir or tenofovir as salvage therapy in HBeAg-negative chronic hepatitis B and therefore no evidence-based recommendations can be made about these agents. Figures 2 and 3 illustrate virologic responses and resistance rates encountered with rescue therapy of lamivudine resistant HBeAg positive and negative patients.

For the management of adefovir resistance, evidence is based on small case reports and in vitro testing (33). These reports suggested that management should be based on the pattern of adefovir-resistant mutations present (16, 34). For patients with the rtN236T mutation, options include switching to or adding entecavir; adding lamivudine; or switching to tenofovir; or switching to the combination of tenofovir plus emtricitabine (off-label use). For the scenario where the rtA181T mutation has developed, options are fewer and include switching to or adding entecavir; or switching to tenofovir; or switching to the combination of tenofovir plus emtricitabine (off-label use). Lamivudine should not be used in this setting due to cross-resistance to the rtA181T mutation.

Data on the management of entecavir resistance are based largely from case reports and in vitro phenotypic testing. On the basis of this limited evidence, two approaches are available: to switch or add adefovir; or switch to or add tenofovir monotherapy; or switch to tenofovir plus emtricitabine (off-label) (24, 35).
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**Outcome of Rescue Therapy in HBeAg (+) Patients with Lamivudine Resistance**

![Graph](image)

**Fig. 2.** Outcomes of rescue therapy for lamivudine resistance in HBeAg-positive patients. (a) Virological response: percent HBV DNA undetectable by PCR assay. (b) Rates of resistance over time either with switching or add-on strategy.

**Outcome of Rescue Therapy in HBeAg (-) Patients with Lamivudine Resistance**

![Graph](image)

**Fig. 3.** Outcomes of rescue therapy for lamivudine resistance in HBeAg-negative patients. (a) Virological response: percent HBV DNA undetectable by PCR assay. (b) Rates of resistance over time either with switching or add-on strategy.

There is limited information on the management of resistance to telbivudine; however, the recommendations would be similar to those for lamivudine resistance based upon the similarities of these two compounds. Scant data are available on the management of multi-drug resistance (36). Every precaution should be taken to prevent this clinical scenario from occurring. Peginterferon may have a role in carefully selected cases and expert advice should be sought in managing these patients.
7. PREVENTION OF ANTIVIRAL RESISTANCE

The prevention of antiviral resistance is a major goal of future management strategies. This should begin with proper patient selection and judicious use of antiviral agents. One should avoid futile/inappropriate antiviral therapy. An antiviral agent with the highest potency and high genetic barrier to resistance should be selected especially in HBeAg-positive patients with high viral loads to prevent the emergence of drug-resistant mutants. Whether initiating therapy with combination therapy will achieve the goal of minimizing the development of antiviral resistance is currently an unanswered question. Since all available antiviral agents have the same target of action, the utility of this approach would have to be proven in clinical trials. Certainly, the 5-year data for entecavir in the treatment of naïve patients would not support this approach. Other unanswered questions include what agents to combine and could one agent be withdrawn after HBV DNA is fully suppressed. It is important to avoid sequential monotherapy, which can lead to multi-drug resistance and avoid use of agents with similar cross-resistance profiles. Monitoring the antiviral response is crucial for the early detection of virological breakthrough, thus permitting early intervention with the prospect of better outcomes. Finally, reinforcement of compliance with a prescribed regimen is of paramount importance.

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Key Principles

- Complementary alternative therapy is a growing market, estimated at $180 billion annually, with at least one-third of liver patients acknowledging their use.
- Patients infected with hepatitis B and C account for the wide use of herbal and non-traditional therapies for viral hepatitis.
- Silymarin is most commonly used for patients with liver disease. In hepatitis C patients, two small trials showed a benefit in the reduction of liver enzymes, without affecting the hepatitis C viral load. Other trials have demonstrated no difference in aminotransferase levels and no reported trial has noted an effect on viral clearance rates with silymarin administration.
- In hepatitis B patients, silymarin showed potential benefit in some trials, but these results could not be reproduced. As such,
silymarin, while safe, cannot be recommended for patients with hepatitis B or C.

- Glycyrrhizin, the active constituent of glycyrrhiza, has showed some benefits in the treatment of those with chronic hepatitis C, with a reduction in aminotransferase levels noted in some trials. The side effect profile including hypokalemia and fluid retention approaching 20% of patients who receive this supplement cautions against its use.

- Small trials using *Phyllanthus amarus* have suggested a higher clearance rate of serum HBsAg when given in combination with interferon. There is insufficient data to routinely recommend its use.

- TJ-9 (Xiao-Chai-Hutang/Sho-Saiko-To) showed benefit in patients with chronic HBV with increased HBeAg seroconversion, as well as an improved survival rate in those with non-HBsAg-related cirrhosis at 5 years. Unfortunately, side effects including liver injury, autoimmune hepatitis, hypokalemia, and hypertension prohibit routine use of this medicine.

- St. John’s wort (*Hypericum perforatum*) showed no efficacy in decreasing viral levels in chronic hepatitis C patients. Significant side effects associated with this medication suggest against its use in patients with liver disease.

- Plantago Asiatic has shown preliminary promising results, suggesting suppression of hepatitis B DNA levels. More studies are required to recommend its use.

- Herbal Medicine 861 showed promising initial results in patients with hepatitis B, reducing the level of liver fibrosis at the 6-month posttreatment interval. More studies will be required to verify this promising result.

- CH 100 showed initial promise in decreasing ALT levels in patients with chronic hepatitis C, but these results were not verified by other studies. More data will be required for it to be recommended in patients with liver disease.

- Liv 52 initially showed benefit in non-alcohol-related cirrhosis. However, in alcohol-related cirrhosis, a randomized, placebo-controlled trial showed increased mortality among the Child Class C patients, leading to the immediate withdrawal of the supplement from the United States.

- The antioxidant *N*-acetyl cysteine (NAC) failed to show a benefit in patients with acute hepatitis A and B.

- *S*-Adenosylmethionine use in patients with liver disease has also failed to show a consistent benefit.

- Vitamin E may show benefit in those with chronic HBV infection though additional studies are required.
1. ECONOMICS OF COMPLIMENTARY ALTERNATIVE MEDICINE

Acute and chronic liver disease due to viral hepatitis is an important cause of morbidity and mortality throughout the world. Over the past two decades, there has been rapid development of specific antiviral therapies in developing countries where chronic liver disease due to viral hepatitis may be prevalent at rates equal to or greater than that seen in developed countries. These therapies have been tested in rigorous randomized trials with objective endpoints. However, cost and limited resources have limited access to these therapies in many parts of the world including continents where specific therapies are available. As an example, anti-viral therapy for chronic hepatitis C (HCV) with pegylated interferon and ribavirin may cost more than $25,000 (US) per year for treatment, and even oral therapies for chronic hepatitis B (CHB) such as lamivudine, adefovir, entecavir, and telbivudine are associated with costs from $2000 to $9000 (US) per year. In addition, these therapies are often administered for greater than 1 year and can be associated with resistance and other side effects. Finally, not all patients with chronic viral hepatitis are candidates for these therapies.

As an alternative to these specific antiviral therapies, herbal and non-traditional therapies for viral hepatitis, which have been used worldwide for thousands of years, remain the backbone of treatment in many parts of the world. They are widely used in countries with access to traditional antiviral agents as well as countries without the resources to provide widespread access to traditional antiviral agents. There are multiple reasons for the widespread use of these agents including lower cost, the perception that these “natural agents” are safer than traditional medicines, and fewer restrictions as these herbal therapies may not require a physician visit or a prescription. However, these agents have rarely been evaluated in a fashion similar to approved antiviral therapies, rather relying on uncontrolled data with subjective endpoints. Moreover, many herbal and non-traditional therapies for viral hepatitis are not single agents but rather combinations of various herbs or other compounds, without stringent guidelines, thus making assessment of efficacy difficult. The complementary alternative medicine (CAM) industry is a growing market among the population in the United States and is currently estimated at $180 billion. Approximately one in six adults taking prescription drugs is currently using CAM (1). Liver disease patients report the use of CAM from 30 to 64%, with silymarin noted as the most commonly used agent by this group (2–6).
2. BURDEN OF VIRAL HEPATITIS

The two diseases responsible for the majority of chronic viral hepatitis worldwide are hepatitis B and hepatitis C. Hepatitis B represents a major concern to public health and of two billion individuals who have been infected worldwide, there are an estimated 400 million persons worldwide who are chronically infected with HBV. Sub-Saharan Africa, the Pacific Islands, and particularly Asia represent most of the HBV disease burden due to the vertical transmission of the disease. Many countries in these continents lack resources to provide traditional medicines for viral hepatitis (7).

Hepatitis C chronically infects an estimated 170 million persons worldwide. There are an estimated 2.7 million people in the United States who have active HCV infection with higher prevalence rates in Europe and Asia (8). Just 15% of patients with acute hepatitis C infection achieve clearance, leaving the large worldwide reservoir in approximately 85% of acutely infected individuals (9). It is these two large reservoirs of patients that account for the wide use of herbal and non-traditional therapies for viral hepatitis. In this chapter, we will review the most commonly used herbal and non-traditional therapies and the data that support their efficacy as well as noting potential toxicities of this class of therapies.

3. SELECTED HERBAL THERAPIES

3.1. Silymarin

Silymarin or milk thistle has been used medicinally for over 2000 years, most commonly for the treatment of liver and gallbladder disorders. There is one specific medical indication, that being therapy for Amanita phalloides poisoning (10). Silymarin is an extract of Silybum marianum (milk thistle) found commonly throughout Europe, Asia, and North America and typically comprises at least 70% of milk thistle. Highly purified extracts of this plant have been available since the 1960s (11,12). Silymarin has been reported to be composed of the flavonolignans silybin, silicristin, silydianin, and isosilybin (a diastereomer of silybin) (13). Silybin is the major active constituent of silymarin and represents 80–90% of its composition, while the rest is undefined (14). Proposed mechanisms of action of silymarin are believed to be through antioxidant properties as can be seen in iron overload and other states with increased oxidative stress (14) and through anti-fibrotic properties (15). Additionally, silymarin is thought to act via anti-inflammatory properties through inhibition of cytokine production by the Kupffer cells (16).
3.1.1. Silymarin Use in Hepatitis C

There is limited data supporting silymarin as a beneficial treatment for HCV infection. A recent Cochrane database review focused on the use of silymarin in patients with alcoholic and viral causes of liver disease (10). Of the 1833 initial references, exclusion criteria reduced the set to 26 references of 13 randomized controlled trials. In this review a potentially beneficial effect of silymarin on alcoholic liver disease was observed, although this finding could not be supported by two high-quality trials. Additionally, the benefit was not observed in a subgroup analysis including patients with HCV infection. Overall, the review concluded that there is insufficient evidence to recommend or refute the use of milk thistle in patients with liver disease of any etiology including HCV, emphasizing the need for more randomized clinical trials (10). A second Cochrane hepatobiliary systematic review of randomized trials evaluating medicinal herbs for chronic HCV yielded similar results for silymarin (17). In only one placebo-controlled trial was silymarin noted to improve AST and γ-glutamyl transpeptidase (GGTP) levels compared to placebo, although there were no effects on the viral load.

In small pilot studies, silymarin showed a possible decrease in ALT levels in patient with chronic HCV without impacting HCV RNA levels (11). Other trials have demonstrated no difference in aminotransferase levels and most importantly, no reported trial has noted an effect on viral clearance rates with silymarin administration (18).

3.1.2. Silymarin Use in Hepatitis B

Currently, no randomized controlled trials have evaluated the efficacy of silymarin in those with CHB. In a review focusing on silymarin in acute viral hepatitis, promising results were noted in a cohort of patients acute hepatitis B (19). In this study, HBV patients treated with silymarin had a shorter hospitalization (23 vs 30 days) and improved liver-associated chemistries as compared to patients with supportive care. However, other studies have not been able to demonstrate a similar benefit in those with acute HBV infection (20). Magnilio et al. (21) did not find a difference in the seroconversion rate in HBV-positive patients treated with silymarin, though they did show an improvement in liver tests, most notably for bilirubin and aminotransferase levels in the silymarin group. While these studies have shown that silymarin does not appear to have any hepatotoxic concerns in acute and chronic viral hepatitis, the current evidence demonstrates that there is no definite indication for using silymarin in either hepatitis B or C viral infection.
3.2. Glycyrrhizin

**Glycyrrhiza glabra** (lichorice root) originates in the Middle East and Mediterranean and in the past has been used for numerous medical purposes, including peptic ulcer disease and as an expectorant. Glycyrrhizin, the active part of glycyrrhiza, is a conjugate of glucuronic acid and glycyrrhetinic acid (22). There are several proposed mechanisms to explain its potential benefit including antioxidant properties, tumor necrosis factor inhibition, and endogenous interferon production (23). In the treatment of HCV with glycyrrhizin, some trials have demonstrated a decrease in aminotransferase levels (23).

Two well-designed studies with glycyrrhizin in 226 patients with chronic HCV failed to show viral clearance in any of the patients. In one study, patients received either ursodeoxycholic acid plus intravenous glycyrrhizin or glycyrrhizin alone. Patients who received the combination therapy showed improvement of liver enzyme levels. Another large non-randomized trial observed a significantly lower prevalence of cirrhosis (21% vs 35%) and HCC (12% vs 25%) in patients treated with Stronger Neo-Minophagen C (SNMC), a derivation of glycyrrhizin, cysteine, and glyceine routinely used for active chronic hepatitis in Japan (24).

Trials with glycyrrhizin in CHB have shown improvement in aminotransferase levels in patients receiving glycyrrhizin. In addition to lacking sufficient detail with respect to nature of liver disease or hepatitis B viral (HBV) DNA levels, none of the trials using glycyrrhizin have reported a benefit with reduction in HBV DNA level (22–24). Importantly, patients who consider the use of glycyrrhizin should be counseled regarding the side effects of fluid retention and hypokalemia, which are seen in up to 20% of patients receiving glycyrrhizin (22). Generally, beneficial effects of glycyrrhizin cease after the cessation of the administration of the medication and in trials that showed benefit in chronic viral hepatitis, it was related to the reduction of aminotransferase levels. There are no studies to our knowledge that assess the addition of glycyrrhizin for patients receiving interferon therapy. However, the side effect profile of this herbal therapy limits its recommendation for routine use in liver disease and patients who take glycyrrhizin should be cautioned regarding the side effects.

3.3. Phyllanthus amarus

*P. amarus* is a herb, which is a part of Indian ayurvedic medicine. *P. amarus* has been used to treat diabetes, kidney and urinary problems, diarrhea, and viral hepatitis and has thus far been limited to chronic hepatitis B. The proposed mechanism of action is believed to act through
the inhibition of polymerase activity, mRNA transcription, and replication (18). A systematic review by Liu et al. of 22 trials noted that most were of small sample size and less than ideal methodological quality. In patients using this supplement, nausea, decreased appetite, and stomach ache were reported with no serious side effects found (25). Five trials were assessed to be of adequate methodological quality. The results of four placebo-controlled trials showed that *Phyllanthus* herb vs placebo had an effect on clearance of serum HBsAg. The beneficial effect of *Phyllanthus* was mainly from an Indian trial, which was not repeated in a later trial by the same author (26). Additionally, in the trial by Liu et al, beneficial effects were noted when patients used interferon in combination with the *P. amarus* with improved clearance of HBsAg noted, as compared to interferon alone (25). No other supplements or herbal therapies for treatment of CHB have been assessed in this manner. Although using *Phyllanthus* for the treatment of patients with CHB is an attractive option, randomized controlled trials need to be conducted to confirm this effect and as such, this herb cannot be routinely recommended for use alone in the treatment of CHB.

### 3.4. **TJ-9 (Xiao-Chai-Hutang/Sho-Saiko-To)**

This herbal supplement has been a part of the traditional Japanese Kampo system and the herbal medicine Sho-saiko-to (TJ-9) is an officially approved prescription drug in Japan, widely administered in Japan to patients with chronic liver disease. TJ-9 consists of a dried mixture of seven herbs including roots of scutellaria, glycyrrhiza, bupleurum, ginseng, pinella tuber, jujube fruit, and the ginger rhizome (27). In animal and cell culture models, TJ-9 has showed benefit by preventing collagen production in cultured stellate cells, as well as inhibiting lipid peroxidation in liver mitochondrial membranes (28). In investigating the mechanism by which TJ-9 activates stellate cells, Kakumu et al. showed that TJ-9 enhanced in vitro production of interferon-γ and production of antibodies to hepatitis B core and hepatitis B e antigens by peripheral blood mononuclear cells from patients with chronic hepatitis B (28).

Similar to many of the herbal therapies, there are limited studies in humans, though one study treated 116 patients with chronic hepatitis with TJ-9. Liver chemistries improved significantly after administration of TJ-9, and in patients with CHB, hepatitis B e antigen (HBeAg) seroconversion was observed with no significant side effects noted (29). Another randomized prospective study in patients with cirrhosis due to CHB or HCV noted an improved survival rate in those with non-HBsAg-related cirrhosis at 5 years (30). However, side effects including
liver injury, autoimmune hepatitis, hypokalemia, and hypertension have been noted, raising concerns regarding the use of this herb, particularly in those with cirrhosis (28–32). At this time, there is insufficient evidence in human studies to recommend the use of this drug though future studies might be desirable in those with HBV-related liver disease.

3.5. St. John’s Wort

Hypericin is a natural derivative of the common St. John’s wort plant, *H. perforatum*. Previous reviews suggested there may be benefit from the use of St. John’s wort in depression, but a Cochrane database review from 2005 contradicted previous reviews from 1995 and 2000 and concluded that in the treatment of mild-to-moderate depression, the data were inconsistent and did not support its use as an antidepressant (33). Regarding the use of St. John’s wort in viral hepatitis, in a phase one trial, Jacobson et al. tested the effect of hypericin in patients with chronic HCV infection. In this study, hypericin demonstrated no detectable benefit as defined by change in HCV RNA levels and caused considerable phototoxicity at the doses studied (34). The side effect profile of St. John’s wort also includes solid organ rejection, as well as a number of other potentially significant interactions with other medications, making its use hazardous as an herbal supplement, and this supplement should be avoided in those with chronic liver disease (35, 36).

3.6. Plantago Asiatic

This Chinese herb’s active component, aucubin, was systematically studied for its potent liver-protective activities using experimental systems of hepatic damage. In animal studies, this plant was found to suppress the HBV replication and in humans, suppression of HBV DNA levels was also noted with the anti-viral effect ceasing after the herb was stopped (37). While encouraging, more trials need to be completed to assess its effect and safety profile in humans.

3.7. Herbal Medicine 861

Herbal medicine 861 is a mixture of 10 extract herbs, including *Salvia miltiorrhiza*, *Astragalus membranaceous*, and *Spatholobus suberectus*. In patients with hepatitis B, three controlled trials showed a reduction in the level of liver fibrosis 6 month post treatment.

All patients remained hepatitis B surface antigen (HBsAg)-positive (11). In vitro studies of this herbal supplement showed a possible reduction in the expression of α-smooth muscle actin mRNA in liver cells. Since the cell proliferation and high levels of α-SMA are associated
with liver fibrosis, HM 861 may be useful in the treatment of those with chronic liver disease with advanced fibrosis and no significant side effects have been reported (20, 38). However, more trials will need to be performed to verify these promising results.

3.8. **CH-100**

CH-100 is a herbal formulation of 19 known ingredients that may work by reducing TNF-α production by lymphocytes (38). This Chinese herbal medication was associated with a significant reduction in alanine aminotransferase (ALT) levels over a 6-month study period with four individuals normalizing their ALT levels on treatment in those with chronic HCV infection, though there was no significant effect on the clearance of serum HCV RNA (38). Similar to many herbal preparations, larger studies with better endpoints will be required to assess whether the reduction in ALT levels noted in these small studies can be reproduced and to allow collection of better safety data in those with viral hepatitis.

3.9. **Liv 52**

Liv 52 is an Indian supplement that has been marketed specifically for the treatment of liver diseases. The extract was reported to improve serum liver chemistry values in rats with toxic liver damage and in humans with acute viral hepatitis (39).

In one trial patients with non-alcoholic-related cirrhosis, patients treated with Liv 52 for 6 months had significantly better Child-Pugh score, decreased ascites, and decreased serum ALT and AST levels compared to those who received placebo (39). However, in another randomized, placebo-controlled trial for over 2 years in 188 patients with alcohol-related cirrhosis, Liv 52 did not affect the survival rate of Child-Pugh Class A and B patients but increased mortality among Child Class C patients. This led to the drug being withdrawn from the US market (18).

4. **ANTIOXIDANTS AND DIETARY SUPPLEMENTS**

Multiple studies have demonstrated that in both acute and chronic viral hepatitis, there may exist high levels of oxidative stress that can be associated with reduced levels of some important antioxidants. In addition, the amount of reactive oxygen species (ROS) found in healthy human livers is significantly lower than values found in livers infected by HBV or HCV (40). This provides a theoretical rationale for the use of antioxidants in those with chronic viral hepatitis. In a trial by
von Herbay et al., high-dose vitamin E was found to improve aminotransferase levels in hepatitis C-infected patients refractory to interferon treatment. The ALT and AST improvement was transient and after the cessation of vitamin E, liver tests returned to their previous levels (41). Andreone et al. evaluated vitamin E supplementation as therapy for CHB in a pilot study including 32 patients (42). Patients were randomly allocated to receive vitamin E at the dose of 300 mg twice daily. At the end of the study period, ALT normalization was observed in 47% patients in vitamin E group and only in 6% of the controls; HBV DNA was cleared in 53% patients in the vitamin E group and 18% in the control group, respectively. These preliminary results suggest that additional trials should be done to establish the beneficial effect of vitamin E in chronic viral hepatitis patients, particularly in those with hepatitis B.

4.1. N-Acetyl Cysteine (NAC)

N-Acetyl cysteine (NAC) is well known as an effective antidote in acetaminophen hepatotoxicity by repleting glutathione stores. In vitro studies demonstrated that NAC administration to HBV-producing cell lines resulted in the reduction of HBV DNA levels (43). However, when NAC was administered to patients with acute viral hepatitis A and B, no effect on aminotransferase levels or bilirubin levels was seen, suggesting little effect in the setting of acute viral hepatitis (44). The role of NAC in acute liver failure due to viral hepatitis is an area currently undergoing study.

4.2. S-Adenosylmethionine (SAM)

S-Adenosylmethionine deficiency may be seen in alcoholic liver disease (ALD) as well as in experimental models of hepatotoxicity and may potentiate liver damage by furthering mitochondrial damage (45). The studies on the benefit of this supplement on mortality in patients with liver disease have not shown a consistent benefit and thus more studies will be required to be able to recommend this supplement (18).

4.2.1. Acupuncture

This method of Chinese traditional treatment requires mention due to its high prevalence among liver patients with as many as 18% of patients with chronic hepatitis receiving acupuncture therapy (46). The transmission of hepatitis B and C is a concern in this method of treatment though the use of universal precautions should minimize this risk (47).
5. DISCUSSION

As noted in the previous review of the peer-reviewed medical literature, none of the herbal/CAM supplements can be recommended for both safe and effective use in patients with acute or chronic viral liver disease. Only silymarin appears to have sufficient data available to have minimal safety concerns, though efficacy data in viral hepatitis remain limited. Tables 1 and 2 summarize common herbal supplements for chronic viral hepatitis, with available data regarding their benefits and risks.

One major issue that stands out for most herbal therapies is the lack of randomized controlled trials supporting their use, as the herbal/CAM industry is not required to conduct such efficacy trials to get their products to market. Manufacturers of herbal/CAM have noted that it would be difficult to recover the high research costs required of a randomized controlled trial, because herbal products can be patented less easily than newly synthesized drugs (48). Other issues of concern with research conducted by the CAM industry include lack of randomization, lack of standardization and quality control of the herbal drugs, use of varying dosages, small patient numbers, lack of placebo, and the wide variation in the time during which the herbal preparations were used. Furthermore, even published research in this clinical area must be interpreted with caution as some have noted that certain countries, including China are known to publish only positive results (49). Moreover, it is well known from clinical trial literature that methodologically less rigorous trials may demonstrate larger treatment effects than those conducted with better rigor. As such, the potential beneficial effects of

<table>
<thead>
<tr>
<th>Plant</th>
<th>Dose/potency</th>
<th>Number of tablets</th>
<th>Administration</th>
<th>Price</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silymarin</td>
<td>200 mg/80%</td>
<td>240</td>
<td>1–3 tablets daily</td>
<td>$43</td>
<td>Nature Purest</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>500 mg/12%</td>
<td>60</td>
<td>1–2 tablets between meals</td>
<td>$14.60</td>
<td>Douglas Laboratories</td>
</tr>
<tr>
<td>P. amarus</td>
<td>250 mg</td>
<td>60</td>
<td>1 tablet with meals</td>
<td>$10</td>
<td>Douglas Laboratories</td>
</tr>
<tr>
<td>St. John’s wort</td>
<td>990 mcg</td>
<td>90</td>
<td>1 tablet with meals</td>
<td>$40</td>
<td>Blackmores</td>
</tr>
</tbody>
</table>
Table 2
Summary of Common Herbal/CAM

<table>
<thead>
<tr>
<th>Herbal/CAM</th>
<th>Efficacy in HCV</th>
<th>Efficacy in HBV</th>
<th>Safety concerns/side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simybarin</td>
<td>No clear benefit</td>
<td>No clear benefit</td>
<td>No hepatotoxic effects</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>Transaminase decrease</td>
<td>Transaminase decrease</td>
<td>Fluid retention, hypokalemia</td>
</tr>
<tr>
<td>Phyllanthus amarus</td>
<td>None</td>
<td>Possible improved viral clearance when used with interferon</td>
<td>Stomach ache, nausea</td>
</tr>
<tr>
<td>TJ-9</td>
<td>None</td>
<td>Possible benefit in HBeAg</td>
<td>Liver injury, autoimmune hepatitis, hypokalemia, and hypertension</td>
</tr>
<tr>
<td>St. John’s wort</td>
<td>None</td>
<td>None</td>
<td>Solid organ rejection, phototoxicity</td>
</tr>
<tr>
<td>Plantago Asiatic</td>
<td>None</td>
<td>None</td>
<td>Insufficient data on side effect profile</td>
</tr>
<tr>
<td>HM861</td>
<td>None</td>
<td>Possibly in liver fibrosis</td>
<td>Insufficient data on side effect profile</td>
</tr>
<tr>
<td>CH100</td>
<td>Transaminase decrease</td>
<td>None</td>
<td>Insufficient data on side effect profile</td>
</tr>
<tr>
<td>Liv 52</td>
<td>None</td>
<td>None</td>
<td>Increased mortality in patients with Child Class C patients</td>
</tr>
</tbody>
</table>

herbal medications can be magnified and the lay public may be influenced into their use without recognizing the limitations of trial designs (50–52).

Still, there is no denying of the widespread appeal that surrounds the CAM industry and clinicians should question patients specifically about their use as the majority of patients will not volunteer their use of herbal supplements (53). Patients perceive that herbal/CAM preparations are natural and therefore possess an inherent safety profile not present in prescription medicines available for chronic viral hepatitis as well as the relative cost factor. In fact, herbal/CAM preparations often are concoctions of various extracts, many of which are unidentified, thus limiting the safety evaluation (54). In China, 50% of clinical trials of Chinese medicinal herbs report adverse effects and one possible explanation is that Chinese practitioners perceive herbs as free of side effects (55).
While not as well known to the lay public, it is well known by practicing clinicians that the use of herbal remedies can pose serious health risks. Besides the direct risks of adverse effects and drug interactions, there is an indirect risk that a herbal remedy without demonstrated efficacy may compromise, delay, or replace an effective form of conventional treatment. There are reports of liver toxicity and other serious adverse events associated with the use of certain Chinese herbal medicines (56–59). A survey of the National Poison Information Service for the years 1991–1995 documented 785 cases of possible or confirmed adverse reactions to herbal drugs given for all indications, not just viral hepatitis, but hepatotoxicity was the most frequent side effect noted (60). In addition, it has been noted that the hepatotoxicity of CAM is underreported due to the lack of safety reporting system as is available for prescription medicines in developed countries (11). Other risks in addition to the hepatotoxicity caused by the direct effect of the herbal medication include misidentification of the plant, selection of a wrong part of the plant, inadequate storage, contamination of the plant by various chemicals, heavy metals, microorganisms, alteration during conditioning, and mislabeling of the final product (60).

The use of CAM is underreported to the extent that 70% of patients will neglect to mention their use to their physicians (53). Some patients believe that physicians may have an underlying bias against or not be knowledgeable on this subject (61), while other patients may not be taking the herbal/CAM preparations for a medical reason and may perceive the relationship as unrelated (62). Physicians need to remain vigilant in taking accurate histories regarding medication use and approach their patients about this topic in an unbiased manner. Given the fact that 12–16% of those on prescription drugs utilize CAM, physicians should familiarize themselves on the use of herbal medications as well as on their side effects and interactions to safely and effectively counsel and treat the vast number of patients who seek therapy for chronic viral hepatitis (62).

REFERENCES


Hepatitis B Reactivation in the Setting of Chemotherapy and Immunosuppression

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and James H. Lewis, MD, FACP, FACG

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Key Principles

- Reactivation of hepatitis B is recognized as the reappearance of active necroinflammatory disease of the liver in a person known to have the inactive hepatitis B surface antigen (HBsAg) carrier state or resolved hepatitis B.
- More recently, hepatitis B reactivation has been defined as a one log or greater increase in the HBV DNA level compared with the preractivation level, or the reappearance of a previously negative HBsAg on two consecutive tests.
- Reactivation may result in acute flares of HBV, with consequent clinical decompensation.
- Patients at risk for hepatitis B reactivation are those with hematological or oncological malignancies who require chemotherapy, patients with non-malignant diseases who require immunosuppression, and patients with rheumatologic diseases or inflammatory bowel diseases treated with biologic response modifiers.
- With the administration of cytotoxic or immunosuppressive agents, the immune response is suppressed, viral replication is enhanced, and widespread infection of hepatocytes ensues. With the discontinuation of the cytotoxic or immunosuppressive therapy, the immune function is restored. This immune reconstitution results in a rapid destruction of hepatocytes infected with hepatitis B.
- Patients who are negative for HBsAg but have evidence of either circulating or intrahepatic hepatitis B viral (HBV) DNA are denoted as having occult HBV. It is well established that chemotherapy can cause HBV reactivation even in HBsAg-negative patients with occult HBV infection.
- All patients should undergo screening for occult or overt HBV prior to the initiation of chemotherapy or immunomodulatory therapy. Seronegative patients should be vaccinated against HBV.
- HBsAg-positive patients should be considered at high risk of reactivation and should be started on preemptive anti-HBV therapy.
- If reactivation of HBV occurs, prompt antiviral therapy should be combined with aggressive supportive therapy in addition to the cessation of chemotherapy.
1. INTRODUCTION

It is estimated that out of the 2 billion people who have been infected with the hepatitis B virus (HBV) worldwide, more than 350 million have chronic infection (1). The prevalence of chronic hepatitis B (CHB) infection varies widely throughout the world, ranging from as high as 25% in endemic countries to less than 0.5% in populations of the western world (2–4). A large reservoir of infected individuals harbor the virus without clinical evidence of liver disease (non-replicative or low-replicative stage). In such individuals, immunosuppressive agents can precipitate an increase in HBV replication followed by a flare of hepatitis B that can be severe and even fatal.

**Reactivation of hepatitis B:** This is defined as the reappearance of active necroinflammatory disease of the liver in a person known to have the inactive HBsAg carrier state or resolved HBV (5,6). Reactivation results in acute flares of HBV, with consequent clinical decompensation.

Spontaneous reactivation occurs more commonly in the setting of HIV infection, concurrent bacterial infection, surgery, or physical and emotional stress.

Many patients with acute flares of HBV are asymptomatic, but some patients experience symptoms including nausea, vomiting, fatigue, and anorexia (7). Hepatic failure can be seen rarely, particularly in patients with underlying cirrhosis (8–10).

Flares have been described in both the replicative phase and the non-replicative phase. Frequently these flares of hepatitis precede clearance of the virus and HBeAg to anti-HBe seroconversion (5, 11). In anti-HBe-positive patients who have no detectable HBV DNA, an elevation of the serum aminotransferases occurs in response to the sudden reemergence of viral replication (5, 12–14). In patients who are in the active replicative stage of the infection, serum HBV DNA levels increase, and biochemical deterioration occurs without subsequent loss of HBeAg (15). These episodes can be viewed as an abortive attempt at seroconversion (12).

2. HISTORY OF HEPATITIS B REACTIVATION

The first reported cases of HBV reactivation in patients receiving chemotherapy were published in 1975. Wands et al. described a decrease in anti-hepatitis B surface antigen (anti-HBs) titers in patients receiving antitumor chemotherapy for myeloproliferative and lymphoproliferative disorders (16). In some patients this decrease was followed by reappearance of hepatitis B surface antigen (HBsAg). Patients who
were positive for HBsAg prior to the initiation of chemotherapy experienced a rise in HBsAg titers associated with an increase in serum aminotransferase levels. Galbraith et al. reported three cases of fulminant hepatic failure in patients with leukemia and choriocarcinoma related to withdrawal of cytotoxic drug therapy (17). In 1977, reports of HBV reactivation in patient undergoing renal transplant were published (18). Many instances of HBV reactivation associated with chemotherapy have been published since. Most of them involved patients with lymphoma and other hematological malignancies from Asia where the incidence of HBV is highest (16, 19). HBV reactivation has also been well recognized with chemotherapy for solid tumors including lung cancer, ovarian cancer, cervical cancer, breast cancer, gastrointestinal cancers, hepatocellular carcinoma, embryonal carcinoma of the testes, and neuroendocrine tumors (19–28). In addition to chemotherapy, hepatitis B reactivation can also be seen with corticosteroid therapy (29) and with antitumor necrosis factor (anti-TNF) therapy for inflammatory bowel diseases or rheumatologic disorders (30–32).

3. INCIDENCE/PREVALENCE OF HEPATITIS B REACTIVATION

The frequency of HBV reactivation in patients receiving chemotherapy varies widely among different reports, ranging from 14% to 67% in HBsAg carriers (20, 21, 24, 33–37), depending on the criteria used to define reactivation. Early reports defined HBV reactivation and flare using clinical features and increasing aminotransferase levels in the absence of other causes, or the reappearance or increase in the level of serum HBsAg in patients with previously undetectable or low levels. More recent publications have defined HBV reactivation as a one log or greater increase in the HBV DNA level compared with the prereactivation level, or the reappearance of a previously negative HBsAg on two consecutive tests (38–40).

The wide range of incidence rates is also due to the differences in the types of malignancy being treated, chemotherapy regimens used, patient demographics, and the phase of chronic hepatitis B infection (i.e., active or inactive replication). Regardless of the exact risk of hepatitis B reactivation, it is clear that the administration of chemotherapy to cancer patients with chronic hepatitis B infection leads to an increased risk of liver-related morbidity and mortality (41). It is well established that anyone with a history of exposure to HBV is at risk of reactivation during immunosuppression, even after clearance of HBsAg. The presence of ostensibly protective anti-HBsAb does not
eliminate the risk of hepatitis B reactivation with chemotherapy (12, 16, 36).

In a recent study using updated diagnostic criteria, 100 Chinese patients who underwent chemotherapy for lymphoma were studied. A high proportion (67% or 18 of 27) of those who were HBsAg-positive had a hepatitis flare. Of these 18, 13 (72%) were confirmed to have positive HBV DNA. Of the patients who were HBsAg-negative, and HBsAb-negative, 7 of 33 patients (21%) had a hepatitis flare, although only one had confirmed hepatitis B reactivation by HBV DNA testing (36). In a series from Greece, out of 50 HBsAg-positive patients receiving chemotherapy, 7 (14%) developed clinical and/or biochemical hepatitis (21). In hematopoietic stem cell transplants, hepatitis B reactivation has been reported in more than 50% of the patients (10, 41, 42). This is probably due to the intense immunosuppression produced by the chemotherapy regimens and due to the coexistence of graft vs host disease (41).

4. RISK FACTORS FOR REACTIVATION

Patients at risk for HBV reactivation are those with hematological or oncological malignancies who require chemotherapy, patients with non-malignant diseases who require immunosuppression, and patients with rheumatologic diseases or inflammatory bowel diseases treated with biologic response modifiers. The various types of malignancies associated with hepatitis B reactivation are given in Table 1. Other risk factors are given in Table 2.

Studies examining the significance of HBV genotypes suggest that they may correlate with clinical outcomes (43). Most of these studies have been performed in Asia, where genotypes B and C predominate. Genotype C is associated with more severe liver disease and a higher rate of progression to cirrhosis compared to genotype B (43–47). Data on the clinical course of patients infected with genotypes other than B and C are scarce. A study of 22 patients who underwent liver transplantation for hepatitis B suggested that patients with genotype A have the lowest risk for HBV recurrence despite having the highest rate of pretransplantation HBV viral replication. Patients with genotype D appeared to have the highest risk for HBV recurrence and mortality (48). This has not been as well studied in the setting of chemotherapy. It has been suggested that HBV reactivation seems to correlate with HBV genotypes B and C, but there are insufficient data to consider these genotypes as separate risk factors for HBV reactivation.
Table 1
Malignancies Reported in Association with Hepatitis B Reactivation

<table>
<thead>
<tr>
<th>Cancer Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myeloid leukemia</td>
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<tr>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
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<tr>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
</tr>
<tr>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Waldenstrom macroglobulinemia</td>
</tr>
<tr>
<td>Plasmacytoma</td>
</tr>
<tr>
<td>Aplastic anemia</td>
</tr>
<tr>
<td>Myelodysplastic syndromes</td>
</tr>
<tr>
<td>Hematopoietic stem cell transplantation</td>
</tr>
<tr>
<td>Breast cancer</td>
</tr>
<tr>
<td>Bladder cancer</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Small cell lung cancer</td>
</tr>
<tr>
<td>Neuroendocrine tumor</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>Cervical cancer</td>
</tr>
<tr>
<td>Embryonal carcinoma of the testes</td>
</tr>
<tr>
<td>Yolk sac tumor of mediastinum</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
</tr>
<tr>
<td>Glioblastoma</td>
</tr>
<tr>
<td>Ovarian carcinosarcoma</td>
</tr>
</tbody>
</table>

4.1. A. Host Risk Factors for HBV Reactivation

Multiple host risk factors for hepatitis B reactivation have been suggested, several of which have been confirmed in clinical trials. In a study of 138 chronic HBV carriers treated with cancer chemotherapy, a multivariate analysis identified factors associated with a higher risk of developing HBV reactivation. These factors were detectable HBV DNA levels pre-chemotherapy, the use of steroids, a diagnosis of lymphoma and breast cancer. Another suggested risk factor was the use of anthracycline agents (daunorubicin, doxorubicin, epirubicin,
idarubicin). However, pretreatment ALT, bilirubin, and albumin levels were not associated with the development of HBV reactivation (49). In contrast, in a study of patients with hepatocellular carcinoma, pretreatment ALT elevation was shown to be a risk factor for HBV reactivation (22). Male sex, younger age, HBeAg positivity, and the presence of lymphoma were significant risk factors associated with HBV reactivation in other studies of HBsAg-positive cancer patients undergoing chemotherapy (20, 36, 50, 51).

Although HBsAb positivity decreases the risk of HBV reactivation (36, 39), it does not confer absolute protection against HBV reactivation. In fact, several reports described a “reversion” with HBsAb going from positive to negative and HBsAg becoming positive. This phenomenon has been recognized since the earliest reports describing HBV reactivation (16).

Following allogenic hematopoietic stem cell transplantation, HBV reactivation has been reported in HBsAg-positive as well as HBsAg-negative patients. The rate of reactivation varies between 14% and 50% (52, 53). The risk appears to be greatest in those treated for graft-vs-host disease and is possibly reduced in recipients from HBsAb-positive donors. HBV reactivation has also been described in patients

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### Table 2

**Host Risk Factors for Hepatitis B Reactivation**

<table>
<thead>
<tr>
<th>Proven</th>
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<tbody>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>Younger age</td>
</tr>
<tr>
<td>HBsAg positivity</td>
</tr>
<tr>
<td>HBeAg positivity</td>
</tr>
<tr>
<td>Detectable HBV DNA</td>
</tr>
<tr>
<td>Use of steroids</td>
</tr>
<tr>
<td>Use of anthracyclines</td>
</tr>
<tr>
<td>Diagnosis of lymphoma</td>
</tr>
<tr>
<td>Diagnosis of breast cancer</td>
</tr>
<tr>
<td>Intensity of immunosuppression</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggested but not proven</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of chemotherapy</td>
</tr>
<tr>
<td>Pretreatment ALT level</td>
</tr>
<tr>
<td>Genotypes B and C</td>
</tr>
</tbody>
</table>
with autologous hematopoietic stem cell transplant, although it may be less likely than in allogenic stem cell transplantation (39). A study of 137 autologous hematopoietic cell transplantation (HCT) patients found that pre-transplant HBV DNA level >10^5 copies/ml was the principal risk factor for HBV reactivation on multivariate regression analysis (39). High HBV DNA levels have been consistently found to be a risk factor for HBV reactivation in breast cancer and hematological malignancies as well (52, 53).

### 4.2. Role of Chemotherapeutic Agents/Steroids/Immunosuppressants

Numerous chemotherapeutic agents have been reported to be associated with HBV reactivation (Table 3), two classes deserve special attention: corticosteroids and anthracyclines. The use of anthracyclines (daunorubicin, doxorubicin, epirubicin, and idarubicin) has been shown in vitro to stimulate HBV DNA secretion (54). The relationship between corticosteroids and HBV replication was observed as early as 1980. Long-term treatment with corticosteroids was noted to cause an increased expression of HBeAg in the serum (55). It was subsequently discovered that HBV DNA polymerase activity increased in association with a decrease in serum AST during a short course of corticosteroid therapy (56), and the opposite effect, a significant increase in AST in association with a decrease in HBV DNA polymerase, was observed after withdrawal of corticosteroid therapy (57). The hepatitis B virus was found to contain a glucocorticoid-responsive element that stimulates viral replication and transcriptional activity (58, 59). One potential means of minimizing the risk of HBV reactivation is the avoidance of corticosteroid therapy as part of chemotherapeutic/antiemetic regimens in HBsAg carriers (60–62). However, a steroid-free chemotherapy strategy may lead to suboptimal therapeutic response and may even jeopardize the patient’s chance of cure. For example, in a prospective study of 50 patients with non-Hodgkin’s lymphoma who were randomized to receive either the standard steroid-containing regimen or a steroid-free regimen, patients receiving a steroid-free regimen had a significantly lower rate of HBV reactivation (73% vs 38%; P = 0.03). However, patients in the steroid-free arm had a significantly lower rate of complete remission and shorter overall survival (68% vs 36%; P = 0.18), presumably due to suboptimal therapy (63).

The degree of immunosuppression could also contribute to the development of reactivation. The intensity of the immune suppression induced by chemotherapy has been suggested to be a risk factor
for reactivation. One study found that the incidence of HBV reactivation was increased in patients receiving second- or third-line chemotherapy (65). On the other hand, patients with gastrointestinal malignancies who undergo cytotoxic chemotherapy, mainly consisting of less-immunosuppressive agents such as 5-fluorouracil for colon cancer, have a lower risk of developing viral reactivation (66). The duration of the chemotherapy has not been proven to be an independent risk factor for HBV reactivation. Hematopoietic stem cell transplantation has been particularly associated with viral reactivation (67–72).

The use of therapeutic monoclonal antibodies against B and T lymphocytes such as rituximab (a chimeric mouse human monoclonal antibody against anti-CD20+ lymphoid cells) and alemtuzumab (a humanized monoclonal antibody against anti-CD52+ lymphoid cells), used alone or in combination with cytotoxic therapy, has been associated with HBV reactivation (73–82). In October 2004, the U.S. Food and Drug Administration reported a possible relationship between fulminant hepatitis and rituximab use (83).

There have been several reports of severe HBV reactivation, including fatalities, in patients who have received infliximab for Crohn’s disease and rheumatic disorders (30–32, 84). No cases of HBV reactivation associated with the use of adalimumab have been reported to date, although the adalimumab package insert carries a warning about the use of the medication in patients with history of hepatitis B infection, implying that patients should be screened for hepatitis B (85). Anti-TNF agents cause profound and long-lasting immunosuppression, which may account for the risk of HBV reactivation following their use.

4.3. Occult HBV

Patients who are negative for the hepatitis B surface antigen but have evidence of either circulating or intrahepatic HBV DNA are denoted as having occult HBV. It is well established that chemotherapy can cause HBV reactivation even in HBsAg-negative patients with occult HBV infection (36, 64).

HBV reactivation in patients who have cleared HBsAg is thought to be due to the persistence of hepatitis B as an occult infection in the hepatocytes as cccDNA (covalently closed circular DNA) (86). HBV DNA has also been found in peripheral blood mononuclear cells recovered from immune-suppressed transplanted patients with a serologic profile anti-HBc-positive/HBsAg-negative/anti-HBs-negative in the absence of HBV DNA in the serum (87).
5. PATHOPHYSIOLOGY OF REACTIVATION

Most episodes of chemotherapy-induced hepatitis B reactivation are seen after chemotherapy is withdrawn and are caused by a change in the balance between the immunologic response to the hepatitis B virus and the extent of viral proliferation.

The persistent necroinflammatory changes in the liver tissue that characterize chronic hepatitis B are caused by a suboptimal or an inadequate cellular immune response to nucleocapsid antigens (88). The greater the deficiency in the cellular immune response to the encoded viral antigens, the more likely the host will be immunotolerant to the virus. The more robust the immunologic response to these antigens is, the greater is the likelihood of substantial inflammatory changes and damage to hepatocytes (12). With the administration of cytotoxic or immunosuppressive agents, the immune response is suppressed and as a result, viral replication is enhanced and widespread infection of hepatocytes ensues (27, 36). With the discontinuation of the cytotoxic or immunosuppressive therapy, the immune function is restored and this immune reconstitution results in a rapid destruction of hepatocytes infected with hepatitis B (89–91). It is believed that the more potent the immunosuppression, the greater the level of viral replication and the greater the liver damage on withdrawal of the immunosuppressive agent (12).

Hepatitis B reactivation that occurs during ongoing chemotherapy or immunosuppression suggests a direct cytopathic effect of the drugs on the hepatitis B virus (92). It has been shown that the glucocorticoid receptor recognizes a specific nucleotide sequence on the hepatitis B virus DNA and induces replication and transcription of the virus (93). Hepatitis B reactivation can also occur in patients with a history of a resolved hepatitis B infection (with positive HBsAb). Persistence of cccDNA (covalently closed circular DNA) is thought to act as a template for transcription of viral RNAs or mRNAs serving either as viral pregenome RNAs or as mRNAs coding for the multifunctional polymerase, core, X, and envelope (S) proteins (94, 95). It has been shown that cccDNA is the major reason for hepatitis B reactivation after stopping anti-HBV therapy (94, 96, 97).

6. CLINICAL MANIFESTATIONS OF HEPATITIS B REACTIVATION

Hepatitis B reactivation can be associated with a wide range of clinical manifestations that vary from mild subclinical elevation of liver enzymes or serological evidence of reactivation to fulminant hepatic
failure and death. When symptomatic, HBV reactivation manifests by the classic symptoms of hepatitis including fatigue, jaundice, ascites, hepatic encephalopathy, and coagulopathy.

As mentioned, hepatitis B virus reactivation typically occurs after cessation of chemotherapy, but it may occur during chemotherapy. When it occurs during treatment, it may be seen as early as 4 weeks to as late as 36 weeks after the initiation of chemotherapy (41, 98). Lethal hepatitis B reactivation has been reported 1 year after the completion of rituximab therapy for a low-grade cutaneous B-cell lymphoma (99). Seroreversion (from anti-HBs to HBsAg) was described 39 months after allogenic hematopoietic stem cell transplantation (100). The first sign of hepatitis B reactivation is the detection of a rising HBV DNA level. The rise in viremia is followed by an elevation in the ALT level, which usually lags behind the increasing viral load by several weeks (0–11 weeks) (98). In fact HBV DNA levels may be declining or even become undetectable by the time the ALT elevation is detected (19, 101). This pattern is explained by the pathophysiology of hepatitis B reactivation. As hepatitis B virus replication is enhanced by immunosuppression, there is an immunologically mediated attack on the hepatocytes infected with HBV, with rapid destruction of hepatocytes resulting in a decrease in the HBV viral load and an elevation in the ALT levels. This serologic and biochemical pattern may complicate the diagnosis of hepatitis B reactivation. If a patient becomes symptomatic or the ALT elevation is noticed after the HBV DNA becomes undetectable, the clinician might be left with a suspicion of HBV reactivation, with no way to confirm the diagnosis (19). Anti-HBc IgM can be detected during a hepatitis B flare, which may lead to a misdiagnosis of acute hepatitis B in patients with undiagnosed chronic hepatitis B or previous exposure to hepatitis B. A liver biopsy is not necessary for the diagnosis of hepatitis B flare; however, in the absence of a documented increase in HBV DNA levels, it may be very helpful in excluding other etiologies of liver injury. As in any patient with chronic hepatitis B, an acute flare may be induced either by HBV mutations such as the precore or DNA polymerase mutation or by a superinfection with another hepatotropic virus such as hepatitis A, hepatitis C, hepatitis D, hepatitis E, EBV, HSV, or CMV. Interaction with HIV or hepatotoxicity of anti-HIV medications may also play a role (102). HBV reactivation can be associated with an increase in α-fetoprotein which raises the concern about the development of hepatocellular carcinoma, but is often secondary to inflammation (36, 103, 104).

Patients with underlying cirrhosis may rapidly decompensate and develop liver failure. Their mortality on chemotherapy ranges between 4% and 41% (36, 41, 65, 105, 106). These patients are at particularly
increased risk of sepsis in the setting of cirrhosis and immunosuppression. Patients undergoing chemotherapy for hepatocellular carcinoma were found to have a poor outcome with frequent cases of severe hepatitis and a mortality of 30% in the setting of reactivation (10, 22–24, 33–35, 41, 42 107–113). This high mortality may be explained by the presence of coexisting cirrhosis (19). It is very important that cirrhosis be recognized before starting chemotherapy as these patients require closer monitoring.

In a series of 100 lymphoma patients from Hong Kong undergoing chemotherapy (36), among the patients who developed hepatitis, it was transient (lasting 1 week to 3 months) in 50% of HBsAg-positive patients and 80% of HBsAg-negative patients. Hepatitis was recurrent (2–4 episodes) in 38% of HBsAg-positive patients during an observation period of 4–12 months. Recurrent hepatitis was not observed in the HBsAg-negative patients. In one patient in each group persistent hepatitis developed (lasting more than 6 months). Fifty percent of the hepatitis episodes were anicteric. Icteric hepatitis was more often seen in HBsAg-positive patients. Hepatic failure with ascites and coagulopathy developed in 7% of HBsAg-positive and 4% of HBsAg-negative patients. Mortality from hepatic failure was 4% for HBsAg-positive and 1% for HBsAg-negative patients (36).

When a hepatitis B flare occurs during chemotherapy, the chemotherapy often has to be interrupted due to the hepatitis. This may jeopardize the patient’s prognosis. In a study of breast cancer patients, over 70% of patients who developed hepatitis B reactivation required either premature termination of chemotherapy or disruption in the treatment schedules, compared to 30% in those without hepatitis B reactivation (23).

7. SCREENING OF PATIENTS PRIOR TO CHEMOTHERAPY OR IMMUNOSUPPRESSION

It seems obvious that all patients should be screened for hepatitis B infection prior to starting chemotherapy or immunosuppression, but the reality is sobering. Fewer than 40% of oncologists were screening their patients in a survey conducted in the Washington, DC area in early 2007 (114). The initial serological profile should include HBsAg, HBsAb, and HBCAb. Seronegative patients should be vaccinated against HBV. HBsAg-positive patients should be considered at high risk of reactivation and should be started on preemptive anti-HBV therapy. These patients should also be tested for HBeAg, HBeAb, and HBV DNA to distinguish between those who are chronically infected and those who are chronic carriers. Those who have chronic infection will need long-term active treatment and follow-up, whereas “carriers” may have their
prophylactic treatment stopped 6–12 months after chemotherapy is discontinued. Following the HBV DNA levels should allow for the diagnosis of a potential HBV reactivation prior to the development of hepatitis.

HBsAg-negative patients who are HBeAb positive are also at risk of HBV reactivation, whether they are HBsAb positive or negative. Although reactivation is much less frequent than in HBsAg-positive patients, fatal cases of hepatic failure have been reported in these patients (115). While there is not enough information to recommend routine prophylaxis for these patients (116) and while adopting a routine prophylaxis strategy might not be cost-effective, the decision to start prophylactic antiviral therapy should be left to the discretion of the clinician and should be made on a case-by-case basis.

8. IMMUNIZATION AGAINST HEPATITIS B

It is recommended that all patients be screened for HBV before starting chemotherapy or immunosuppression. HBV vaccine should be administered when appropriate. Frequently, in cancer patients, the urgent administration of chemotherapy does not allow the completion of the usual three-dose regimen over 6 months. In these cases, an effort should be made to administer three doses of the vaccine within a 3–4-week interval. An additional dose can be delayed and given a few months after the completion of the chemotherapy. Rapid seroconversion has been demonstrated using this accelerated vaccination schedule (0–7–21–360 days) (117–120).

Decreased response to the HBV vaccine is frequent in cancer patients, whether due to disease-induced or treatment-induced immunosuppression. Modified dosing regimens, including doubling the standard antigen dose or administering additional doses, might increase response rates (121–123).

9. MANAGEMENT OF ESTABLISHED HBV REACTIVATION

Until recently, aggressive supportive therapy and discontinuation of cytotoxic chemotherapy had been the mainstay of treatment for hepatitis B reactivation while on chemotherapy. Interferon has been shown to control hepatitis B during chemotherapy (65). However, its use may be limited by side effects such as thrombocytopenia and leucopenia and by the possibility of fatal hepatitis flare via augmentation of the host’s immune response to HBV, resulting in increased destruction of hepatocytes. Interferon is also contraindicated in cirrhosis and decompensated liver disease. Lamivudine, the first in the class of oral nucleoside
Table 3
Chemotherapeutic Agents Associated with Hepatitis B Reactivation

<table>
<thead>
<tr>
<th>Class</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylating agents</td>
<td>Cyclophosphamide; temozolomide; ifosfamide; chlorambucil; carboplatin</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>Cytarabine; flouaracil; gemcitabine; mercaptopurine; methotrexate; thioguanine</td>
</tr>
<tr>
<td>Antitumor antibiotics</td>
<td>Daunorubicin; doxorubicin; epirubicin, idarubicin; Bleomycin; mitomycin C, actinomycin D</td>
</tr>
<tr>
<td>Anthracyclines Others</td>
<td>Vincristine; vinblastine</td>
</tr>
<tr>
<td>Plant alkaloids</td>
<td>Prednisolone; dexamethasone; methylprednisolone</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Asparaginase; procarbazine; docetaxel; etoposide; fludarabine; imatinib mesylate</td>
</tr>
</tbody>
</table>

analougues with anti-HBV activity, was initially utilized in 1998 in two studies that documented resolution of reactivation in one case of fulminant hepatitis following chemotherapy for lymphoma and in a case of acute icteric hepatitis in an allogenic bone marrow transplant patient receiving immunosuppression for graft-vs-host disease (124, 125). The effectiveness of lamivudine in the treatment of chemotherapy-induced HBV reactivation has been shown in several additional reports (25, 124–126); and lamivudine became the main agent for the treatment of HBV reactivation. It was suggested, however, that some of the reported cases of response to lamivudine in the setting of HBV reactivation might have been a coincidence, as the spontaneous resolution of viral reactivation was a common outcome, even before the widespread availability of lamivudine (19). And despite lamivudine therapy, the mortality associated with HBV reactivation has been reported in up to 20% of HBsAg-positive patients which was not different from untreated patients (127). Mortality rates as high as 40% have been reported when therapy was initiated after severe hepatic injury had already occurred (19, 20, 105, 128). Such studies emphasize the importance of starting lamivudine therapy as soon as a flare is diagnosed, preferably when a rise in the HBV DNA is detected and before irreversible hepatic damage occurs. The detection of HBV reactivation at a very early stage requires an intense monitoring regimen with frequent testing of HBV viral loads and liver enzymes. Such a program may be difficult to implement and may not be cost-effective. Along with prompt antiviral therapy,
aggressive supportive therapy should be instituted in addition to the cessation of chemotherapy and the withdrawal of any potentially hepatotoxic agents. Once the reactivation is under control with normal aminotransferases, chemotherapy can be restarted, if it had been held.

10. PREEMPTIVE ANTIVIRAL THERAPY AGAINST HBV REACTIVATION

Since viral replication manifested by a rise in HBV viral load precedes ALT elevation and clinical hepatitis, it has been shown that early treatment with antiviral therapy prior to hepatic injury is superior to deferred intervention (127). The use of lamivudine or other oral anti-HBV agents prophylactically prior to the administration of chemotherapy has been recommended (129). Indeed, with the low side effect profile, tolerability, and once-a-day dosing of lamivudine and other nucleoside analogues, therapy for chronic HBsAg carriers receiving immunosuppression or chemotherapy has shifted from treatment after the diagnosis of reactivation toward prophylaxis with the initiation of chemotherapy.

The first case series of primary prophylaxis utilized lamivudine and was published in 2001 (130). It included 20 patients with hematological malignancies treated with different chemotherapy regimens, the majority of which included steroids. With a rate of HBV reactivation of 5%, the role of lamivudine for prophylaxis among HBsAg carriers appeared to be indicated. Since then the efficacy of preemptive lamivudine therapy has been demonstrated in several additional retrospective series (37, 90, 91, 131–135).

More recent studies have been conducted prospectively (Table 4) (91, 130, 136–139). A systematic review of nine prospective trials and one randomized-controlled trial showed a four- to sevenfold decrease in the rate of hepatitis and hepatitis B virus reactivation in patients who received lamivudine prophylaxis (140). Of patients receiving prophylaxis, 0–24% developed hepatitis B virus reactivation, compared with 29–56% of controls. Three reactivation-related mortalities were reported (one receiving prophylaxis, two controls).

A meta-analysis of 11 studies included 220 patients who received lamivudine prophylaxis and 400 patients who did not receive prophylaxis (141). Patients given lamivudine prophylaxis had an 87% decrease in HBV reactivation compared to patients not given prophylaxis. The number needed to treat to prevent one reactivation was 3. The lamivudine prophylaxis group was also associated with a 70% reduction in reactivation-related mortality compared with controls. There was a
<table>
<thead>
<tr>
<th>Author</th>
<th>Diagnosis</th>
<th>No. of Patients in Lamivudine Group</th>
<th>Duration of Lamivudine Therapy</th>
<th>No. of Controls (No Prophylactic Lamivudine)</th>
<th>Number of HBV Reactivation Cases (Lamivudine vs Control)</th>
<th>Number of HBV-Related Deaths (Lamivudine vs Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Randomized-controlled trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lau et al. (2003)</td>
<td>Lymphoma</td>
<td>15</td>
<td>Until 6 wk after CT and when WBC &gt; 4.0 (\times 10^9/L)</td>
<td>15</td>
<td>0 vs 8</td>
<td>0 vs 0</td>
</tr>
<tr>
<td>Jang et al. (2006)</td>
<td>HCC</td>
<td>36</td>
<td>Until 12 mo after CT</td>
<td>37</td>
<td>1 vs 15</td>
<td>0 vs 1</td>
</tr>
<tr>
<td><strong>Prospective trials with historical controls</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dai et al. (2004)</td>
<td>Breast cancer</td>
<td>11</td>
<td>Until 4 wk after CT</td>
<td>9</td>
<td>0 vs 5</td>
<td>0 vs 1</td>
</tr>
<tr>
<td>Idilman et al. (2004)</td>
<td>Hematological malignancies</td>
<td>8</td>
<td>Until 12 mo after CT</td>
<td>10</td>
<td>0 vs 5</td>
<td>0 vs 0</td>
</tr>
<tr>
<td>Yeo et al. (2004)</td>
<td>Various malignancies</td>
<td>65</td>
<td>Until 8 wk after CT</td>
<td>193</td>
<td>5 vs 23</td>
<td>0 vs 5</td>
</tr>
<tr>
<td>Yeo et al. (2004)</td>
<td>Breast cancer</td>
<td>31</td>
<td>Until 8 wk after CT</td>
<td>61</td>
<td>2 vs 19</td>
<td>0 vs 0</td>
</tr>
<tr>
<td>Yeo et al. (2005)</td>
<td>Nasopharyngeal cancer</td>
<td>16</td>
<td>Until 8 wk after CT</td>
<td>21</td>
<td>0 vs 6</td>
<td>0 vs 1</td>
</tr>
<tr>
<td>Li et al. (2006)</td>
<td>Lymphoma</td>
<td>40</td>
<td>Until 8–64 wk after CT</td>
<td>116</td>
<td>7 vs 60</td>
<td>0 vs 6</td>
</tr>
<tr>
<td><strong>Prospective trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rossi et al. (2001)</td>
<td>Hematological malignancies</td>
<td>20</td>
<td>Until 4 wk after CT</td>
<td>NA</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dai et al. (2004b)</td>
<td>Breast cancer</td>
<td>6</td>
<td>Until 4 wk after CT</td>
<td>NA</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>El-Sayed et al. (2004)</td>
<td>Hematological malignancies</td>
<td>10</td>
<td>Until last day of CT</td>
<td>NA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vassiliadis et al. (2005)</td>
<td>Hematological malignancies</td>
<td>10</td>
<td>Continued throughout follow-up</td>
<td>NA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hui et al. (2005)</td>
<td>Hematological malignancies</td>
<td>46</td>
<td>Until 3 mo after CT and when WBC &gt; 4.0 (\times 10^9/L)</td>
<td>NA</td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>

CT, chemotherapy; HCC, hepatocellular carcinoma; mo, months; wk, weeks; WBC, white blood cell count. Trials with newer nucleotide/nucleoside agents are currently lacking.
reduction in treatment delays and premature termination of chemotherapy in the lamivudine prophylaxis arm.

All prospective adult trials have used equivalent daily doses of lamivudine, i.e., 100 mg daily (33, 53, 98, 130, 136, 142). Three studies using higher doses of lamivudine have shown similar and not superior efficacy in preventing HBV reactivation (132, 143, 144). Few data exist to recommend dosing lamivudine on a regimen other than 100 mg/day. The use of the newer nucleoside/nucleotide analogues in this setting has not been well studied to date; although with more potent agents such as entecavir and tenofovir, the rate of reactivation may be lower.

The optimal timing for beginning preemptive anti-viral therapy has not been established.

In many instances, lamivudine was started 1 week prior to chemotherapy. Idilman et al. and Jang et al. started their patients on lamivudine at the start of chemotherapy. We recommend starting preemptive therapy at least 1 week prior to chemotherapy and definitely not later than the first day of chemotherapy.

The duration of nucleoside analogue therapy after completion of chemotherapy has varied widely among studies with HBV reactivation rates increasing in incidence following withdrawal of chemotherapy and prophylaxis. The HBV reactivation rates tended to be higher in the prospective trials with longer follow-up, and the only HBV reactivation-related mortality among the prospective trials occurred following discontinuation of lamivudine (53). Idilman et al. continued lamivudine for 1 year following chemotherapy and reported no HBV reactivation or reactivation-related mortality (91). Vassiliadis et al. continued lamivudine prophylaxis indefinitely and observed no events of HBV reactivation or reactivation-related mortality (142). Given that HBV reactivation has been reported up to 1 year after completion of chemotherapy (99), and lamivudine therapy is well tolerated, an argument can be made to continue lamivudine or one of the newer nucleotide or nucleoside agents indefinitely. This strategy might be reasonable and cost-effective in high-risk patients, such as those diagnosed with high HBV viral loads or liver transplant patients undergoing liver transplant for HBV. Continuing therapy 6–12 months after completion of chemotherapy might be more appropriate in lower risk individuals.

A major clinical concern regarding prophylactic therapy with lamivudine is the development of lamivudine-resistant mutant strains of HBV (145–147). In the case of chronic HBV infection, prolonged therapy with lamivudine has been associated with an increased likelihood of treatment-resistant HBV variants with YMDD mutations (148, 149), from 24% at 1 year to nearly 70% at 4 years (150, 151). The incidence of lamivudine-resistant YMDD mutations among chronic HBsAg
Fig. 1. Algorithm for the prevention of hepatitis B reactivation associated with chemotherapy.

carriers receiving lamivudine as prophylaxis rather than treatment has not been well studied. Low rates of YMDD mutations have been reported (152), but this is likely due to the short duration of therapy. Among case series of HBsAg carriers following liver transplantation or receiving stem cell transplantation, emergence of YMDD mutants was significantly increased by lamivudine prophylaxis, observed in 21% of patients at 1 year and 34% at 2 years (153, 154). In case of treatment emergent YMDD mutation, the addition of newer antivirals such as adefovir, entecavir, or tenofovir can be used, all of which are associated with lower rates of resistance (155). Figure 1 presents a possible algorithm to patients who are at risk for HBV reactivation.

11. COST-EFFECTIVENESS OF PREEMPTIVE THERAPY

It is clear that a preemptive strategy is effective in preventing HBV reactivation related to chemotherapy and immunosuppression. However, recommending the widespread implementation of such a strategy carries with it a certain economic burden that must be balanced against the risks and costs of reactivation. Saab et al. used a decision analysis model to simulate the clinical outcomes, effectiveness, and costs associated with lamivudine prophylaxis for hepatitis B reactivation in patients receiving chemotherapy (156). They applied the model to a cohort of 1000 patients, with 500 patients in each treatment strategy (prophylaxis vs no prophylaxis). Lamivudine prophylaxis increased the cost
approximately $1,500 from $17,177 to $18,707 per patient. However, prophylaxis was effective in reducing the number of HBV reactivations (48 vs 219), liver-associated deaths (0 vs 20), and cancer-associated deaths (39 vs 47). Prophylaxis was also associated with prolonging life years from 0.876 in the no-prophylaxis group to 0.922 in the prophylaxis group. The incremental cost-effectiveness ratio (ICER) was $33,514 per life year, which was below the generally accepted price ceiling of $50,000 per life year saved. This study provided strong pharmacoeconomic support to the use of lamivudine prophylaxis in patients undergoing chemotherapy. Newer nucleoside analogues are somewhat more expensive but have fewer treatment failure rates and lower resistance, which would likely offset their higher costs.

12. AWARENESS OF HBV REACTIVATION AMONG ONCOLOGISTS

The risk of HBV reactivation with chemotherapy is being increasingly recognized. However, many oncologists have not seen this complication or are not aware of the current recommendations for HBV prophylaxis. Farhadi et al. administered a questionnaire to 131 Hematology–Oncology physicians in the Washington, DC area, asking them about HBV reactivation (114). While most oncologists (78%) were aware of the potential risk of HBV reactivation, only 30% have ever seen a reactivation and most (62%) were not screening patients for HBV prior to chemotherapy. Fewer than half of the responders were aware that prophylactic antiviral agents are available, and even fewer were aware that prophylaxis has been shown to reduce HBV flares, and most were not sure what agent to select. A majority would, however, seek the recommendations of a gastroenterologist or a hepatologist. A similar survey from Los Angeles found nearly identical results (157). These surveys demonstrate that there is still a need to improve awareness of HBV reactivation and antiviral prophylaxis in the Hematology–Oncology community.

13. ADDENDUM

In September 2008, the U.S. Centers for Disease Control and Prevention (CDC) released new guidelines for health-care providers to increase routine testing for chronic hepatitis B. The CDC now recommends serologic testing for all markers of HBV infection (HBsAg, anti-HBc, and anti-HBs) in persons needing cytotoxic or immunosuppressive therapy, including chemotherapy, immunosuppression related to organ transplantation, and immunosuppression for rheumatologic
or gastroenterologic disorders. Chronically infected (HBsAg positive) patients are at elevated risk of fulminant hepatitis once suppressive therapy is initiated and should be referred for medical management and anti-viral treatment. Those with resolved infection (anti-HBc positive, HBsAg-negative) are at risk for reactivation and should be monitored closely with blood tests for liver enzymes (158).

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Support of Patients During Antiviral Therapy for Hepatitis B and C

Alyson N. Fox and Thomas W. Faust

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Key Principles

• The mainstay of treatment for the viral hepatitides are immunomodulators (interferons) and antiviral agents.
• While many of these therapies are effective at suppressing and eliminating the virus, their use can be inhibited by the side effects that they cause. Treatment for chronic hepatitis B and C can therefore be complex and requires a strong therapeutic alliance between the patient and the health-care provider.
• The standard of care for the treatment of hepatitis C is centered around combination therapy with pegylated interferon-α and ribavirin. Prior to initiating treatment, it is crucial for the provider to screen the patient for contraindications to therapy and to outline potential medication-related side effects for the patient.
• Hematologic side effects are common with interferon-based therapy and may require the use of growth factors. Other side effects

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experienced with hepatitis C therapy include psychiatric, ophthalmologic, endocrine, and dermatologic events.

- There are now several approved oral agents used to treat chronic hepatitis B (CHB). Unlike the treatment for hepatitis C, the treatment for hepatitis B can be indefinite, so recognition of a management strategy for side effects is paramount. Fortunately, most of these therapies appear safe and well tolerated.
- The therapies for chronic hepatitis B and C have dramatically evolved over the last decade. Principal in treating patients for chronic hepatitis is choosing a regimen that is suitable for an individual patient, disclosing the expected and potential side effects, and early identification and management of adverse effects.

1. INTRODUCTION

Worldwide, over 300 million people are affected by chronic viral hepatitis. On a yearly basis, millions of those affected die from progression of end-stage liver disease and hepatocellular carcinoma. In the last decade, our understanding of and treatments for chronic hepatitis B and C have dramatically improved. While the goals of hepatitis care have remained unchanged – to halt the progression of disease and prevent long-term complications – our ability to accomplish those goals has improved.

The mainstay of treatment for the viral hepatitides are immunomodulators (interferons) and antiviral agents. While many of these therapies are effective at suppressing and eliminating the virus, their use can be inhibited by the side effects that they cause. Treatment for chronic hepatitis B and C can therefore be complex and requires a strong therapeutic alliance between the patient and the health-care provider. The goal of this chapter is to discuss the current standard of care therapies for hepatitis B and C, treatment considerations, side effects, and their management.

2. CHRONIC HEPATITIS C

The standard of care for the treatment of hepatitis C is centered around combination therapy with pegylated interferon-α and ribavirin. In multiple clinical trials, it has been established that the highest levels of efficacy at achieving a sustained viral response have been with pegylated interferon and ribavirin combination therapy (1, 2). Combination therapy with pegylated interferon and ribavirin can produce a variety of side effects that can often be treatment limiting and may require dose reduction or cessation of the medications. Due to the fact that viral
Support of Patients During Antiviral Therapy for Hepatitis B and C

suppression is compromised when the medications are dose reduced or withdrawn, it is of primary importance to have a working management strategy for side effects in order to prevent unnecessary treatment cessation.

Prior to initiating treatment, it is crucial for the provider to screen the patient for contraindications to therapy and to outline potential medication-related side effects for the patient. Absolute contraindications to pegylated interferon/ribavirin treatment are known drug allergy, the presence of any major comorbid medical or neuropsychiatric condition, decompensated liver disease, history of a solid organ transplant other than the liver, uncontrolled autoimmune disease, pregnancy and active substance abuse. Ribavirin is renally cleared and thus contraindicated in those with renal disease; it is not cleared during dialysis, so is likewise contraindicated in those on hemodialysis. Side effects of hepatitis C therapy can potentially be avoided by adequate pretreatment screening to identify those most at risk for the development of certain adverse effects. The goal of this section is to acquaint the prescriber with the most common side effects experienced by patients on pegylated interferon and ribavirin combination therapy for HCV in a systems-based approach.

2.1. Hematologic Side Effects

2.1.1. Anemia

The anemia experienced with pegylated interferon and ribavirin combination therapy is primarily caused by a ribavirin-mediated hemolysis and secondarily by an interferon-induced bone marrow toxicity. The mechanism of ribavirin toxicity is ribavirin phosphate accumulation within erythrocytes and the red cells’ inability to breakdown these phosphates, thus causing red cell oxidative injury and cell lysis (3). Typically, the development of anemia occurs within the first months of therapy. In a trial comparing peginterferon alfa-2a plus ribavirin, standard interferon alfa-2b and ribavirin, and peginterferon alfa-2a alone, Fried et al. found that maximal hemoglobin decreases were 3.7 g/dL, 3.6 g/dL, and 2.2 g/dL, respectively, and returned to close to baseline after the completion of treatment (1). A similar decrease in hemoglobin was seen in a comparison of peginterferon alfa-2b and ribavirin with standard interferon alfa-2b and ribavirin, where mean hemoglobin decreases were reportedly 2.5 g/dL (2).

Since the anemia seen with ribavirin treatment is dose dependent, the standard approach to managing the anemia is dose reduction of ribavirin from the standard 1,000–1,200 mg/day dose to 600 mg/day for
hemoglobin values less than 10 g/dL and discontinuation if hemoglobin falls under 8.5 g/dL (4).

The use of epoetin alfa in the management of ribavirin-induced anemia was evaluated in a trial of 185 chronic hepatitis C-infected patients with hemoglobin values ≤12 g/dL while on combination therapy (5). The authors found that in those patients randomized to receive weekly subcutaneous erythropoietin injections, the average hemoglobin increased by 2.2 g/dL and ribavirin doses were maintained in 88% of patients versus only 60% in the placebo group. As a secondary end-point, the authors also looked at quality of life. Those in the erythropoietin group had a significantly better quality of life assessment, which may have implications for the sustenance of therapy and ultimately the achievement of a sustained viral response. Similar findings have been reported with the use of darbepoetin, which is given on a biweekly basis instead of weekly.

Recommendations:

- Recombinant erythropoietin is not currently FDA approved to treat ribavirin-mediated anemia; however, trials suggest that it can be used to raise hemoglobin, maintain ribavirin dose, and improve quality of life in those being treated for hepatitis C.
- If it is used, the goal of erythropoietin therapy should be to maintain hemoglobin in the 11–12 g/dL range.

2.1.2. THROMBOCYTOPENIA

Thrombocytopenia is a typical complication of chronic hepatitis C and is largely due to viral-mediated bone marrow inhibition, splenic sequestration of platelets, and decreased thrombopoietin production by the impaired liver. Thrombocytopenia can be exacerbated by the interferon-mediated bone marrow suppression and can limit treatment for patients. Typically, the thrombocytopenia can be managed by interferon reduction or discontinuation depending on the severity of the thrombocytopenia. The manufacturers of pegylated interferon alfa-2a and alfa-2b have recommended dose reduction and/or discontinuation for given platelet values listed in their respective dosing guides (available at www.pegasys.com and www.peginteron.com).

A recent phase II study was conducted by McHutchinson et al. to evaluate the efficacy of eltrombopag, an oral thrombopoietin receptor agonist in patients with hepatitis C-related cirrhosis and platelet counts between 20,000 and 70,000/mm³ (6). Seventy-four patients were randomized to receive placebo or three graduated doses of eltrombopag. At 4 weeks of treatment, 95% of those getting the highest dose of eltrombopag (75 mg) had platelet counts of 100,000/mm³ or
greater, while none of the patients in the placebo arm had greater than
100,000/mm³ platelets. Antiviral combination therapy was commenced
for 12 weeks in those with platelet counts greater than 70,000/mm³.
Although platelet counts were found to decrease during antiviral ther-
apy, those receiving concurrent eltrombopag maintained higher platelet
counts than those in the placebo group.

Recommendations:

- Thrombocytopenia that is exacerbated by interferon therapy is managed
  by dose reduction or discontinuation of interferon.
- Thrombopoietin receptor agonists may provide an alternative to medici-
  nation reduction or cessation but are currently under investigational use
  only.

2.1.3. NEUTROPENIA

The neutropenia that accompanies peginterferon/ribavirin treatment
is related to an interferon-mediated bone marrow toxicity. When
compared on the basis of the development of neutropenia, up to 48% of
patients receiving pegylated interferon developed absolute neutrophil
counts lower than 1,000 cells/mL as compared to 9% of patients receiv-
ing standard interferon (7). The danger of neutropenia is the subsequent
development of infection. In a recent study to evaluate the relationship
between neutropenia and risk of infection while being treated with
pegylated interferon and ribavirin, the authors found that the incidence
of infection was 41 per 100 person years and the development of
infection had a greater association with age than with the presence
of or the severity of neutropenia (8). These findings are consistent
with previous data suggesting that interferon-mediated neutropenia did
not confer increased risk of sepsis; however, despite several trials the
manufacturers of both peginterferon alfa-2a and alfa-2b recommend
dose reductions in peginterferons when the absolute neutrophil counts
fall below 750 cells/mm³ and discontinuation when ANC falls below
500 cells/mm³.

The use of granulocyte colony-stimulating factor (G-CSF) to boost
neutrophil counts in an effort to maintain peginterferon therapy has had
only limited evaluation. Koirala et al. examined a group of 163 patients
being treated for hepatitis C with pegylated interferon and ribavirin (9).
Thirty of the 163 developed neutropenia and were started on weekly
G-CSF, the remainder did not develop neutropenia. Of those who got
G-CSF, 70% had an undetectable end of treatment viral response as
compared to 90% in the non-G-CSF group. The findings of this study
suggest that adjuvant G-CSF may allow treatment continuation in
patients who develop neutropenia; however, further investigation is
necessary to demonstrate the efficacy of G-CSF for routine use.
Recommendations:

- Given the lack of evidence for increased rates of infection or sepsis development with interferon-mediated neutropenia and the lack of evidence regarding the routine use of G-CSF, there are no firm recommendations for the use of G-CSF.
- We would, however, advocate its use in order to maintain counts so that patients can continue antiviral therapy.

2.2. Psychiatric Side Effects

Twenty-one to fifty-eight percent of patients treated with interferon alfa will experience significant depressive symptoms ranging from mood alteration to neurovegetative disturbance. Those at highest risk for the development of these symptoms are those with preexisting mood conditions. The options for management of interferon-induced depression are to pretreat with antidepressants or to initiate antidepressant therapy should depressive symptoms occur. For those patients already being treated with an antidepressant, therapy should be continued. Pretreatment should be highly considered for those patients at greatest risk for developing depression (10). Initiation of antidepressants for those who develop depression is indicated and has been proven effective at providing symptom relief and allowing for continuation of antiviral therapy (11). Most studies on treating interferon-based depression have used selective serotonin reuptake inhibitors as the antidepressant agent. Rarely, patients have experienced suicidal ideations while being treated with interferon and ribavirin. Clearly, if depressive symptoms are extreme, consideration should be given to the discontinuation of treatment.

Although depression is the most common neuropsychiatric side effect of interferon therapy, mania can also emerge during treatment. Since mania is considered a psychiatric emergency, it is recommended that antiviral therapy be discontinued and urgent psychiatric evaluation be obtained.

Recommendations:

- Recognize depression as a common side effect of interferon therapy.
- Prescreen patients for psychiatric disorders prior to initiating interferon therapy.
- Consider pretreatment in those at highest risk for the development of depression.
- Consider initiation of antidepressants in those who develop symptoms.
- Recognize the potential for the development of mania and stop antiviral treatment and seek psychiatric consult.
2.3. Ophthalmologic Side Effects

The most common ophthalmologic side effect of interferon-α and ribavirin treatment is an interferon-induced retinopathy that occurs as a constellation of cotton wool spots, microaneurysms, and retinal hemorrhages. The risk of developing this retinopathy is likely increased in those patients with preexisting diabetes or hypertension. Reported ophthalmologic events include neurovisual impairment, exophthalmia, subconjunctival hemorrhage, papilledema, retinal artery occlusion, and retinal vein thrombosis (12).

In 2006, a prospective trial was published by d’Alteroche et al. to identify ophthalmologic complications and their frequency in those being treated for viral hepatitis. Of the 156 patients followed, serial exams revealed the development of retinopathy in 24%. The authors also reported that more than twice the cases of retinopathy occurred in those receiving pegylated interferon in contrast to standard interferon. Of note, all lesions regressed with either withdrawal or completion of therapy. Management of ophthalmologic side effects must be tailored to symptoms; if asymptomatic, one can continue interferon at the same dose with close ophthalmologic monitoring; however, if lesions become symptomatic, consideration should be given to the withdrawal of interferon.

Recommendations:

- Regular ophthalmologic exams in those predisposed to retinal disease.
- Ophthalmologic evaluation and dose reduction or withdrawal in anyone who develops symptoms.

2.4. Pulmonary Side Effects

The pulmonary complications of hepatitis C therapy are very rare and most are found only as case reports. Pulmonary complications are primarily due to an interferon-mediated lung toxicity; however, ribavirin can cause dry cough and anemia-related dyspnea. The reported interferon-attributable lung complications include asthma exacerbations, pleural effusions, bronchiolitis obliterans with organizing pneumonia, or most commonly an interstitial pneumonitis. Typically, these complications have occurred months into interferon therapy. Kumar et al. reviewed the literature on the topic and found that by 2004, there were 32 reported cases of parenchymal lung disease in patients receiving interferon for hepatitis C (13). Almost half of the cases they reviewed were of interstitial pneumonitis, which is marked by the presence of dry cough, dyspnea, inspiratory crackles, and patchy infiltrates and/or consolidation on radiography. After interstitial pneumonitis, the
most commonly reported pulmonary complication of interferon treat-
ment was a sarcoid-like reaction.

Given the infrequent nature of pulmonary complications of HCV therapy, there are no formal recommendations on management in this setting. In the practice of pulmonary medicine, the typical treatment of interstitial pneumonitis is to remove the inciting agent; therefore, consideration must be given to the withdrawal of therapy. Many of the reported interstitial pneumonitis cases were treated with steroids; however, almost all of these patients recovered with or without corticosteroid treatment (14).

Recommendations:

- Pulmonary complications of combination therapy are rare; however, when they occur can cause significant parenchymal lung disease.
- Pulmonary adverse effects usually occur months into interferon and ribavi

2.5. Endocrine Side Effects

The two major endocrine side effects of interferon/ribavirin treat-
ment are the development of diabetes and thyroid dysfunction. Both increased insulin resistance and insulin dependence have been reported. Those with a family history of type I or II diabetes may be at higher risk for developing diabetes. Schreuder et al. followed 189 nondiabetic patients being treated with pegylated interferon alfa and ribavirin and found that 2.6% developed type I diabetes mellitus, while 1.6% developed type II diabetes mellitus (18). Based on these findings, the authors suggested routine monitoring of blood glucose levels for patients undergoing antiviral therapy for chronic hepatitis C.

An interferon-induced thyroiditis has been well described in the lit-
erature and is thought to consist of an autoimmune subtype and nonau-
toimmune subtype of disease (19). Typically, disease develops 6–12
months into therapy and patients can present with classic hyperthyroid or hypothyroid symptoms. The emergence of thyroid disease while on interferon/ribavirin therapy can usually be managed with symptomatic therapy or thyroid hormone replacement but can necessitate consulta-
tion with an endocrinologist for definitive treatment.

Recommendations:

- Both type I and type II diabetes can develop during combination therapy for chronic hepatitis C.
- Routine monitoring of blood glucose should occur during follow-up visits.
Patients undergoing therapy for chronic hepatitis C should be screened for thyroid disease by obtaining a serum TSH at 3–6-month intervals. Symptoms of thyroid disease should be assessed and if they develop should be investigated in consultation with an endocrinologist.

2.6. Dermatologic Side Effects

In the landmark trial comparing peginterferon alfa-2b and ribavirin to standard interferon and ribavirin, over 18% of patients experienced a dermatologic side effect (2). The reported dermatologic complications of interferon/ribavirin treatment include injection site erythema/induration, an eczematous reaction, xerosis with pruritus, hyperpigmentation, photosensitivity, and alopecia (15). These reactions most commonly occur with interferon/ribavirin combination therapy; however, ribavirin is thought to be responsible for the most common rash, a generalized xerosis associated with pruritus, while interferon is responsible for injection side reactions.

In the aforementioned peginterferon alfa-2b trial (2), 29% of patients experienced some degree of alopecia. The alopecia seen with interferon and ribavirin treatment is reversible with completion or withdrawal of therapy. Kartal et al. reported a case of alopecia universalis during treatment with peginterferon alfa-2b and ribavirin with hair regrowth beginning within 3 months after therapy completion (16).

Management of these dermatologic complications has primarily been handled conservatively. Depending on the lesion or symptoms, patients have been treated with topical emollients, steroids, oral antihistamines, or even oral corticosteroids for more generalized reactions.

Recommendations:

- Over 18% of patients experience a dermatologic side effect while being treated with interferon and ribavirin.
- Many of these side effects can be managed conservatively with topical steroids/emollients and oral antihistamines.
- The alopecia experienced during combination treatment is reversible.

2.7. General Side Effects

While generalized symptoms such as fatigue, nausea, vomiting, anorexia, and flu-like symptoms are rarely the cause for medication discontinuation in patients being treated for hepatitis C, they can impose a significant quality of life burden on patients. Up to 80% of patients on interferon therapy will experience the flu-like symptoms of low-grade fevers, fatigue, and myalgias. These symptoms are most common during the first month of therapy and occur predictably within 48 hours
of interferon injection. These symptoms should be managed conserva-
tively with acetaminophen and NSAIDS.

Anorexia, nausea, and vomiting are most likely attributed to inter-
feron. These effects can be dose dependent but unless they are
extremely severe, antiviral therapy should not be interrupted for
those symptoms. Most commonly, antiemetic medications and dietary
supplements can be prescribed to combat these symptoms. Results
from a small retrospective cohort study recently suggested that oral
cannabinoid-containing medications can be used to manage anorexia
and nausea in patients being treated for chronic hepatitis C (20).
The authors found that 64% of patients who were given the oral
cannabinoid-containing medication had subjective improvement in
symptoms. Likewise, the treatment group had a higher proportion of
patients completing a full course of antiviral therapy and a higher per-
centage achieving a sustained viral response.

Recommendations:

- Flu-like symptoms are experienced by most patients during interferon
  therapy and can be managed conservatively.
- Anorexia, nausea, and vomiting should likewise be managed conserva-
tively with the aid of antiemetic medications and dietary supplements.
  There is investigation into the use of appetite stimulants; however, none
  are routinely utilized to combat anorexia in patients being treated for
  hepatitis C.

3. HEPATITIS B

There are seven approved agents used to treat chronic hepatitis B
(CHB). Selection of a singular agent or a combination of agents must
be individualized and based on a variety of patient factors, disease fac-
tors, and the severity of liver disease. Unlike the treatment for hepatitis
C, the treatment for hepatitis B can be indefinite, so recognition of a
management strategy for side effects is paramount. The goal of this
section is to outline the common side effects and management of the
approved CHB therapies in a medication-based approach.

3.1. Standard Interferon Alfa-2b or Pegylated
Interferon Alfa-2a

Both standard and pegylated forms of interferon-α have been eval-
uated in clinical trials and are efficacious in decreasing hepatitis B
DNA levels, causing loss of hepatitis B e antigen(HBeAg) and hepatitis
B surface antigen(HBsAg), and conversion to hepatitis Be antibody
(HBeAb) (21, 22). Interferon treatment for CHB carries with it all of
the interferon-mediated side effects discussed in the hepatitis C section with the additional potential for a significant hepatitis flare.

Approximately 25–40% of patients treated for CHB with interferon will experience a hepatitis flare, which can be life threatening (23). The interferon-mediated hepatitis flare typically occurs during the first few months of therapy and can indicate seroconversion from HBeAg to HBeAb. Mechanisms of a flare are thought to be related to interferon stimulation of cytotoxic T cells or an immune-mediated hepatocyte lysis. Depending on the severity of the flare, interferon therapy may need to be curtailed or stopped.

3.2. Lamivudine

Lamivudine is a nucleoside analogue that incorporates into the viral DNA via HBV reverse transcriptase and results in chain termination. It is effective as a solo agent for the treatment of replicative hepatitis B and has also been used in combination with interferon and other antiviral agents; however, the long-term efficacy of those combinations remains under investigation. In general, lamivudine is a safe and effective medication; however, it has been associated with the development of a severe lactic acidosis, severe hepatomegaly with steatosis, emergence of viral resistance, posttreatment hepatitis flares, and pancreatitis. Given the frequent development of resistant viral mutants, the efficacy of lamivudine may be limited as a long-term therapy. Depending on the severity of these reactions, discontinuation of therapy may be necessitated.

3.3. Adefovir Dipivoxil

Adefovir is a nucleotide reverse transcriptase inhibitor which acts by interfering with HBV DNA polymerase and inhibits viral replication. Adefovir has been proven effective in the treatment of both hepatitis B e antigen-positive and antigen-negative patients (24, 25). Like lamivudine, adefovir can cause a severe lactic acidosis, severe hepatomegaly with steatosis, viral resistance, and posttreatment hepatitis, which usually occur within 12 weeks of drug discontinuation and can occur in up to 25% of patients. The limiting toxicity of adefovir is that it can cause renal impairment at high doses and when used for a prolonged period. In the trial evaluating the efficacy of adefovir in HBeAg-positive patients, those receiving 30 mg had a higher rate of renal abnormalities than those receiving 10 mg (24). When HBeAg-negative patients were followed on adefovir for 5 years, 3% developed a \( \geq 0.5 \text{ mg/dL} \) increase in creatinine over baseline values (27).
3.4. Entecavir

Entecavir is another nucleoside reverse transcriptase inhibitor that was approved by the Food and Drug Administration (FDA) in 2005 for the treatment of CHB. Entecavir has been proven efficacious in treating lamivudine-resistant CHB and also as a primary agent to improve virologic, biochemical, and histologic disease (27, 28). The quality of entecavir that makes it most desirable is its very low evidence of viral resistance. Similar to the other reverse transcriptase inhibitors, its most dangerous side effects are the occurrence of lactic acidosis, hepatomegaly with steatosis, and posttreatment hepatitis flare. There has been a warning issued with regard to the development of HIV resistance in those HIV-positive patients not on highly active antiretroviral therapy who are being treated with entecavir as HBV monotherapy (29).

3.5. Telbivudine

Telbivudine is a nucleoside reverse transcriptase inhibitor approved to treat hepatitis B. In clinical trials, it has shown improved HBV clearance when compared with lamivudine and adefovir (30, 31). The major side effects are the same as seen with the other RTIs as well as a myopathy in association with elevated creatine kinase levels. There are emerging reports of increased rates of peripheral neuropathy when used in conjunction with pegylated interferon alfa-2a.

3.6. Tenofovir

Tenofovir is the newest nucleotide analogue to be approved by the FDA. Its side effect profile is similar to that of adefovir. It has a propensity to cause renal dysfunction and needs to be dose reduced in patients with impaired creatinine clearance. Like the other agents described, it may cause hepatomegaly and lactic acidosis. Gastrointestinal side effects occur in about 10% of users and are usually mild.

Recommendations:

- Standard interferon-α, pegylated interferon alfa, lamivudine, adefovir dipivoxil, entecavir, and telbivudine are the approved treatments for chronic hepatitis B.
- All of the nucleotide/nucleoside reverse transcriptase inhibitors carry a risk of lactic acidosis, hepatomegaly with steatosis, and posttreatment hepatitis flare.
- Lamivudine is associated with a high degree of viral resistance.
- Adefovir can cause renal impairment when given at higher doses or for a prolonged period of time.
- Entecavir has a very low rate of viral resistance but may be associated with HIV resistance in HIV-positive patients not on HAART.
• Telbivudine has initially demonstrated increased antiviral activity when compared to adefovir and lamivudine; however, it may be associated with the development of a myopathy and when used in conjunction with peginterferon may cause a peripheral neuropathy.
• Tenofovir is a potent antiviral with an excellent resistance profile. It may cause azotemia when used for prolonged periods of time.

4. CONCLUSION

The therapies for chronic hepatitis B and C have dramatically evolved over the last decade. With the addition of new antiviral agents, we are seeing rates of viral suppression and elimination that have previously been unachievable. Principal in treating patients for chronic hepatitis is choosing a regimen that is suitable for an individual patient, disclosing the expected and potential side effects, and early identification and management of adverse effects. Despite the myriad side effects of these medications, we move closer to the goal of eradicating virus and preventing long-term sequelae of hepatitis infection.

REFERENCES

Viral Hepatitis, A Through E, In Pregnancy

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Key Principles

- Pregnancy causes physiological changes in liver function and biochemical testing, which should be recognized before embarking on an extensive workup of liver disease in pregnancy.
- Viral hepatitis A through D does not affect the pregnant woman any differently. However, hepatitis E develops into fulminant hepatic failure in 25–70% of pregnant women.
- The treatment for acute infection during pregnancy is similar to the treatment of adults who are not pregnant and consists mainly of supportive care.
- It is recommended that all pregnant women undergo testing for HBsAg during an early prenatal visit even if they have been previously tested or vaccinated.

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- Perinatal transmission represents one of the most common routes of transmission of chronic hepatitis B worldwide and probably accounts for more than half of the world’s chronic carriers.
- A number of strategies have been employed to prevent mother-to-infant transmission. The most effective strategy is the combination of hepatitis B immune globulin (HBIG) and hepatitis B virus (HBV) vaccination within 12 h of birth followed by the completion of the vaccine series at 1 and 6 months of age.
- Nucleos(t)ide analogue therapy with an FDA-approved anti-HBV agent is appropriate for managing CHB infection in mothers whose serum tests positive for HBsAg and who have high serum HBV DNA concentrations.
- Vertical transmission of hepatitis D virus (HDV) has been reported but it is uncommon. The therapeutic strategies to prevent perinatal transmission of HBV are also effective in preventing the transmission of HDV.
- Vertical transmission of hepatitis C virus (HCV) is less common than in HBV infection but has been well documented. The treatment for acute and hepatitis C infection in pregnant women is supportive.
- Breastfeeding is not contraindicated in women with hepatitis A virus (HAV) infection or chronic viral hepatitis B, C, or D. It is unclear at this time whether HEV transmission occurs while breastfeeding and therefore no formal recommendations can be made.

1. INTRODUCTION

Jaundice during pregnancy may be the result of liver disease unique to pregnancy or a disease unrelated to pregnancy. Liver diseases unique to pregnancy include the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), acute fatty liver of pregnancy, and cholestasis of pregnancy. In the United States, viral hepatitis is the most common cause of jaundice in pregnancy. Viruses which uniquely cause hepatitis are hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), hepatitis D or delta hepatitis (HDV), and hepatitis E (HEV). Other viruses which may be associated with hepatitis include cytomegalovirus, Epstein–Barr virus, herpes simplex virus, varicella-zoster virus, human parvovirus B19, and adenoviruses. The clinical course of hepatitis E is distinctive in pregnant woman. Otherwise, viral hepatitis does not differ between pregnant and nonpregnant populations (1).
2. EFFECT OF PREGNANCY ON LIVER FUNCTION

In evaluating the pregnant woman, one must be aware of how the gestational state affects liver function and other biochemical tests. There is a progressive increase in alkaline phosphatase to 1.5–2 times the nonpregnant values because of the release of placental and bone alkaline phosphatase. Also, leucine aminopeptidase levels progressively increase because of release by placental enzymes. There may be a slight increase in serum bilirubin because of mild impairment of bilirubin transport with decreased hepatic clearance caused by estrogen’s effect on organic anion-transporting pumps. Also, with impaired hepatic transport and biliary secretion caused by estrogen’s effect on bile-salt transport pumps, serum bile acids increase to 200–300% of nonpregnant values. Serum aminotransaminases do not change, nor does the prothrombin time (2, 3). Gamma-glutamyl transpeptidase levels are normal to low because of impaired release. Serum albumin levels decrease 10–60% because of hemodilution by expanded plasma volume and decrease in synthesis. Changes in physical examination occur during pregnancy as well. The liver, which is normally palpable 1–2 cm below the right costal margin, may become more difficult to palpate because of the expanding uterus within the abdominal cavity. Large amounts of estrogen and progesterone are produced by the placenta. As the liver cannot metabolize these hormones quickly, telangiectasia and palmar erythema, suggestive of liver disease in nonpregnant woman, may develop in up to 60% of normal pregnancies.

3. VIRAL HEPATITIS IN PREGNANCY

Acute hepatitis, presumably viral, accounted for 40% of 654 cases of jaundice during pregnancy reviewed in the 1950s and 1960s (2, 4–6). A review of hepatitis in pregnancy from Parkland Hospital in Dallas noted an incidence of 1 in 700 deliveries (7).

Viral hepatitis A through D does not manifest differently in pregnant women compared to men or nonpregnant women. Also, the laboratory manifestations are similar in pregnant and nonpregnant populations (1). In developed countries, maternal mortality from fulminant hepatic failure is low, about 1.2% (2, 8). However, reports from India (9), the Middle East (10), and Africa (11) describe disease of greater severity, often leading to fulminant hepatitis, particularly in the third trimester, with maternal mortality rates of about 30%. Studies in Israel have shown a reduction in mortality among pregnant women with viral hepatitis during pregnancy as socioeconomic class in the country has risen (12). The higher mortality in underdeveloped countries may be due to poor
nutritional status and inadequate prenatal care. Hepatitis E develops into fulminant hepatic failure in 25–70% of pregnant women. Many series report mother and infant mortality rates of almost 100% in hepatitis E-induced fulminant hepatitis. Acute viral hepatitis is associated with premature birth in about 25% of cases (range 8–54%) and a fetal/neonatal death rate of 8% in developed countries such as the United States, Australia, and Israel and about 50% in India and the Middle East (2).

3.1. Hepatitis A

Hepatitis A (HAV) virus was identified in 1973. It is a single-stranded RNA virus belonging to the family Picornaviridae (13). The incidence of hepatitis A varies globally. According to the CDC, high areas of prevalence include Greenland, Mexico, Central America, South America, Africa, the Middle East, and all of Asia. Intermediate prevalent areas include Eastern Europe, territories formally known as the Soviet Union and the Siberian peninsula. Low prevalence areas include the United States, Canada, Western Europe, Scandinavia, Australia, and New Zealand. The incidence in the United States in 2005 was 1.5 per 100,000, which is the lowest reported rate in United States history (14).

The main mode of transmission is fecal-oral and most patients that acquire the illness have had direct contact with an infected person. Rare cases of parenteral transmission have been reported. In the United States, a higher incidence of hepatitis A occurs in areas where sanitation and/or hygiene are poor. Outbreaks have occurred with contaminated water, food, such as imported green onions from Mexico, contaminated frozen strawberries or shellfish, and within neonatal units or daycare centers (15–17). Vertical transmission of hepatitis A is extremely rare.

The incubation period is usually 30 days, with a range of 15–40 days. Among children and adolescents, 80% are asymptomatic and without jaundice. However, more than 80% of infected adults develop jaundice. The presentation does not differ with pregnancy; it begins with acute constitutional symptoms of fevers, chills, fatigue, nausea, vomiting, anorexia, right upper quadrant pain, and occasionally diarrhea, progressing to dark urine, acholic stool, jaundice, and pruritus. The disease is self-limited with no long-term sequelae. Relapsing disease has been reported and no unique risk factors have been identified for individuals who relapse after full recovery. Rarely, intrahepatic cholestasis with prolonged jaundice and pruritus may occur. Hepatitis A rarely causes fulminant hepatitis and the fatality rate is <0.1% (18, 19). There is an increased incidence of fulminant hepatitis occurring during the third trimester of pregnancy; however, fulminant hepatitis has been reported only in populations where prenatal care is not as available or nutrition
not as plentiful, such as India, the Middle East, and Africa (9–11). Atypical presentations of hepatitis A occurring in pregnancy have included acalculous cholecystitis in a rare case report (20).

Cases that have been identified during pregnancy are usually a result of the pregnant mother having direct contact with an infected person. There are no reported cases of transmission through breast milk. Perinatal transmission of hepatitis A from an infected mother to the fetus has been described in two case reports. The mothers are described as being infected within the first trimester and the fetuses subsequently develop meconium peritonitis from perforation of the terminal ileum. The fetuses recovered fully after postdelivery bowel resection. The authors suggest serial ultrasonographic examinations after maternal hepatitis A for detection of fetal ascites and meconium peritonitis (21, 22).

HAV can be isolated from the stool and other body fluids, which typically can be identified before the identification of antibodies in the serum. The gold standard for diagnosis is serum anti-HAV IgM with clinical symptoms of acute infection. The anti-HAV IgM can persist for months and a positive result does not necessarily mean active infection. Anti-HAV IgG can be detected as early as the convalescent phase and persist for decades (23–25).

If a pregnant woman reports exposure, two options are available to prevent the acute infection: hepatitis A vaccination and immune serum globulin. The immune serum globulin is most useful if given within 2 weeks of known exposure. The administration of immune serum globulin gives months of immunoprotection, while the vaccination will produce immunity that lasts for a lifetime. The vaccine is more than 85% effective in preventing acute infection. Vaccination is not routine in the United States, unless the woman falls into a high-risk population, has known exposure, has underlying liver disease, or has a travel history to an endemic area. The vaccine is considered safe in pregnancy, since it contains an inactivated form of the virus. The administration of immune serum globulin does have side effects and carries risk of infection with other viruses since it is derived from a pool of donors. Therefore, it should be reserved for patients that have allergies to the vaccine (26–29).

The treatment for acute infection during pregnancy is similar to the treatment of adults who are not pregnant, which is supportive care. Antiemetics with potential teratogenic properties should be avoided or minimized. Hospitalization becomes necessary if the woman develops severe hepatitis manifested by markedly elevated aminotransaminases, usually greater than 1000 IU/L, elevated prothrombin time, hypoglycemia, jaundice, or acute mental status changes. In this case,
the woman should be admitted to a hospital where liver transplantation is available. Other possible reasons to hospitalize pregnant women with acute illness include inability to self-hydrate.

Vertical transmission of hepatitis A is rare. Infants born to mothers with acute hepatitis A may be given immune serum globulin and the first dose of the hepatitis A vaccine immediately after delivery and this may be repeated at 5–6 months of age (30, 31). There have not been any reported cases of transmission of hepatitis A with breastfeeding, which is safe.

3.2. Hepatitis E

Hepatitis E virus (HEV) is an enterically transmitted hepatitis virus that causes large-scale epidemics and sporadic cases of acute viral hepatitis in developing countries. As with hepatitis A, hepatitis E causes acute hepatitis; chronic infection never develops. Prior to the identification of hepatitis E, this virus was called “waterborne or enterically transmitted non-A and non-B hepatitis.” It is a single-stranded RNA virus that has characteristics similar to the Caliciviridae family (32, 33). The highest incidences of acute hepatitis E occurs in Asia, Africa, the Middle East, and Central America. Sporadic cases have been documented in Western countries. The main source of transmission of HEV is drinking water contaminated with sewage causing large waterborne outbreaks. Infection among household contacts indicates that person-to-person spread may also occur and transmission with blood transfusions has been reported. The mean incubation time is about 45 days, with a range from 15 to 60 days (34, 35).

In men and nonpregnant women, the disease is usually self-limited with a mild disease presentation. Most common symptoms include nausea, vomiting, abdominal pain, fever, and jaundice. Other signs and symptoms include diarrhea, arthralgias, pruritus, and urticarial rash. Fulminant hepatitis can occur, although it is rare. The overall fatality rate associated with men and nonpregnant women with HEV infection is <0.1%. However, in pregnant women, HEV infection is more severe, often leading to fulminant hepatic failure and death in up to 15–20% of cases. Acute infection has not been associated with the development of chronic hepatitis. Laboratory abnormalities include elevated serum bilirubin, alanine aminotransaminase (ALT), and aspartate aminotransaminase (AST). Resolution of these laboratory abnormalities can take 1–6 weeks (36–40). The diagnosis of acute hepatitis E is made with a positive anti-HEV IgM and anti-HEV IgG is detectable in the convalescent phase or after the acute infection has resolved.
A 2007 report from a tertiary hospital in India followed 220 consecutive pregnant women presenting with jaundice caused by acute viral hepatitis. Infection with HEV caused acute viral hepatitis in 60% of included women (41). Fulminant hepatic failure (FHF) was more common [relative risk, 2.7 (95% CI, 1.7–4.2); P = 0.001] and maternal mortality was greater [relative risk, 6.0 (CI 2.7–13.3); P <0.001] in HEV-infected women than in non-HEV-infected women. The overall maternal mortality rate was 41% (54/132) in HEV-infected women and 7% (6/88) in non-HEV-infected women and occurred exclusively in women with FHF. FHF was more common among HEV-infected women than non-HEV-infected women [73 of 132 (55%) vs. 18 of 88 (0%)]. FHF particularly affected HEV-infected women in their third trimester [46 of 88 (52%) vs. 11 of 71 (15%) non-HEV-infected women]. The frequency of FHF did not differ between HEV-infected women and non-HEV-infected women in their second trimester. More HEV-infected women died, had obstetric complications, or had worse fetal outcomes than did women who had jaundice and acute viral hepatitis caused by other hepatitis viruses.

The mechanism of vertical transmission is unclear at this time. Previous studies have demonstrated vertical transmission in five newborns whose mothers had developed HEV in the third trimester and delivered vaginally. Vertical transmission was also documented in 26 cases of HEV RNA-positive women by testing for HEV RNA in cord blood or newborn blood. However, the possibility of contamination of cord blood with the maternal blood could not be excluded. It is unclear at this time whether transmission occurs while breastfeeding and therefore no formal recommendations can be made.

The reasons for the high frequency of FHF, in pregnant women with HEV infection, are unclear. A shift in the Th1–Th2 balance toward a Th2 response in pregnant but not in nonpregnant women with HEV infection may point toward a primary immunologic cause of severe disease in pregnancy. Also, hormones of pregnancy, i.e., estrogen and progesterone, may impair cellular immunity by triggering an adapter protein (ORF3 of HEV), which could facilitate viral replication and lead to release of cytokines and liver cell apoptosis (42–44).

Treatment for HEV infection is supportive care. There are current clinical trials evaluating vaccines for prevention and treatment, which have been shown to prevent transmission in 96% of their participants. Intravenous immune serum globulin has also been used for prophylaxis and initial treatment after exposure. However, it has not been shown to decrease mortality or duration of symptoms (45, 46).

Because of the high mortality rate and lack of effective treatment of acute infection, prevention of HEV infection in pregnant women is of
utmost importance. Pregnant women should be advised to avoid travel to endemic areas, to avoid drinking water of unknown purity, uncooked shellfish, and uncooked fruits and vegetables from endemic areas.

3.3. Hepatitis B

Hepatitis B virus (HBV) is a double-stranded DNA virus in the family Hepadnaviridae (47). Hepatitis B is a major global health problem. Of the 2 billion who have been infected, more than 350 million are chronic carriers (48–50).

3.3.1. Perinatal Transmission

This is the major mode of disease acquisition in endemic areas, accounting for 90% of the affected population. The risk of perinatal transmission is associated with maternal hepatitis B e antigen status and viral load. The HBV transmission rate of HBeAg-positive mothers to their infants is higher, 70–90%, compared with HBeAg-negative, anti-HBe-positive mothers, 5–20%. Similarly, a high maternal viral load, greater than 5 pg/ml, was associated with a higher rate of perinatal transmission (51, 52).

The mode of perinatal transmission is presumed to be via exposure to maternal blood during passage through the birth canal or immediately postdelivery, or from exposure in utero. Studies examining rates of HBsAg, HBeAg, or HBV DNA detection in cord blood or infant sera within 24 h of delivery suggested that in utero infection was not a significant mode of transmission, accounting for less than 5% of cases of perinatal transmission (53, 54). Rates of in utero transmission may be increased during acute HBV infection in the third trimester of pregnancy or if a history of threatened preterm labor was present (54). Bhat and Anderson (55) have shown that HBV has the ability to translocate across the trophoblastic layer of the early placenta during the first trimester, which is a possible mechanism of intrauterine infection.

3.3.2. Prophylaxis to Prevent Perinatal Transmission

According to the Centers for Disease Control and Prevention (CDC) in the United States, in an infant born to a mother who is positive for both HBsAg and HBeAg, the risk of chronic HBV infection is 70–90% by the age of 6 months without the use of postexposure immunoprophylaxis. In mothers who are HBsAg-positive and HBeAg-negative, the risk for chronic infection is <10% in the absence of postexposure immunoprophylaxis (56, 57). Cases of fulminant hepatitis B as a result of perinatal transmission have been reported, although much less so than the development of chronic disease. A report by Friedt et al.
proposed that selection for an HBeAg-negative strain is responsible for the rare development of fulminant hepatitis in the fetus (62).

A number of strategies have been employed to prevent mother-to-infant transmission: the use of HBIG alone, HBV vaccine alone, or the combination of HBIG plus HBV vaccine. All strategies have varying success in preventing acquisition of HBV in the newborn, but the most effective strategy is the combination of HBIG (.06 ml/kg IM) plus HBV vaccination within 12 h of birth followed by the completion of the vaccine series at 1 and 6 months of age. This strategy was 89–98% effective at preventing acute and chronic hepatitis B in infants born to HBsAg/HBeAg-positive women (58,59).

3.3.3. SCREENING OF PREGNANT WOMEN

A long-term study of the prevalence of hepatitis B seropositivity among pregnant women in the United States was reported by Euler et al. in 2003. They found that of 10,523 women in four urban centers, 0.97% of blacks, 0.6% of whites, 0.14% of Hispanics, and 5.79% of Asian-Americans were seropositive for the HBV with a positive hepatitis B surface antigen (51). In the United States, the Advisory Committee on Immunization Practices (ACIP) recommended testing of all pregnant women for HBsAg during an early prenatal visit, even though previously tested or vaccinated (60). Women who were not screened perinatally, or who engaged in high-risk behavior, had an HBsAg-positive sex partner, or those treated for sexually transmitted disorders should be tested at the time of admission to the hospital for delivery. If HBsAg testing is not available, it is recommended to vaccinate the neonate within 12 h of birth and to complete the vaccine schedule according to that recommended for infants born to HBsAg-positive mothers.

3.3.4. POSTEXPOSITION PROPHYLAXIS DURING PREGNANCY

Postexposure prophylaxis using hepatitis B immune globulin (HBIG) and active vaccination during pregnancy appears to be safe for both mother and fetus (61–67).

The Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis reported that there was no apparent risk for adverse effects toward developing fetuses when hepatitis B vaccine was administered to pregnant women (56). Therefore, postexposure prophylaxis should be offered to all pregnant women.

Breastfeeding and HBV: Before the availability of HBV vaccine and HBIG, several case series have shown transmission of HBV through breast milk. Although the mechanism of transmission by breast milk was unclear, many physicians did not recommend breastfeeding to
mothers with HBV. Now, breastfeeding is considered safe whether the mother has acute HBV, chronic HBV infection, or a carrier HBV state as long as the infant receives a full course of the hepatitis B vaccine series with the first dose being given within 12 h of birth in combination with hepatitis B immunoglobulin. These recommendations are based on prospective longitudinal studies done by Hill, Sheffield et al. (63). Neither the World Health Organization (WHO) nor the CDC discourages breastfeeding between a mother with HBV and her infant as long as the HBV vaccine series was given at birth or HBIG and the vaccine was given within 12 hours of birth.

3.3.5. MATERNAL OUTCOME IN PREGNANCY

A recent investigation of women infected with hepatitis B who had 38 pregnancies between 1998 and 2006 revealed that there was an increase in liver disease activity in 45% of the women in the months following delivery (64). Predicting who was going to have an exacerbation during pregnancy was not possible using HBV DNA level, ALT, or HBeAg status. Even in those women treated with lamivudine during the last trimester of pregnancy, this exacerbation occurred 62% of the time. The researchers concluded that a significant increase in liver inflammation can often occur after pregnancy, perhaps due to reactivation of the immune system after delivery. Women should be monitored closely after delivery and treated if necessary.

3.3.6. TREATMENT OF HBV IN PREGNANCY

A strategy to reduce vertical transmission of HBV infection is administering lamivudine therapy during pregnancy (65). The first report of lamivudine therapy during pregnancy was by van Zonneveld M et al. in The Netherlands (68). They treated eight mothers with HBV infection and high viral loads, >1.2 × 10^9 genome Eq/mL, with lamivudine 150 mg daily during the last month of pregnancy. HBV DNA level, hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), and hepatitis B core antibody (HBCAb) of the infants were measured at birth and at 3, 6, and 12 months, respectively. Twenty-four children born to untreated HBsAg-seropositive mothers with HBV-DNA levels greater than or equal to 1.2 × 10^9 genome Eq/L served as historical controls. All the children received passive–active immunization with HBIG 30 IU at birth and hepatitis B vaccine at 2, 3, 4, and 11 months of age. Seven of the eight mothers who received lamivudine had a reduction in serum HBV DNA concentration; serum HBV concentrations were less than 1.2 × 10^8 genome Eq/mL in five of these mothers. Maternal ALT levels were normal or near normal at the time of delivery (range, 20–49 U/L). At follow-up 1–7 months post delivery, ALT levels had increased
in seven women but remained below five times the upper limit of normal in all eight participants. In the lamivudine-treated group, one of the eight (12.5%) infants who tested positive for HBsAg had a detectable serum HBV DNA concentration at the age of 12 months. Among the 24 untreated historical control subjects, perinatal transmission occurred in 7 (28%) infants. There were no obvious adverse effects from lamivudine in the mothers or in the infants born to mothers in the treatment group.

A multicenter, randomized, double-blind, placebo-controlled study from Xu et al. (69) strongly suggests that the use of lamivudine in the third trimester of pregnancy is more effective than passive and active immunization alone in reducing vertical transmission in mothers with high serum HBV DNA concentrations. One hundred and fourteen HBV-infected mothers with HBV DNA greater than 1000 Meq/mL were randomized to receive either lamivudine 100 mg daily (n = 68) beginning at the 32nd week of gestation and continuing until 4 weeks postpartum or matched placebo (n = 52). All of the infants received standard prophylaxis with HBIG within 24 h of birth and vaccination with recombinant HBV vaccine; three injections over the first 6 months of life. The proportion of mothers with serum HBV DNA levels less than or equal to 1000 Meq/mL at the time of delivery was higher in the lamivudine than the placebo group [55/56 (98%) vs. 18/59 (31%), respectively]. Infants born to mothers with a lower serum viral load had a lower likelihood of HBsAg positivity at 1 year of age (84 and 61%; p = 0.008). The incidence of adverse effects in the mothers and infants was not significantly different in the two groups (17, 9). The authors suggested that lamivudine should be considered for use in the third trimester in HBV-infected mothers with high serum HBV DNA concentrations.

Thus, nucleos(t)ide analogue therapy with an FDA-approved anti-HBV agent is appropriate for managing HBV infection in mothers whose serum tests positive for HBsAg and who have high serum HBV DNA concentrations. The five oral nucleos(t)ide analogues are listed as either a category B or a category C agent by the FDA. Category B drugs are those that, according to the results of animal studies, carry no teratogenic or embryonic risk and for which there have been no controlled human studies to refute these findings. Category C drugs are those that exert teratogenic or embryocidal effects on animals and for which there are no controlled studies in humans. Lamivudine, adefovir, and entecavir are designated category C drugs; telbivudine and tenofovir are category B drugs (65).

In her review of the Antiretroviral Pregnancy Registry 2006, Terrault (65, 70) reports that more than 4000 women have been treated with lamivudine, making it the anti-HBV therapy most extensively used in
pregnancy. This report notes that between 1989 and 2006, the rate of birth defects among 1662 women who were exposed to lamivudine during the first trimester of pregnancy was 2.7% [95% confidence interval (CI), 2.0–3.6%]. Among 3000 women who were exposed during the second or third trimester, the rate of birth defects was 2.4% (95% CI, 1.9–3.1%). These birth defect rates are similar to the rate of major birth defects in live births in the general populations (3%, according to the Center for Disease Control and Prevention). There has been only limited use of the other nucleos(t)ide analogues, adefovir, entecavir, telbivudine, or tenofovir during pregnancy. Thus lamivudine is considered the treatment of choice for pregnancy women with HBV infection, although the use of any agents remains controversial (65).

Terrault notes there are no consensus guidelines for the treatment of hepatitis B in women who become pregnant during the course of antiviral therapy. For patients who become aware of their pregnancy during the first several weeks of gestation, one option is to stop therapy, monitor HBV DNA levels and ALT throughout the pregnancy, and restart therapy during the postpartum period. Other options are to continue therapy or to switch to lamivudine or to a category B agent.

For pregnant women with treatment-naïve HBV infection, prophylactic therapy may be considered during the third trimester of pregnancy. However, Keefe et al. (71) caution that therapy should be postponed until after pregnancy in women with mild liver disease. All women of childbearing age and infected with HBV should be counseled about the various treatment options for HBV and the associated risks of each. For mothers with serum HBV DNA concentrations of $10^8$ or higher, antiviral therapy during the third trimester may be considered as a means of reducing the risk of perinatal HBV transmission (65).

3.4. Hepatitis D (Delta)

Delta hepatitis is caused by an RNA virus that can only replicate in the presence of HBV, which acts as a helper virus. Infection occurs only in persons with hepatitis B, with hepatitis D being acquired as a co-infection, i.e., simultaneous infection with HBV and HDV, or as a super-infection in a person who is already HBsAg-positive. HDV infection in chronic HBV carriers is associated with more active and progressive disease and cases of fulminant hepatic failure have been reported. The modes of transmission are the same as with HBV (72, 73).

Vertical transmission of HDV has been reported but it is uncommon. The therapeutic strategies to prevent perinatal transmission of HBV are also effective in preventing the transmission of HDV. Since HDV cannot
be transmitted in the absence of HBV, routine vaccination of all new-
born infants with the HBV vaccine will protect against HDV (74).

3.5. Hepatitis C

Hepatitis C virus (HCV) is one of the leading known causes of liver
disease in the United States (75, 76).

The prevalence among pregnant women varies from 1 to 5%, with
more cases seen in the urban population. There is ethnic variation, with
HCV being more prevalent among African-Americans (6.1%) than non-
Hispanic Caucasians (3.1%) and Hispanics (2.8%) (77, 78).

HCV is not spread by breastfeeding, even in mothers whose milk
contains HCV RNA, casual contact, sharing eating utensils or drinking
glasses, food, or water (79). The World Health Organization and Center
for Disease Control recommend breastfeeding. Infected mothers should
put breastfeeding on hold if their nipples are cracked or bleeding.

3.5.1. Screening of Pregnant Women

In the United States, routine screening of pregnant women for HCV
is not recommended. Pregnancy screening for HCV is recommended
only in women who have a history of IV drug use or a partner with
drug use, have a history of receiving blood, blood products, or organ
transplant before 1992, have received hemodialysis, have HIV or hepa-
titis B infection, have elevated liver enzymes, or have a history of tat-
tooing (80, 81). Initial screening is performed by identification of the
hepatic C antibody by enzyme immunoassay. This test is 97% sensitiv-
e and 99% specific. Confirmation of a positive antibody test may
be performed with the recombinant immunoblot assay (RIBA) against
specific antigens. The antibody may not develop in some infected indi-
viduals until 2–8 weeks after evidence of liver injury and may not be
present in patients with initial signs and symptoms of disease. Active
infection is diagnosed with a reverse transcriptase polymerase chain
reaction (RT-PCR) assay to determine viral load (82).

3.5.2. Vertical Transmission of HCV

Mother-to-infant, vertical, transmission of HCV is less common than
in HBV infection, but transmission has been well documented. The
risk of vertical transmission is approximately 2% for infants of hep-
atitis C antibody-positive mothers. When a pregnant women is HCV
RNA-positive at delivery, the risk increases to 4–7%. Regardless of
HIV status, a viral load of greater than $1 \times 10^5$ copies/mL has been
shown to increase the rate of transmission (83, 84). The highest rate of
vertical transmission occurs in mothers who have HCV with HIV coin-
fection. Transmission rates increase to 20% in this coinfectcd group.
This increased risk is likely due to high viremia (85). The pathogenesis of transmission is unclear. It seems to occur in utero and perinatally; there have been no documented cases of postnatal transmission between mother and infant. Among 54 HCV-infected children, 17 (31%) were positive in the first 3 days of life and were assumed to have acquired the infection in utero. These children with evidence of intrauterine infection were significantly more likely to be of lower birth weight and infected with genotype 1 (58% vs. 12%, p = 0.01). HIV coinfection did not significantly increase the risk of in utero transmission of HCV. Twenty-seven of the children (50%) were first HCV RNA PCR-positive at 3 months, nine (5%) were first HCV RNA PCR-positive after 3 months, and one had no further tests (86).

The mode of delivery has not been shown to affect the vertical transmission rate (3.5). Some observational studies show a decreased rate of transmission between vaginal and caesarean section; however, most studies do not (87). In the European Pediatric Hepatitis C Virus Network study, the women with HCV infection showed no difference in transmission rate with caesarean vs. vaginal delivery (odds ratio = 1.46; p = 0.16). In women coinfected with HCV and HIV, caesarean delivery is protective against HCV transmission (odds ratio = 0.36; p = 0.01), but in those who received antiretroviral treatment, this protective effect is not evident. A cost-effective analysis by Plunkett et al. noted that an elective caesarean section is cost-effective only if it reduces the risk of perinatal transmission by greater than 77%. Studies have not shown this reduction in the risk of HCV perinatal transmission with caesarean section (88). Steiniager et al. reported an increased risk of transmission of HCV with intrapartum exposure to virus-contaminated maternal blood during vaginal delivery (89). Other possible risk factors are ruptured membrane >6 h prior to delivery, internal fetal monitoring or fetal blood sampling, and the presence of HCV in maternal mononuclear cells (78). Avoiding fetal scalp monitoring and prolonged labor after rupture membranes may reduce the risk of transmission to the infant. Finally, a Cochrane collaboration review by McIntyre et al. does not recommend one mode of delivery over the next, based on the fact that no randomized clinical trials have been done and that in published studies on this subject, HIV- and HCV-coinfected mothers were combined with HIV-negative mothers (90).

Vertically acquired HCV infection predominantly is asymptomatic in infancy, although elevated alanine aminotransaminase levels in the first 6–12 months of life have been observed. Infants born to HCV-positive mothers should be tested for HCV infection by serum HCV RNA tests on two occasions between the ages of 2 and 6 months and/or have HCV antibody tests after 15 months of age. Positive HCV antibody in infants
prior to 15 months of age may be due to transplacental transfer of maternal HCV antibody (82). The long-term outcome of vertically infected infants is unknown. Limited data indicate that less than 10% of the infected children develop chronic hepatitis and less than 5% develop cirrhosis (78).

The treatment for acute and hepatitis C infection in pregnant women is supportive. The Federal Drug Administration approved treatment for chronic HCV is ribavirin and pegylated interferon. Ribavirin has been shown to be teratogenic in rodents and is considered a category X for use in pregnancy. Interferon has been shown to increase the incidence of fetal loss and low birthweight in some studies (91). On the other hand, there are several case reports of pregnant women being treated with interferon during the early part of pregnancy, without any problems being reported in the fetus; however, the cases are few (92–95). There are no data to determine whether antiviral therapy reduces perinatal transmission of HCV and treatment is not recommended.

There is currently no active or passive immunization available for HCV. The virus genome mutates rapidly making vaccine development difficult. In addition, in chimpanzees, primary infection does not protect against subsequent infection to the same viral strain or a heterologous strain. To date, there are no trials to conclude that intravenous immunoglobulin can decrease transmission of infection.

4. CONCLUSIONS

The clinical presentation of acute viral hepatitis in a pregnant woman is not different than that in a man or a nonpregnant woman. Acute viral hepatitis increases the risk of premature delivery and fetal/neonatal death rate, especially in developing countries. Infants whose mothers have acute hepatitis A at delivery can be protected against infection with HAV vaccination or immune serum globulin. HEV infection in pregnancy is associated with a high maternal and infant mortality rate and there is no effective treatment. HEV vaccine development shows promising results but is not available for widespread use. Infants can be protected from acquiring HBV and HDV from mothers with acute or chronic HBV infection or HBV plus HDV coinfection with hepatitis B vaccine and hepatitis B immune serum globulin. This active plus passive immunization may fail when the mother has high serum HBV DNA levels. Infants may contract HCV infection from their mothers in the perinatal period. The risk is highest in women with high serum HCV RNA levels, which is more commonly seen in those mothers with HIV coinfection. There is no active or passive immunization available against hepatitis C.
If treatment guidelines are used, i.e., passive or active immunization and treatment for HIV infection in those mothers with coinfection, caesarean section does not decrease the risk of transmission of viral hepatitis to the infant. Breastfeeding is not contraindicated in women with HAV infection or chronic viral hepatitis B, C, or D. It is unclear at this time whether HEV transmission occurs while breastfeeding and therefore no formal recommendations can be made.

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Chronic Viral Hepatitis and Liver Transplantation

Kirti Shetty, MD, FACG

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Key Principles

- Chronic hepatitis B and C are the most common etiological factors worldwide for cirrhosis and hepatocellular carcinoma (HCC).
- Liver transplantation (LT) is the only option for those with complicated cirrhosis and early HCC.

• Outcomes of LT for viral hepatitis are compromised by recurrent viral infection, resulting in allograft failure.
• Combined passive immunoprophylaxis and the use of oral antiviral agents have dramatically improved outcomes in hepatitis B virus (HBV) related LT.
• Persistent challenges related to LT for HBV include the costs of immunoprophylaxis and the emergence of drug-resistant mutants.
• Hepatitis C virus (HCV) recurrence is encountered universally following LT. In an attempt to manage recurrence, interferon-based therapies are used pre-LT, preemptively post-LT, and for treatment of established disease.
• Present therapeutic regimens for post-LT HCV are limited by poor tolerability and limited efficacy. However, emerging evidence suggests a long-term beneficial effect in those patients achieving a sustained virological response (SVR).
• The role of hepatitis C immunoglobulin, and orally available small molecules such as polymerase and protease inhibitors, is not well studied in the post-LT population.
• Future strategies in HCV-related LT will require to focus on combinations of drugs that will effectively eradicate the virus and prevent long-term graft failure.

1. INTRODUCTION

Chronic viral hepatitis is an important contributor to liver disease worldwide. Globally, the two hepatotrophic viruses, hepatitis B and C, together constitute the most common etiological factor for cirrhosis and hepatocellular carcinoma (HCC) (1, 2).

Liver transplantation (LT) is the only option for those with advanced cirrhosis or early HCC which satisfies Milan criteria (3). In the United States, listing for LT is currently under the auspices of the United Network for Organ Sharing (UNOS), and organ allocation is based on the model for end-stage liver disease (MELD) score (4). Hepatitis C (HCV) cirrhosis is the most common indication for LT in this country, comprising 27% of all liver transplants performed, while hepatitis B (HBV) accounts for 4–6% of transplants (5).

Advances in immunosuppression and surgical technique have dramatically improved outcomes of LT over the past two decades. However, outcomes following LT for viral hepatitis have lagged behind those for conditions such as cholestatic and alcoholic liver disease. Compromised post-LT survivals are mainly attributable to a high rate of recurrence of the primary viral infection (5). This challenge has been
 admirably met in HBV with the development of highly efficacious preventative and therapeutic strategies. HCV, on the other hand, has continued to evade efforts to prevent post-LT recurrence and is the focus of intense ongoing study.

2. LIVER TRANSPLANTATION FOR VIRAL HEPATITIS B

In patients who develop HBV cirrhosis, 5-year survival rates range from 84% in those with compensated liver disease to 14% in decompensated cirrhosis (6). Thus, all patients with complications of HBV cirrhosis should be considered for LT. However, the initial LT experience for HBV was disappointing. Reinfecction, manifesting as detectable hepatitis B surface antigen (HBsAg), occurred in 80% of recipients (7, 8). Left untreated, cirrhosis developed within 1–2 years of reinfection, and 3-year patient survival rates were unacceptably low at under 50% (8, 9). Fortunately, rapid advances in antiviral therapy and immunoprophylaxis have dramatically decreased the rate of HBV reinfection to under 10%. Current 5-year patient survival rates following LT for HBV are in the range of 70–75% (10), transforming our attitudes toward a disease previously considered to be a relative contraindication for LT.

3. POST-LT HBV RECURRENCE

3.1. Mechanisms of Recurrent Hepatitis B

Without prophylaxis, reinfection of graft tissue generally occurs within 16 months of transplantation, with a range of 2–37 months (11). Various mechanisms of reinfection have been identified. Immunosuppressant therapy, in particular steroids and azathioprine, has been demonstrated to have a stimulatory effect on viral replication in vitro. Steroid therapy enhances the glucocorticoid-responsive element in the HBV genome, resulting in enhanced HBV replication (12). Another contributory factor is the presence of extrahepatic reservoirs of HBV such as the spleen, peripheral mononuclear cells, and thyroid (13, 14). LT recipients may also develop HBV reinfection due to the emergence of resistant and immune escape mutants (15, 16). A point mutation in the S gene results in the disruption of the antigenicity of HBsAg, allowing escape from neutralization by Hepatitis B Immune Globulin (HBIG). These mutations have been described in patients who received HBIG alone or HBIG plus lamivudine monotherapy after LT (16, 17).
3.2. Patterns of Recurrent Hepatitis B

HBV reinfecion typically first manifests by elevations in alanine aminotransferase (ALT) and detectable serum HBV DNA, which often precedes detection of HBsAg (18). Reinfecion presents along a spectrum of hepatic injury, ranging from asymptomatic transaminase elevation to chronic hepatitis, subacute graft failure, and cirrhosis (19). A severe variant of HBV-associated liver injury occurs in 5–10% of LT recipients and is termed fibrosing cholestatic hepatitis (FCH) (20). This entity is characterized histologically by prominent cholestasis and fibrosis, minimal necroinflammation, and intense expression of HBsAg and HB core antigen. The mortality of FCH in the pretreatment era was 100%.

3.3. Factors Affecting HBV Recurrence

Several studies have explored risk factors that may impact on the severity and timing of HBV recurrence (Table 1). The best-studied recipient variable is pretransplantation HBV DNA. Several studies have demonstrated that elevated viral load at the time of transplantation representing active viral replication portends a poor outcome (21, 22). In a review of 177 patients by Marzano et al. (21), a linear relationship was

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<td>Risk Factors Associated with HBV Recurrence After</td>
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<td><strong>High risk</strong></td>
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<td>HBV DNA &gt;100,000 copies/mL prior to transplant</td>
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<td>Evidence of antiviral resistance prior to transplant</td>
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<td>HBeAg positivity in recipient</td>
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<td>HBIG monotherapy</td>
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<td>Shorter interval to HBV DNA detection after transplant</td>
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<td>Anti-HBc positivity in donor organ</td>
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<td>High-dose immunosuppression after transplant</td>
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<td><strong>Low risk</strong></td>
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<td>Combined HBIG and antiviral prophylaxis</td>
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noted between HBV DNA level and recurrence risk. Other recipient factors affecting outcomes adversely are pre-transplant antiviral resistance, HBeAg positivity, HBIG monophylaxis, shorter interval to HBV DNA positivity after transplantation, and the presence of HCC (11, 23, 24, 25). Factors predicting a lower risk of recurrence include hepatitis D coinfection, fulminating hepatitis B infection, and HBeAg negativity (26). There is little data to suggest age and race as independent risk factors for compromised outcomes; several case series have demonstrated that Asian race does not predict poor outcomes as originally believed (27, 28).

4. ANTIVIRAL THERAPY

The primary goal of antiviral therapy in patients with chronic HBV awaiting LT is to maintain viral suppression. The secondary goals are to improve or stabilize cirrhosis complications and to decrease the rate of HBV recurrence post-LT.

Following LT, the goal of therapy is to prevent reinfection of the graft. If reinfection occurs, the goal then becomes to treat the infection adequately so as to prevent graft failure and the need for retransplantation.

4.1. Treatment Prior to LT

Antiviral therapy has been demonstrated to provide clinical benefit in patients with HBV cirrhosis. According to guidelines from the American Association for the Study of Liver Diseases (AASLD) (29), treatment should be reserved for cirrhotic patients who have HBV DNA levels >2000 IU/mL or an elevated ALT associated with HBV DNA levels between 200 and 2000 IU/ml. Patients with decompensated cirrhosis should be referred for LT evaluation. A separate treatment algorithm proposed by an US expert panel (30) liberalizes treatment to all patients with decompensated cirrhosis, irrespective of HBV DNA level (Fig. 1).

**Antiviral Agents in the Management of HBV Cirrhosis:** Five nucleotide/nucleoside analogues (lamivudine, adefovir, entecavir, telbivudine, and most recently, tenofovir) and the interferons (peginterferon alpha-2a and standard interferon alpha-2b) are approved by the Food and Drug Administration (FDA) for the treatment of chronic hepatitis B (CHB).

**Interferon alpha-2b/Pegylated interferon:** The interferons are contraindicated in patients with cirrhosis, as they may exacerbate underlying liver disease.
**Lamivudine:** This was the first orally administered antiviral agent approved for the treatment of CHB and the first agent demonstrated to have utility in those with decompensated HBV cirrhosis. A retrospective analysis of 154 North American patients with decompensated HBV cirrhosis who were treated with lamivudine demonstrated a biphasic survival pattern, with most mortalities (78%) occurring within the first 6 months of initiating lamivudine therapy (31). For those who lived beyond 6 months, the 3-year survival approximated 88%. It appears that those with advanced HBV cirrhosis may be stratified into two groups: those who will require expedited liver transplantation and those who will enjoy prolonged survival with antiviral therapy. The three major risk factors associated with 6-month mortality were baseline serum bilirubin, creatinine, and HBV DNA \( >7 \times 10^5 \) copies/ml. Stabilization or improvement in liver biochemistry was demonstrated in a multi-center trial, which showed that the actuarial survival of treated patients was better than that of untreated historical controls (32) This was also confirmed in a single-center series (33).

The main limitation of lamivudine is the emergence of drug-resistant mutants, which increases to 65% after 5 years of treatment (34). Those with advanced liver disease have cumulative rates of lamivudine resistance approximating 15%–20% annually (35, 36).

With the advent of newer oral antiviral agents with demonstrably lower resistance rates, the utility of lamivudine monotherapy is limited. This drug can no longer be recommended as first-line therapy.
**Adefovir dipivoxil** is a nucleotide analogue of adenosine monophosphate and can inhibit replication of wild-type (37) and lamivudine-resistant HBV (38). A large multicenter trial (39) studied a total of 324 patients (128 pre-LT and 196 post-LT) with lamivudine-resistant HBV. In the pre-LT population, addition of adefovir was associated with undetectable serum HBV DNA levels in 81% of patients. No resistance was identified after 48 weeks of therapy. Follow-up data on 226 patients showed that viral suppression was maintained in 65% of patients after 96 weeks of treatment with accompanying improvement in liver function (and Child-Pugh scores) (40).

Resistance to adefovir develops at a lower rate compared to lamivudine. Recent studies have demonstrated that adding adefovir to lamivudine rather than switching to adefovir is more efficacious and results in lower resistance rates (41).

The major concern with adefovir use in cirrhosis is the potential for nephrotoxicity. At the approved dose of 10 mg daily, nephrotoxicity (which is defined as an increase in creatinine to 0.5 mg/dL above baseline) was observed in 2% of treated patients but did not prompt discontinuation of the medication (39). The extent to which renal dysfunction was solely attributable to adefovir is debatable, given the multiple other etiologies for renal disease in this population. However, dosing interval modification is recommended for those patients with creatinine clearance under 50 mL/min.

**Entecavir:** This is an oral nucleoside analogue that inhibits hepatitis B viral polymerase, reducing viral DNA synthesis. This drug has more potent antiviral activity than lamivudine. It is effective in suppressing lamivudine-resistant HBV but has less antiviral activity in this subgroup as compared to wild-type HBV. Clinical trials in patients with compensated liver disease have shown an excellent safety profile (42). However, its use has not been well studied in those with decompensated cirrhosis. Resistance to entecavir has not been observed up to 2 years of treatment in nucleoside-naïve patients but has been reported in 7% of patients with prior lamivudine resistance. The possibility of cross-resistance makes entecavir an unattractive choice for monotherapy in lamivudine-resistant patients.

**Telbivudine:** Telbivudine is a nucleoside analogue and a potent inhibitor of HBV DNA polymerase. The GLOBE trial, a randomized phase III study, established the superiority of telbivudine to lamivudine in HBeAg-positive and HBeAg-negative patients after 1 and 2 years of therapy (43). Resistance to telbivudine has been reported as 4.4% and 21.6% in HBeAg-positive patients and 2.7% and 8.6% in HBeAg-negative patients after 1 and 2 years of therapy, respectively (43). Cross-resistance of telbivudine-resistant mutations to lamivudine
makes telbivudine unsuitable for use as monotherapy or in the treatment of lamivudine-refractory HBV. Its use in decompensated cirrhosis has not been studied.

**Tenofovir:** Tenofovir is structurally related to adefovir and has been in clinical use for the treatment of human immunodeficiency virus (HIV). It was recently approved by the FDA in August 2008 for treatment of CHB. It was more potent than adefovir in achieving viral suppression defined as <400 copies/mL (76% versus 13%), histological improvement (67% versus 12%), and higher rates of HBsAg loss (3.2% versus 0%) at 48 weeks in patients with HBeAg-positive CHB (44). Both adefovir and tenofovir were well tolerated in these studies, with no evidence of significant renal toxicity. No resistance to tenofovir has been detected to date. Tenofovir has been shown to be effective against lamivudine- and adefovir-resistant HBV. Its use in decompensated cirrhosis has not been studied.

**Emtricitabine:** Emtricitabine is a nucleoside analogue structurally similar to lamivudine that is currently approved for use with other antiretroviral drugs in the treatment of HIV infection, but not for HBV. It demonstrates activity against HBV and shares the same drug-resistant mutation as lamivudine (M204V/I ± L180M). A 48-week course of therapy with emtricitabine is associated with a 62% improvement in fibrosis and inflammation compared to 25% in the placebo group (45). Viral suppression to <400 copies/mL was achieved in 54% versus 2% in the emtricitabine and placebo groups, respectively. Resistance development occurred in 9% of patients after 1 year and 13% after 2 years of therapy. The rate of emergence of resistance and cross-resistance with lamivudine limits the use of emtricitabine as monotherapy in the management of CHB. However, it is a good candidate for use with other antiviral agents and is currently being tested in combination with tenofovir in the management of CHB.

### 4.2. Management of Hepatitis B in Liver Transplant Recipients

#### 4.2.1. Prevention of Hepatitis B Following Liver Transplantation

**Hepatitis B Immune Globulin Prophylaxis for HBV Reinfection**

In 1993, the European Concerted Action on Viral Hepatitis study demonstrated for the first time that perioperative and long-term administration of hepatitis B immunoglobulin (HBIG) following LT resulted in decreased reinfection rates and improved survival (46). This study demonstrated that HBIG administered over a longer term (>6 months) led to a 33% recurrence rate as opposed to the 75% recurrence rate in
those receiving no immunoprophylaxis. Improved patient survival was also noted in the long-term treatment arm (46). Recurrent disease, however, was frequent when HBIg was discontinued. Subsequent protocols used high-dose antibody therapy for longer or indefinite periods, with even more efficacious results. In recent years, combination therapy of HBIg and nucleoside/nucleotide analogues has been proven to be most effective.

**HBIg Preparations**

HBIg is prepared from human plasma containing high titers of antibodies to hepatitis B surface antigen (anti-HBs). Donors documented to be HBsAg-negative are immunized with FDA-licensed plasma-derived recombinant hepatitis B vaccine. High responders are selected for repeated plasmapheresis. After additional processing and sterilization procedures, this product is aliquoted into 5-ml vials for storage. The principal manufacturer in the United States currently provides a virologically safe product (NABI-HB, NABI – Rockville, MD) that is licensed for intramuscular OMLT use.

Despite the central role of HBIg in the management of post-LT HBV patients, its use was not officially sanctioned until recently. In March 2008, the FDA granted orphan drug exclusivity status to HepaGam B™ (Cangene Corporation, Winnipeg, Manitoba, Canada), a solvent and detergent-treated sterile solution of purified gamma globulin (5% or 50 mg/ml) containing >312 IU/mL of anti-HBs.

**Efficacy of HBIg**

The mechanisms whereby passive immunization prevents recurrent HBV infection are unknown. It is hypothesized that the exogenously administered antibody binds to virus in the serum, which limits viral entry into the new allograft (47, 48). Viral eradication does not seem to occur, as evidenced by the detection of HBV DNA in the liver, plasma, and peripheral blood mononuclear cells of HBsAg-negative patients (47).

HBIg monotherapy had been utilized in the past, and cumulative experience showed that the median rate of recurrent HBV infection was about 20% in those receiving long-term HBIg (49). The standard of care in the post-LT care of HBV-infected patients is now the use of HBIg in combination with nucleoside/nucleotide analogues, which halves recurrence rates to approximately 10%.

It has become increasingly clear that the determinant of efficacy is the titer of protective hepatitis B surface antibody (anti-HBs). Using “prevention of detectable HBsAg” as a treatment endpoint, pharmacokinetic studies showed that the anti-HBs titer needed to provide effective pro-
phylaxis was >500 U/L during the first week, >250 IU/L during weeks 2–12, and >100 IU/L after 12 weeks (50). Patients who were HBeAg-positive required more HBIG to achieve these target anti-HBs titers, especially in the first week post-LT.

Protocols for HBIG administration vary by transplant center. In general, most protocols utilize high-dose HBIG initially (10,000 IU) in the anhepatic phase, followed by 10,000 IU daily for the first post-LT week (48–50). After the early post-transplant period, frequency of HBIG administration is based on two different approaches. The first method is based on a fixed dosage of HBIG that estimates anti-HBs levels to be well above target levels (50). This method requires less frequent monitoring but is more expensive. The second method is based on adjusting the dosage and frequency of HBIG based on protective titers (48). This method saves costs by preventing excess doses of HBIG but requires more frequent monitoring.

**Limitations of HBIG Therapy**

The primary limitation of HBIG prophylaxis is cost. The estimated total cost per patient varies by report, but most regimens can range anywhere from $80,000 to $120,000 in the first year, followed by $50,000 to $60,000 in subsequent years (47, 51).

Other limitations include the need for parenteral administration (which adds significantly to cost), the frequency of side effects such as back or chest pain, headache, and flushing. The newer intravenous formulation of HBIG (NABI-HB) contains a lower protein content and is associated with a lower frequency of these side effects.

Another major limitation is HBV reinfection, despite treatment with HBIG. Two patterns of treatment failure are noted. The first is allograft infection with wild-type HBV in the early post-operative period and is presumably caused by inadequate doses of HBIG or high levels of viral replication pre-LT. The second type is noted after at least 6 months of HBIG therapy and is presumed to be due to the emergence of mutant virus. These mutants have been noted to revert to wild-type HBV following cessation of HBIG therapy (52, 53).

**Combination Therapy with HBIG and Lamivudine**

The combination of lamivudine and HBIG may be synergistic. Since lamivudine is a potent inhibitor of viral replication, the viral binding capacity of HBIG is less likely to be overwhelmed. Overall, HBV recurrence rates with combination therapy are less than 10% at 2 years following LT, with HBV DNA levels by PCR being undetectable in 90% of patients (54, 55). A recent meta-analysis of six high-quality studies examining the use of a combination of HBIG and lamivudine confirmed
the efficacy of such an approach. The cumulative rate of HBV recurrence in the HBIG and lamivudine combination therapy group versus HBIG alone was 4.1% versus 36.1%, respectively; the HBV-related death and all-cause mortality rates were 0.8% versus 15.1% and 5.1% versus 22.2%, respectively (56).

**Combination Therapy with HBIG and Adefovir**

For those patients who develop lamivudine-resistant mutants while receiving the drug in the pre-transplant setting or who become primarily infected with a lamivudine-resistant mutant, the use of lamivudine for post-LT prophylaxis is inappropriate. Adefovir has been demonstrated to improve post-LT outcomes in such patients. A study of 241 LT recipients with lamivudine-resistant HBV who were treated with adefovir, achieved HBV DNA loss of 65% at 96%. The cumulative rate of adefovir resistance was 2% at 96 and 144 weeks (40). Another study of 16 lamivudine-resistant patients examined response to lamivudine and adefovir, with or without HBIG. All patients cleared HBV DNA with no evidence of recurrence (57). This suggests that add-on adefovir is the preferred approach in patients with demonstrated lamivudine resistance. An area of future investigation is whether adefovir should be utilized in combination with lamivudine and HBIG. The theoretical benefits of a combined approach would be that breakthrough mutations to either drug are significantly diminished.

**Use of Intramuscular HBIG**

Several centers have used intramuscular instead of intravenous HBIG, after the first week post-LT. Intramuscular HBIG administration has several unique characteristics. The intramuscular route has a depot effect with maximal intravascular levels of antibodies reached between 3 and 5 days following the injection. Also, only 40–45% of the antibodies administered pass into the intravascular compartment. However, levels of anti-HBs > 100 IU/L are achieved in most patients and overall, this approach is far less expensive. Gane et al. in a recent study examined a group of 147 patients, of which 85% were HBV DNA positive prior to transplantation (58). These patients received low-dose IM HBIG (400 IU) daily for 1 week and monthly thereafter, combined with daily lamivudine. Five-year survival and HBV reinfecion rates were 88% and 4%, respectively, very comparable to results achieved with intravenous HBIG.

**Discontinuation of Therapy**

Several studies have examined the effect of discontinuing HBIG. In a study of 21 patients, HBIG was discontinued at a median of 26 months
after LT; lamivudine and/or adefovir was continued (59). At 48 weeks after HBIG cessation, two patients developed HBV recurrence, one of whom was noncompliant with the oral antiviral agent. This small study suggests that HBIG discontinuation with ongoing maintenance therapy using oral antivirals may be effective in preventing HBV recurrence. However, there is a lack of high-quality controlled data examining this issue, and no current guidelines exist in terms of discontinuing post-LT HBIG prophylaxis.

4.2.2. Treatment of Recurrent Hepatitis B Post-LT

Post-LT hepatitis B infection is defined as the detection of HBsAg, measurable HBV DNA, and elevated ALT. Retransplantation in hepatitis B is associated with high mortality if graft failure is caused by recurrent viral disease, rather than other etiologies (60). It is therefore important to treat recurrent hepatitis B aggressively before graft failure occurs. Lamivudine has been used in this setting for several years with excellent efficacy and safety profile (61–63). In a large multicenter trial involving 52 recipients with recurrent HBV, the use of lamivudine for 1 year led to HBV DNA suppression and HBeAg loss in 60% and 30% of cases, respectively (63). Unfortunately, YMDD mutations developed in 27–30% of recipients as early as 7 months after treatment initiation. Adefovir has been shown to be effective against lamivudine-resistant HBV. Successful rescue therapy with the addition of adefovir to lamivudine has been shown to reverse acute graft failure and severe fibrosing cholestatic hepatitis due to lamivudine- and HBIG-resistant HBV (64, 65).

Entecavir may be useful in adefovir-resistant and lamivudine-resistant HBV, but due to its cross-resistance with lamivudine is not optimal. Tenofovir has shown excellent potency against wild-type lamivudine- and adefovir-resistant HBV. However, scanty data exist regarding the role and efficacy of both entecavir and tenofovir in the post-transplant setting. An early report raised concerns of interferon-induced allograft rejection in this population, and interferon is not commonly used, given the variety of alternative therapeutic choices (66).

5. Liver Transplantation for Viral Hepatitis C

Based on current estimates, the incidence of HCV-related cirrhosis and hepatocellular carcinoma is expected to peak by the year 2008, leading to an increased demand for transplantation in HCV-infected individuals (67, 68). Unfortunately, reinfection of the hepatic allograft by hepatitis C is unavoidable, with a high incidence of accelerated liver injury and subsequent graft failure. The management of recurrent HCV
is one of the most daunting challenges faced by those who care for LT recipients.

6. POST-LT RECURRENCE OF HCV

6.1. Natural History of Recurrent Hepatitis C

Individuals who are viremic at the time of transplantation experience universal virological recurrence of disease. Some evidence of histologic recurrence seems inevitable but the natural history of recurrent HCV is variable and incompletely understood. One-third of patients will have minimal hepatic fibrosis at 5 years of follow-up. In others, progression of liver injury is alarmingly rapid, with cirrhosis developing in 10–25% within 5 years (69). Fibrosis progression in recurrent hepatitis C appears to be more rapid than in nontransplant patients and is nonlinear. Patients with high histologic activity within the first year post-transplant are at greatest risk of progressive fibrosis and cirrhosis (70). Most of those with cirrhosis develop decompensation and death within 1 year. In contrast, only 20% of immune-competent patients develop cirrhosis after 20 years of HCV infection, and only 3–6% of these decompensate each year. The negative impact of recurrent HCV on post-LT survival has been illustrated in a retrospective cohort study of over 11000 transplant recipients (71).

6.2. Kinetics of Post-Transplant HCV Infection

Negative-strand HCV RNA, the most reliable indicator of HCV replication, has been detected in the first week following liver transplantation. A detailed report of HCV kinetics found that HCV RNA concentrations dropped dramatically during the anhepatic phase and again 8–24 h after reperfusion (72). Viral levels increased at 24–72 h post-transplant and peaked between months 1 and 4. At 1-year post-LT, HCV RNA levels were $1-\log_{10}$ higher than pre-transplant levels.

6.3. Patterns of Recurrent hepatitis C

Approximately 70% of patients develop acute hepatitis, usually in the first 3–6 months after LT, which then evolves to chronic hepatitis. Chronic hepatitis without a preceding acute hepatitis occurs in about 20% of patients and has a less aggressive course. A severe and rapidly progressive form of the disease is denoted as the fibrosing cholestatic variety (73), characterized by histological features of ballooned hepatocytes, confluent necrosis, bile duct proliferation, and cholestasis. While fibrosing cholestatic hepatitis C has been historically associated with
dismal outcomes, recent studies have suggested prolongation of survival with the use of antiviral agents.

6.4. Factors Affecting HCV Recurrence

A number of studies have attempted to identify the factors that impact on the severity and timing of HCV recurrence (Table 2). These may broadly be classified as follows:

1. **Viral Factors**: Pre-transplant viral titers have been associated with compromised patient and graft survival (74). Multiple studies have, however, demonstrated a lack of correlation between post-transplant viral titers and histologic severity of disease. HCV genotype and quasispecies have a variable effect on recurrence.

2. **Recipient Factors**: Several recipient variables have been associated with more severe recurrence. The most reliable of these include advanced recipient age and non-Caucasian race (75).

3. **Donor Factors**: Those associated with negative outcome include donor age >50 years (75) and high donor fat content. Prolonged warm and

<table>
<thead>
<tr>
<th>Factor</th>
<th>Association</th>
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<tbody>
<tr>
<td><strong>Recipient-related factors</strong></td>
<td></td>
</tr>
<tr>
<td>Female gender</td>
<td>Established (survival)</td>
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<tr>
<td>Age</td>
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<tr>
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<td>Established (survival, severity)</td>
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<tr>
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<td>Established (survival, severity)</td>
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<tr>
<td><strong>Donor-related factors</strong></td>
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<tr>
<td>Age &gt; 50 years</td>
<td>Established (severity, survival)</td>
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<td>Allograft fat content</td>
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<tr>
<td><strong>Viral factors</strong></td>
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<tr>
<td>Pre-OLT viral load</td>
<td>Established (severity)</td>
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<tr>
<td>Genotype 1b</td>
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<tr>
<td>Diversity of quasispecies</td>
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<tr>
<td><strong>Clinical factors</strong></td>
<td></td>
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<tr>
<td>CMV infection</td>
<td>Established (severity)</td>
</tr>
<tr>
<td>Treatment of acute rejection (OKT3, corticosteroids)</td>
<td>Established (severity)</td>
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cold ischemic time may be risk factors for early recurrence but are not well established. The utilization of living donors for HCV-infected recipients is controversial. Theoretically, living donors may offer significant advantages including the opportunity for pre-transplant antiviral therapy, shortened liver allograft cold ischemic time, and younger donor age. However, biological mechanisms that may potentiate adverse outcomes in this setting include enhanced HCV proliferation due to rapidly regenerating hepatocytes, histocompatibility leukocyte antigen homology between donor and recipient, and a higher rate of biliary complications, which may act synergistically to damage the allograft.

While two studies (76, 77) suggest that living donation may be associated with more rapid HCV recurrence, the majority of studies point to no differences in graft and patient survival or histological findings (78, 79). Emerging data from the multicenter NIH-sponsored study of living donor liver transplantation (LDLT) suggest a neutral effect on allograft survival (80). In an era marked by a shortage of donor organs, LDLT should not be withheld from potential HCV-positive recipients until the outcome data are better defined.

4. Clinical Factors:

(a) CMV infection has been strongly associated with increased severity of recurrence (81)

(b) Impact of immunosuppression

(i) Corticosteroids: if used for acute cellular rejection, steroids are associated with a significant rise in HCV RNA levels and with increased mortality/grant loss (relative risk = 2.7–2.9, p = 0.04) (82).

(ii) Calcineurin Inhibitors: there appear to be no significant differences in the severity of histological recurrence of hepatitis C among recipients receiving tacrolimus when compared to those receiving cyclosporine (83).

(iii) Mycophenolate mofetil (MMF): a recent analysis of over 1100 liver transplant recipients demonstrated that MMF triple therapy at discharge was associated with a reduced risk of death, graft loss, acute rejection, and death from infectious complications (84). While other smaller studies have suggested a slightly higher risk of HCV recurrence associated with MMF, the overall impact of MMF appears to be neutral or beneficial.

(iv) T-cell Depleting Therapies: steroid-resistant rejection and OKT3 administration appear to be significant risk factors for the rapidity and severity of histological recurrence of HCV (85). Data on the impact of thymoglobulin on HCV recurrence are lacking but this agent should probably be used with great caution in this setting. Interleukin-2 receptor antibodies have been reported to be associated with more severe HCV recur-
rence in small single-center studies. Long-term results from a large randomized controlled study of these agents in HCV-infected recipients are awaited.

7. ANTIVIRAL THERAPY

The current standard of therapy for chronic hepatitis C is pegylated interferon (PEG-IFN) weekly and daily ribavirin (RBV) orally. Unfortunately, this therapy is poorly tolerated even in the nontransplant patient and offers many challenges in a transplant population. Interferon is associated with bone marrow depression, increased susceptibility to infection, and may precipitate acute rejection. RBV causes hemolytic anemia, which may be severe if dose adjustment is not made for renal disease.

However, given the absence of alternative therapies, and the recognized impact of HCV recurrence on graft and patient survival, interferon-based therapies have been utilized in a number of different strategies as outlined in the following section.

Therapeutic intervention may be offered at three specific time points (Fig. 2).

7.1. Prior to LT (Pre-Transplant Antiviral Therapy)

It aims to eradicate or suppress virus prior to engraftment.

Pre-Transplant Antiviral Therapy: A pilot study of the tolerability and efficacy of pre-transplant antiviral therapy (86) randomized patients with HCV cirrhosis to varying doses of nonpegylated interferon, combined with RBV. Of the 15 who were randomized, none achieved a sustained virological response (SVR). The majority (86%) experienced side effects, and the study was terminated prematurely due to the unac-

Fig. 2. Time points for therapeutic intervention in HCV patients undergoing LT.
ceptably high occurrence of serious adverse events, including a fatal infection.

A larger single-center study from the University of Colorado (87) utilized the principle of a low accelerated dose regimen (LADR). SVR was achieved in only 11% of patients infected with genotype 1, and two deaths were noted during the treatment period. Encouragingly, of 10 patients who underwent LT after achieving SVR, none experienced recurrent HCV.

These studies offer several important insights into antiviral therapy directed at those with HCV cirrhosis. Those with advanced liver disease are at risk for life-threatening complications including accelerated liver failure and severe infections. Suitable candidates for therapy therefore include patients with adequately compensated liver disease (Child-Pugh class A or B, MELD score 18). These patients often require hemopoietic growth factors in order to maintain full dose therapy. Close attention should be paid to renal function which if compromised, may contribute to RBV toxicity.

7.2. Treatment of LT Recipients

(i) **Preemptive Therapies**: administered immediately post-OLT to prevent infection of the allograft.

(ii) **Prophylactic Therapies**: administered in an attempt to neutralize the infection of the allograft.

(iii) **Therapy of recurrent disease**: instituted after documentation of established disease.

(i) **Preemptive Therapy**: this refers to the initiation of therapy in the early post-transplant period when HCV viral loads are lower than pre-transplant levels, and histologic disease is absent or minimal. Even though there is a theoretical advantage to such an approach, tolerability is a marked limitation.

An uncontrolled Italian trial utilizing combination standard IFN alpha-2b and RBV within 3 weeks after transplantation and continuing for 52 weeks reported an SVR of 33% (88). Two randomized controlled US studies have examined the use of preemptive pegylated interferon therapies. Shergill et al. utilized standard IFN 3 MU thrice weekly or PEG-IFN alpha-2b 1.5 mcg/kg/wk alone or in combination with RBV and continued treatment to a total of 48 weeks (89). Chalasani and associates studied PEG-IFN alpha-2b 180 mcg weekly for 12 months. SVR was noted in only 8–9%, with dose reductions and discontinuations required in 85% and 37% of those treated (90).

LDLT recipients offer an attractive group within which to offer preemptive therapy, as they often have relatively well-preserved liver dis-
ease. One uncontrolled study (91) of 21 HCV-infected LDLT recipients demonstrated an SVR of 43% on combination therapy with IFN alpha-2b and RBV.

Overall, it is not clear as to whether preemptive therapy of recurrent HCV offers improved outcomes. Proponents of such an approach argue that HCV recurrence should and can be treated before graft damage has occurred. Efficacy is believed to be enhanced by treatment at an early stage. However, opponents of early treatment express concern that the risk of graft rejection may be increased and that early treatment does not permit segregation of patients into those with mild versus those with more aggressive graft reinfection.

(ii) Prophylactic Therapies: Preclinical and retrospective cohort studies suggest that HCV antibodies have neutralizing effects and may attenuate the risk of HCV infection. Studies in chimpanzees using polyclonal anti-HCV-enriched immune globulin have demonstrated delay or prevention of acute infection when administered to the animal before inoculation of an infectious isolate (92).

In humans, the neutralizing antibodies generated in individual patients are isolate-specific and are directed against epitopes encoded within the hypervariable region of the HCV envelope gene. Two prospective studies have tested the efficacy of a polyclonal immune globulin preparation enriched in anti-HCV. A preliminary report of the Canadian hepatitis C immunoglobulin (HCIG) study found no benefit, with similar levels of viremia and histologic disease between treated and untreated groups (93). A second multicenter trial in the United States (94) evaluated 18 patients – 6 received high-dose HCIG (Cicavir), 6 received low-dose therapy, and 6 were untreated. No difference in serum HCV RNA was noted between the groups, although a decrease in hepatic HCV RNA post-LT was observed in the group treated with high-dose HCIG.

A third study utilizing a fully human monoclonal antibody that binds to the E2 envelope protein of HCV (HCV-AbXTL68) has also been reported (95). In this multicenter, randomized phase 2 study, 24 HCV-positive patients were administered varying-dose infusions initiated immediately post-LT. None of the patients achieved complete viral suppression, although high-dose therapy did produce a transient median decline from the baseline viral load. The lack of a meaningful clinical effect in any of the HCIG studies suggests the need for further refinement of what may prove to be a promising approach.

(iii) Antiviral Therapy for Established Recurrent HCV: Potential advantages of waiting to treat HCV until documented histological or clinical disease include the following: lower doses of immunosuppression, improved tolerance of therapy, lower risk of acute rejection, and cost savings.
Results of antiviral therapy for established recurrence have been disappointing with SVR rates significantly lower than in nontransplant populations. Most studies evaluating IFN-based therapies are uncontrolled, use variable doses and duration of IFN and RBV therapy, and are of a sample size too limited to perform multivariate analyses to identify predictors of response. Adverse events are common with treatment withdrawal required in about 50% of patients.

In the trials enrolling more than 20 patients evaluating the efficacy of pegylated interferon with RBV in liver transplant recipients, SVR rates have ranged from 12 to 45% (90, 96–103).

Carrion et al. reported an important randomized controlled trial, which was the first to assess the impact of combination therapy on the development of fibrosis and hepatic venous pressure gradient (HVPG) (102). The only variable independently associated with fibrosis improvement/stabilization was treatment (odds ratio [OR] =3.7, 95% confidence interval [CI] 1.3–10, P = 0.009). This data demonstrates that antiviral therapy slows disease progression (particularly in sustained virological responders), as shown by its effects on liver histology and on HVPG. Berenguer et al. (103) have demonstrated that SVR was associated with enhanced survival.

Several important lessons may be learned from these studies. As in the nontransplant setting, PEG-IFN with RBV achieves higher response rates than PEG-IFN monotherapy or standard IFN with RBV. At least one retrospective study suggests that the presence of fibrosis in the transplant population does not significantly reduce the likelihood of virologic response (100), and so an approach of waiting for progression is appropriate. Whether empiric use of growth factors should be used initially to allow full dosing from the start requires further analysis. As reduced doses of PEG-IFN are not associated with reduced efficacy in the nontransplant setting, it seems reasonable to start liver transplant recipients at these lower doses. RBV dosing is much more difficult. Since RBV levels are likely to be more important than doses and are the determinants of response, dose adjustments of RBV according to levels seems logical, although not always clinically feasible.

### 7.3. IFN and the Risk of Rejection in Liver Transplant Recipients

A potentially serious and somewhat controversial complication of antiviral therapy in transplant patients is rejection. Two uncontrolled treatment trials (104, 105) of recurrent disease showed acute cellular rejection rates between 11 and 30%, much higher than in randomized controlled trials. This data suggests that interferon therapy may be a risk factor for rejection, an issue which requires further study. Data
from the University of Colorado (106) suggest that close monitoring of calcineurin inhibitor levels is necessary during antiviral therapy as a greater proportion of antiviral responders experience rejection than nonresponders, presumably since improved hepatic function leads to enhanced biotransformation and lower immunosuppression levels.

**Retransplantation for recurrent hepatitis C virus:** The only definitive treatment for graft failure is retransplantation. At present, retransplants account for 8–9% of all LTs, with recurrent HCV infection comprising approximately 40% of retransplant volumes. Outcomes of single-center studies comparing re-LT outcomes demonstrate inferior rates of success for HCV-associated retransplants, as compared to retransplantation for other indications (107, 108). Predictors of poor outcome after retransplantation include recipient age over 50 years, serum creatinine >2.0 mg/dL, serum bilirubin >10 mg/dL, and poor physical condition. Additionally those with a longer period of time from first to second transplant seem to have a better outcome. Retransplantation for recurrent HCV disease remains a controversial issue. Most transplant programs use a selective approach in limiting this option to patients with favorable clinical characteristics.

8. THE USE OF VIROLOGICALLY COMPROMISED ORGANS IN LIVER TRANSPLANTATION

The emergence of LT as the standard of care in complicated cirrhosis has predictably resulted in a widening disparity between organ availability and demand. In an effort to expand the pool of utilisable organs, many centers will use less than perfect or extended donor criteria organs. Among other criteria, these include organs from patients infected with HCV or exposed to HBV.

**8.1. Use of HCV-Infected Grafts**

Several studies (109–111), including analyses of the UNOS database, have confirmed that the use of HCV-positive grafts in HCV-infected recipients has similar outcomes as compared to the transplantation of HCV-negative grafts. The analysis of donor liver histology is mandatory and only organs with little or no fibrosis are utilized.

**8.2. Use of Anti-Hepatitis B Core (Anti-HBc-Positive) Grafts**

This has become an accepted clinical option, but the selection of recipients for these potentially infectious organs deserves close attention. Those undergoing LT for HBV cirrhosis should, of course, receive primary consideration for such organs since the post-LT immuno-
prophylaxis that they receive would prevent de novo HBV infection. Recipients who are anti-HBc-positive, with or without anti-HBs, are at low risk of de novo HBV (range 0–13%), as are recipients who are anti-HBc-negative, anti-HBs-positive (range 0–11%) (112–115). The highest risk of de novo HBV occurs in those who are negative for both anti-HBc and anti-HBs (116–118) and these patients should be offered anti-HBc-positive organs only in case of extenuating circumstances such as HCC or limited life expectancy without LT. There is a variability in prophylactic practices across transplant centers. Several centers utilize nucleoside/nucleotide analogues along with HBIG in high-risk recipients (119). However, the optimal regimen has not yet been established.

9. LIVER TRANSPLANTATION IN HIV COINFECTED INDIVIDUALS

Patients coinfected with HIV and either HCV or HBV are living longer due to the beneficial effects of highly active antiretroviral therapy (HAART). Unfortunately, complications of end-stage liver disease now account for a large proportion of deaths in this group (120). While HIV was initially considered a contraindication for LT, a pilot study by Stock was pivotal in reassessing the role of LT in this population (121). A recent analysis of the UNOS database suggested that patients with HIV and viral coinfections (especially HCV) had compromised survival as compared to HIV-negative individuals (122). Currently the National Institutes of Health is sponsoring a prospective multicenter trial to analyze outcomes by enrolling 125 recipients who satisfy strict listing criteria at 20 transplant centers in the United States (www.HIVTransplant.com). Until results from this study are available, HIV-coinfected patients should not be denied LT unless other clear contraindications exist.

10. CONCLUSIONS

Liver transplantation is a life-saving option in those with complications of chronic viral hepatitis. However, recurrent viral infection in the allograft is a significant concern and impacts on post-LT outcomes and graft function. The use of immunoprophylaxis and oral antiviral agents has dramatically altered the treatment paradigm in patients transplanted for HBV, and this entity is now associated with excellent post-LT outcomes. Future efforts will have to focus on the optimal duration and dosing of immunoprophylaxis, prevention and management of multidrug-resistant HBV. Unfortunately, HCV
recurrence continues to be a significant clinical burden, associated with compromised post-LT outcomes. The role of hepatitis C immunoglobulin and protease/polymerase inhibitors has not yet been established in this population. Future challenges include an urgent need to gain insight into the pathogenesis of recurrent HCV and formulate effective and safe therapies. We will then be in a position to offer these patients liver transplantation not just as a temporizing measure but as a definitive therapy for their liver disease.

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Treatment of Viral Hepatitis in Children – 2008

Zachary R. Schneider and Parvathi Mohan

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Key Principles

- Chronic hepatitis B and C are still detected in infants and children despite widespread screening of blood donors, high-risk groups, and pregnant women.
- Incidence of hepatitis B has reduced significantly since the implementation of universal vaccination.
- Children with chronic hepatitis B and C are relatively asymptomatic, but may occasionally present with severe sequelae such as liver failure.

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A high degree of suspicion and careful evaluation are essential for the diagnosis and management of these children.

- Treatment options are limited, suboptimal, and should be considered on a case-by-case basis.
- Short- and long-term follow-up of infected children are needed to detect early signs of progression and decompensation of liver disease.
- Availability of newer drugs for more optimal and safer therapy, especially through clinical trials, is the best hope for the immediate future.

1. HEPATITIS B: INTRODUCTION

A 12-year-old girl presented at my institution with a history of recent jaundice and detection of elevation of liver functions. The mother had died of complications of hepatitis B and the child was recently found to be hepatitis B positive, but remained asymptomatic. Further testing including a sonographic study of the liver showed a large mass in the liver with extension to the inferior vena cava and an alpha fetoprotein (AFP) of over 20,000 ng/ml. The tumor was inoperable and the caregiver refused any diagnostic testing or treatment. The patient was lost to follow-up for 4 months, returned in profound multi-system failure and died shortly afterward. The total period of illness from the onset of jaundice to her demise was about 8 months.

This case illustrates an unfortunate, but fortunately rare scenario in a child with perinatally acquired hepatitis B (HBV) infection leading to devastating sequel. Hepatitis B, a small enveloped DNA virus from the family hepadnaviridae, causes acute and chronic liver disease in children just as in adults. Childhood infection appears to differ from that in the adult in terms of epidemiology, clinical features, and natural progression. The age of acquisition of the primary infection determines the propensity to develop chronic liver disease or a carrier state, and therefore, infants and children are at greater risk of a protracted course than adults. Transmission characteristics also differ by population and geographic regions of the world (1, 2, 3). In highly endemic areas of Asia and Africa, majority of infection occurs via vertical or perinatal transmission whereas in countries such as the United States, it is mainly by horizontal transmission through contact with body fluids, as among household contacts or institutionalized children, and sexual contact and intravenous drug use, particularly in the adolescents. HBV infection is preventable by vaccination and universal vaccination is becoming a worldwide priority. Thus, there has been a steady decrease in the incidence of HBV infection (from 1982 to 1998) with a 73% decline in
the adolescents (3). The incidence of acute infection has decreased to about 8–10% of new cases per year among children from 0 to 20 years. This has been achieved through routine screening of pregnant women, increasing use of vaccination, and improving practices among intravenous drug users (IVDU) (4). Nonetheless, HBV infection continues to be a major public health problem in endemic areas with a carrier rate approximating 15–20% and adds to the incidence of new cases in the United States through infected immigrants or adoptees from those areas (5, 6). The risk of developing hepatocellular carcinoma (HCC) is high in hyperendemic areas such as Southeast Asia and the Pacific islands although HCC develops frequently only in the presence of cirrhosis. Effective vaccination has controlled the incidence of HCC even in those areas and the annual incidence of HCC in children below 10 years has dropped from 0.7 per 100,000 to 0.36 per 100,000 between 1986 and 1990 (7).

2. NATURAL HISTORY

About 90% of perinatally infected infants develop chronic HBV infection and the rate of infection drops to 25–50% in toddlers and to 6–10% in older children, i.e., the younger the age of infection the higher the propensity for chronic infection (3, 4, 8). Transmission occurs mostly during birth, but is not related to the mode of delivery and is higher (90%) if mother is hepatitis B antigen (HBeAg) positive or if infected in the third trimester; this may be related to high maternal viral load and immaturity of the neonatal immune system (5, 6). The natural course of HBV infection is now recognized to consist of four phases: (a) an immune tolerant phase (presence of HBeAg, high HBV viral load, normal aminotransferase, and minimal liver disease); (b) an immune clearance phase (HBeAg positive chronic liver disease); (c) inactive HB surface antigen (HBsAg) positive carrier state (HBeAg negative, low HBV DNA, normal aminotransferases, and minimal liver histology changes); and (d) reactivation/HBeAg-negative chronic hepatitis B (negative HBeAg and positive HBe antibody (HBeAb), detectable HBV DNA and active liver inflammation with elevated aminotransferases) (8). The first phase may last for a varying period of time, even into adulthood unless the infection is acquired later in life, beyond infancy. Although sero-conversion to HBeAb is rare during this phase, children do well with minimal liver involvement, but treatment may not be effective in the face of normal or near normal aminotransferases (1, 8). HBeAg negative hepatitis is very rare in children.

Children with chronic HBV infection are generally asymptomatic and may be unaware of their infected status which increases the risk
of horizontal transmission. Liver disease is relatively mild during childhood and biopsies show mild inflammation and fibrosis although bridging fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC) have been reported in pre-teen children (9, 10, 11) Progression to HCC usually occurs over 30 years in 10–40% of those with HBV-related cirrhosis (12). In a study from Taiwan, rapid development of cirrhosis and HCC was found to occur early in life even in HBV carriers with low-positive levels of HBeAg and HBC antibody indicating an early seroconversion to HBeAb (13). Natural seroconversion may vary from 10 to 20% depending on host factors, but is found only in about 2–5% infants and children in endemic areas (7, 13, 14, 15).

3. SCREENING AND PREVENTION

Age at acquisition of HBV infection, is therefore, a major determinant of chronicity and, thus, early identification, appropriate prevention, therapy, and counseling are vital. Over the past two decades, this has been achieved through implementation of active immunization strategies in the United States spearheaded by the Center of Disease Control (CDC). Universal immunization of all infants at birth, routine screening of all pregnant women and immuno-prophylaxis of the newborns of infected or potentially infected mothers, routine immunization of children, adolescents, and adults previously not vaccinated have reduced the incidence of chronic HBV and prevented acute HBV infections significantly (16, 17). Groups at high risk for developing HBV infection include individuals born in areas of high or intermediate endemicity, close contacts of HBsAg positive individuals, IVDU, all pregnant women, inmates of correctional facilities, and those on hemodialysis (16, 18). Details of active and passive prophylaxis of HBV are beyond the scope for discussion in this chapter.

Management of chronic HBV infection in children should include the following considerations (1):

1. Establish evidence for chronic HBV infection with at least two positive HBsAg testing 6 months apart with a positive HBeAb (core antibody) IgG (may be occasionally a false positive).
2. Establish ALT (alanine aminotransferase) elevations for at least 3–6 months in children over 2 years.
3. Monitor yearly HBeAg and anti-HBeAg positivity in those with normal ALT.
4. A liver biopsy to assess extent of histological disease is desirable but not mandatory.
5. Monitor for clinical evidence of decompensated liver disease.
6. Immunize against hepatitis A.
7. Ensure HBV immunization of household/social contacts.
8. Check serum alpha-fetoprotein and liver sonogram yearly as screening tools for HCC.
9. Monitor for a period of time to ensure that there is no spontaneous seroconversion to HBeAb which precludes need for treatment.

4. TREATMENT

Treatment strategies for children with chronic HBV should aim to inhibit the viral replication, prevent active liver disease, and induce HBeAg seroconversion, and ideally, to eradicate the HBsAg. Children with chronic HBV infection can develop active liver disease at any time with potentially severe sequelae later in life and the currently available therapies, while not totally effective in eradicating the virus, may still contain or reduce the progression. But, children in the early immune tolerant phase typically maintain a high serum viral load and normal aminotransferases which are factors against successful outcome of treatment. The antiviral therapy is effective only in <5% in those with normal aminotransferases, 5–10% in those with ALT >1.5–2 times the upper limit of normal (ULN), and 25% if ALT is above 5 × ULN (5, 18). Currently, there are only a few options to treat HBV in children and they have major limitations and dubious success. Patients who develop chronic infection need to be evaluated individually and a strategy to treat should be developed based on age, laboratory values, and the phase of immunity as detailed above (1). Drugs approved for therapy in children, namely interferon (IFN), lamivudine (LAM), and recently, adefovir (ADV) do not consistently fulfill the goals of therapy; therefore, appropriate patient selection is critical to the success of the treatment. For the physician and the parent, it is often a complex and difficult decision to make, to treat or not to treat, especially if the practice of medicine should be based on the principle – “Physician! Do no harm”.

This chapter discusses the key studies on treatment of hepatitis B infected children, the main concerns/questions and future directions in this area. Through the years several guidelines have been developed which are helpful to the practicing pediatricians and hepatologists in making a decision to treat a child with chronic HBV (1, 5, 12). In 1999, a series of consensus statements were released by 18 researchers from Europe who pooled their experience in treating a large group of HBV-infected children. They agreed that (1) children with HBV DNA and HBeAg positivity should be considered for treatment to accelerate the clearance of HBeAg; (2) only children above 2 years should be treated because of potential growth retardation from IFN; (3) children should
maintain elevated ALT levels indicative of active hepatitis in order to have a good response to treatment; (4) the viral load should be low or intermediate; (5) a liver biopsy should be considered as an important tool to assess severity of inflammation and fibrosis; (6) treatment is contraindicated in children with decompensated liver disease, cytopenia, severe renal cardiac, or significant autoimmune diseases; hepatitis C and HIV were not excluded; and (7) A standard dose of 5 MU/m² of interferon three times weekly for 6 months should be used (12). Some of these principles are still applicable when considering treatment with newer, safer oral therapies.

5. IMMUNOMODULATORY THERAPY

5.1. Interferons

Interferon (IFN) alfa was the first drug used to treat chronic HBV in both adults and children. It has antiviral, anti-tumor, and immunomodulatory activities (6). Review of published data on treatment of HBV patients with IFN reveals a positive response in up to 58% of treated adult and pediatric patients in the western hemisphere whereas the response rate is much lower at 3–17% in the Asian continent which may be due to genetic and racial factors (4, 18). Elevated aminotransferases seem to reduce the racial differences in response to therapy and offer about 20–25% seroconversion even in endemic areas, when coupled with a younger age at treatment and low HBV DNA levels. Seroconversion may occur during or up to 12 months of completion of treatment. Doses ranging from 5 to 10 MU/m² (high) to 3–6 MU/m² (low) three times a week for 6–12 months have been used in various published reports and response rates are similar in children and adults and vary from 8 to 50% (Table 1). Response may be affected by the route of acquisition, ethnic origin, histological activity in the liver, and viral factors (Table 2). IFN may occasionally cause flares of hepatitis or elevated aminotransferases during treatment which should not be an indication to stop therapy (20).

Data derived from the various studies on IFN therapy in children are conflicting and not reassuring. One of the earlier, notable studies was a large multinational randomized controlled trial in 149 HBV infected children from 18 centers in Europe and the North American continent (23). Children between 1 and 17 years who had ALT levels X 2 ULN were treated with IFN alpha for 24 weeks. At the end of the study period, there was loss of HBeAg and DNA in 26% vs. 11% untreated controls and loss of HBsAg in 10% vs. 1% of controls; baseline ALT levels, viral load, or liver histology did not affect the response. Torre
Table 1
Treatment of Hepatitis B in Children – Key Studies

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Treated #</th>
<th>Age in years range/mean</th>
<th>Dose million U/m² (TIW)</th>
<th>Duration (weeks)</th>
<th>HBe conversion Rx/control (%)</th>
<th>HBsAg loss Rx/control (%)</th>
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<td>50</td>
<td>9 ± 3</td>
<td>3.6–7.2</td>
<td>12–24</td>
<td>36/10</td>
<td>——</td>
<td>21</td>
</tr>
<tr>
<td>Narkewitz 1995</td>
<td>9</td>
<td>5.7</td>
<td>5–6</td>
<td>16–24</td>
<td>26/11</td>
<td>10/1</td>
<td>22</td>
</tr>
<tr>
<td>Torre 1996</td>
<td>127</td>
<td>1.5–17</td>
<td>3–10</td>
<td>12–48</td>
<td>23/10</td>
<td>1.6/0</td>
<td>24</td>
</tr>
<tr>
<td>Sokal 1998</td>
<td>70</td>
<td>1–17</td>
<td>6</td>
<td>24</td>
<td>26/11</td>
<td>10/1</td>
<td>23</td>
</tr>
<tr>
<td>Bartolotti 2000</td>
<td>107</td>
<td>8.5</td>
<td>3–7.5</td>
<td>12–24</td>
<td>32/29</td>
<td>25/0</td>
<td>25</td>
</tr>
<tr>
<td>Gurakan 2000</td>
<td>30</td>
<td>10.6 ± 3</td>
<td>5–10</td>
<td>24</td>
<td>33–60 (2 doses)/..*</td>
<td>7/..*</td>
<td>26</td>
</tr>
<tr>
<td>Diem 2005</td>
<td>37</td>
<td>5.2 ± 3.8</td>
<td>6</td>
<td>16–24</td>
<td>47/33</td>
<td>9/4</td>
<td>27</td>
</tr>
<tr>
<td>Hsu 2008</td>
<td>21</td>
<td>14</td>
<td>3</td>
<td>24</td>
<td>86/89</td>
<td>9.5/4.8</td>
<td>28</td>
</tr>
</tbody>
</table>

*No placebo control group reported.
Table 2
Variables Associated with Treatment Success in Children with Chronic HBV Infection (Modified with permission from Elisofon et al. (1))

<table>
<thead>
<tr>
<th>Agent</th>
<th>Factors offering a higher response</th>
<th>Factors not affecting response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon</td>
<td>ALT &gt; twice normal for age&lt;br&gt;Female gender&lt;br&gt;HBV viral load &gt;10^5 copies/mL&lt;br&gt;Younger age at treatment (&lt;5 years) (29)&lt;br&gt;Older age at acquisition of infection&lt;br&gt;Active inflammation on liver biopsy&lt;br&gt;HBV genotype (19)</td>
<td>Ethnicity&lt;br&gt;Body surface area</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>Elevated ALT 9 &gt; twice normal for age&lt;br&gt;Active inflammation on baseline liver biopsy (high HAI score)</td>
<td>Baseline Viral load&lt;br&gt;Race/ethnicity/age/gender&lt;br&gt;Baseline BMI/weight&lt;br&gt;Previous interferon treatment</td>
</tr>
<tr>
<td>Adefovir (43)</td>
<td>Age &gt;12 years&lt;br&gt;Elevated ALT &gt;2.3 times normal for age&lt;br&gt;HBV DNA &lt; 8.8 log_{10} copies/ml</td>
<td></td>
</tr>
</tbody>
</table>

and Tambini performed a meta-analysis of six randomized controlled clinical trials involving 240 children from Europe and concluded that prolonged therapy over 6 months, a higher baseline ALT, but not a higher dose of IFN, led to a favorable response (24). In contrast, a higher dose of IFN was found to offer a higher response rate in another smaller study (26). In another large study of 107 children treated for 3–6 months and followed for 69 months, there was a high rate of HBeAg seroconversion (32% total, 15% during therapy and 17% in the follow-up period) indicative of short-term benefit from therapy, but no long-term benefits were observed in maintaining HBe seroconversion as compared to untreated children (25). Kobak et al. reported a favorable response from IFN therapy in clearing the HBsAg and HBeAg in children below 5 years (29). In a retrospective study on 22 children by chart review, 78% under 5 years vs. 23% over 5 years of age responded to therapy and aminotransferase levels did not affect the out-
come. Steroid priming has not been found to be effective (30). Recently Hsu et al. studied the short- and long-term effects of IFN therapy in young children with early acquisition of HBV infection. They found that the cumulative rate of virologic response at 1 year and 6 years after the end of treatment (with and without steroid priming) did not differ between treated and untreated patients. A lower pre-treatment viral load seemed to provide some beneficial effect on therapy (28). Overall, response to IFN alone has been disappointing.

5.2. Limitations

Limited efficiency and adverse effects restrict use of IFN in the treatment of children. The profile of adverse events is similar in children and adults. Side effects include, fever, flu-like symptoms, myalgia, headaches, and more serious ones such as neutropenia, thyroiditis, retinal changes, seizures, and depression. There is potential danger of worsening autoimmune disorders and compensated cirrhosis. The use of IFN in younger children has been of concern due to its potential effect on growth, although growth failure is reversible, but use of IFN under 2 years of age is not advisable (1, 31). Contraindications to the use of IFN include decompensated cirrhosis, renal disease, neutropenia, seizures, cardiac disease, autoimmune disease, and clinical depression – patients with these concomitant conditions should be either excluded from IFN treatment or should be treated with very close monitoring.

5.3. Oral Antiviral Agents

More recent antiviral agents many of which are already approved for use in adults with HBV infection are being considered for treatment of children with HBV, either as approved drugs or through multi-center-controlled studies. They include nucleoside and nucleotide analogues. These drugs are phosphorylated into their active forms within the infected cells and are incorporated into HBV-infected hepatocyte inhibiting viral multiplication (19). These include lamivudine (LAM) and adefovir (ADV) with an attractive safety profile and ease of administration, but with significant deficiencies in the long-term outcome of treatment.

5.4. Lamivudine

Lamivudine (LAM) is a pyrimidine nucleoside analogue which prevents replication of HBV in the hepatocyte, leading to chain termination and thereby inhibition of viral reverse transcription, with a rapid decrease in viral load in the early phase of treatment. Histological improvement has been noted on LAM therapy in various trials in adults
LAM is now approved for use in children by the Federal Drug Administration (FDA) in the United States, at an oral dose of 3 mg/kg (a maximum of 100 mg/day). This was supported by the outcome of a large multi-center double blind, placebo-controlled trial for 52 weeks which was initiated in 2002 in the United States and Europe in which 288 children participated (34). Children who were HBsAg positive for 6 months or more, with HBeAg and DNA positivity and elevated ALT >1.3 times the ULN, but lower than 500 IU/L and histological evidence for chronic liver disease, were given LAM 3 mg/kg or placebo. Twenty-three percent of children cleared HBeAg and HBV DNA at 52 weeks as compared to 13% of untreated children. The response rate was higher, 35% in those with ALT >2 X ULN. Non-responders, treated with open-label LAM showed a cumulative virologic response in 35% over a 3-year period. But loss of HBeAg occurred only in 3%. A minimum of 1 year of treatment with LAM is now recommended and should continue for at least 6 months after clearance of HBeAg (3). In a follow-up study, 19% children showed LAM resistance (YMDD mutant) within the first year of treatment which increased over time if treatment was continued beyond 1 year, resulting in lower response rates (35). Viral response (VR) was maintained in most patients who had initially responded to LAM in the first 12 months. One hundred and fifty one children from this study were followed for 2 more years (36). Durable VR in the form of HBeAg clearance was seen in 82% and over 90% in those who was treated for 52 weeks and at least 2 years, respectively.

Hom et al. reported that higher baseline ALT, higher HAI score, and lower DNA levels may be predictive of a positive viral response, but age or ethnicity did not have any impact on the result of therapy, whereas Choe et al. reported a better response in young school age children when treated with LAM as compared to IFN (37, 38). Thus, cumulative data from various studies point to varying durability of HBe seroconversion, rare HBsAg clearance, and concerns regarding the development of LAM resistance on long-term use (37, 38, 39).

5.5. Combination Therapy

Combination therapy with IFN and LAM is an attractive idea and has been used in a limited number of pediatric studies (4). No improved outcome has been reported. But, a more recent pilot study holds promise especially in children with normal aminotransferases. Twenty-three children infected as infants, most of them with normal transaminases, were treated with LAM alone for 8 weeks followed by LAM and interferon alpha 5 MU/m² for 10 months. Seventy percent children cleared HBV at the end of treatment with a 22% seroconversion to HBeAb with
17% achieving sustained HBsAg clearance after 40 months (40). Diciki et al. reported 30 children who were treated simultaneously with large doses of both drugs (IFN 10 million units) (LAM 4 mg/kg) for 6 months vs. 12 months; 60% of those treated for 12 months and 30% of those treated for 6 months achieved HBeAg clearance (41).

5.6. Limitations

Although the safety profile of LAM is excellent, relapse following cessation of treatment and development of resistance due to viral mutations limit the use of LAM and defines a possible role in combination therapies with either IFN or other nucleoside/nucleotide analogues (19).

5.7. Adefovir

Adefovir dipivoxil (ADV) is another nucleoside analogue currently approved for use in adults with chronic HBV infection. The mechanism of action includes inhibition of HBV DNA reverse transcriptase and DNA polymerase activity, with efficacy against both the wild- and LAM-resistant mutant type of the virus (1, 42). Viral resistance has been much less as compared to LAM (about 3% in 3 years). A recent multi-center, randomized, double-blind, placebo-controlled study conducted in the United States and Europe explored the safety, efficacy, and pharmacokinetics of ADV in 173 treatment naïve and previously treated children with chronic HBV infection, stratified based on age to three categories (2–7, 8–12, 12–18 years). ADV was given at doses of 0.3 mg/kg, 0.25 mg/kg, and 10 mg daily, respectively, vs. placebo (2:1) for 48 weeks (43). The racial distribution was similar for both Caucasian vs. Asian. The primary end point was serum HBV DNA <1000 copies/mL and normal ALT at week 48. This was achieved in 23% of patients between 12 and 17 years on ADV vs. 0% in controls (p <0.009), similar to its efficacy in adult patients. There was no difference in viral clearance between treated and untreated younger patients. Across all age groups, there was a greater number of patients on ADV who seroconverted to HBeAb (15.9% vs. 5.3%) although this was not statistically significant. No patient developed an ADV-associated mutation (rtN236T or rtA181V). The drug was safe and well-tolerated by all age groups. Treatment-related adverse effects were unusual and included asymptomatic elevations of creatinine and elevation of hepatic enzymes in a few patients. The relative lack of response of younger children could not be explained. Overall, the study indicated that higher ALT and lower HBV DNA at baseline were associated with better outcome with the primary endpoints (43).
5.8. Newer Therapy

Entecavir is an oral guanosine nucleoside analogue which inhibits HBV polymerase activity at various steps and is now licensed for use in adults in the United States since 2005. A pharmacokinetic and safety/efficacy study in children is being initiated in Europe and the United States (1). Tenofovir has just been licensed for use in adults and clinical trials in children are anticipated. Pegylated interferon-α 2a (PEG-IFN) is now approved for treatment of adults with HBeAg positive and negative infection and promises to offer a better virologic and histological response as a single agent or in combination with LAM. While there was a 7% loss of HBsAg with PEG alone there was no benefit from combined therapy with LAM (1). PEG-IFN is not yet approved for use in children. Other drugs in trials in adults are telbivudine, entricitabine, and tenofovir especially in the setting of HIV co-infection, but there are no data on their use in children.

6. SUMMARY AND RECOMMENDATIONS

Infants affected perinatally have a greater propensity to develop chronic HBV infection. These children do not seem to benefit from therapy with interferon or the newer oral nucleoside or nucleotide analogues which are generally ineffective in those with low ALT and high viral loads. Indefinite treatment is costly and may prompt the development of resistant strains. Interferons, despite their side effects, offer the best chance of clearance of HBsAg or seroconversion to HBeAb and a finite duration of therapy in those with elevated aminotransferases and abnormal liver histology. Careful selection of pediatric patients for treatment should be based on the capability for close monitoring and regular follow-up, considerations such as co-morbidity, progression or active liver disease, familial, social and compliance issues, and definite end points in treatment. During treatment with any agent, patients should be assessed for markers of viral activity and immune-mediated liver injury. Patients’ growth, weight, body mass index (BMI), and liver functions should be closely monitored and health-related quality of life and psychological factors should not be ignored.

7. HEPATITIS C: INTRODUCTION

Hepatitis C virus (HCV) is a small, single-stranded RNA virus belonging to the family Flaviviridae which has become the most significant cause of chronic liver disease of infectious etiology in the United States. Data from the Centers for Disease Control and Prevention
(CDC) have shown the seroprevalence of HCV infection is 1.8% in the general population of the United States (44). Among children, the seroprevalence is 0.2% for those <12 years of age and 0.4% for those 12–19 years of age. In adults, the highest rates of HCV antibody are found among those with repeated percutaneous exposures (IVDU) and patients with hemophilia who have received multiple blood transfusions (60–90%). This is followed by hemodialysis patients (20%) and even lower rates among parenteral or mucosal exposures such as patients with high-risk sexual behaviors, household contacts of infected persons, and health-care workers (1–10%) (17, 44). Before 1986, transfusion-associated hepatitis occurred in 5–13% of recipients. Following the introduction of HCV antibody screening in 1992 and development of a more sensitive second generation test in 1992, the rate of transmission decreased to 0.001% per unit transfused. Some transfusion-related infections continue to occur due to the failure of the second generation assay to detect anti-HCV in approximately 5% of infected persons and rare cases of blood donations made between the time of acquisition of infection and seroconversion – the “window period” (4, 44, 45).

Subsequent to the introduction of HCV screening techniques, vertical transmission has become the leading source of infection for children (45). Seroprevalence among pregnant women in the United States has been estimated at 1–2% with the risk of perinatal transfer from viremic mothers averaging 5–6% (16). Maternal co-infection with immunodeficiency virus (HIV) and maternal IVDU have been associated with an increased risk of perinatal transmission of HCV (46). Breastfeeding and the mode of delivery (cesarean section or vaginal delivery) do not show any difference in transmission rates. Viral factors such as genotype and viral load have not been consistently measured across studies and, therefore, their roles as risk factors in mother-to-infant HCV transmission remain to be proven (46).

8. NATURAL HISTORY

Perinatal acquisition of HCV has been shown to lead to a high incidence of viremia and relatively high ALT levels during the first few years of life which subsequently normalize in a significant percentage of patients. In one prospective study of 70 infants born to HCV-infected women in five European centers between 1990 and 1999, 93% were shown to have a wide range of ALT elevations in the first year of life with subsequent normalization of ALT and HCV RNA clearance in 19% by 30 months of age (47). Vogt et al. reported a greater prevalence of HCV RNA clearance in 30 of 67 (45%) children with post-transfusion HCV 20 years after cardiac surgery. However, Vogt et al.
did not report the timing of HCV RNA clearance, so that it is difficult
to determine the proportion of children expected to clear viremia later in
life (48). In a second retrospective study of 200 HCV-infected children
(45% HCV perinatally acquired) in seven European centers, followed
for 1–17.5 years, 6% achieved sustained viral clearance and normaliza-
tion of the ALT level (49). Overall, persistent infection develops in at
least 85% of infected newborns even in the absence of biochemical evi-
dence (44). Most studies indicate that spontaneous clearance of HCV
virus is rare beyond infancy.

Chronic HCV infection is generally asymptomatic. Most children
are detected to have chronic HCV infection by history of transfusion,
perinatal HCV positivity, or occasionally elevated ALT and its silent
nature lends itself to failure of detection of the infection in a large
number of patients. Of obvious concern are the long-term effects of
chronic HCV on children. Children generally show mild histological
changes on liver biopsy even in their late adolescence; however, signif-
ica nt fibrosis, cirrhosis, and HCC have been reported (50, 51, 52). In
a study of 60 HCV-infected children (68% transfusion acquired, 13%
perinatally acquired), followed for 13 years after infection, patients
were mostly asymptomatic with fatigue and diffuse abdominal pain
being the most frequent reported symptoms. Overall, 42 liver biopsy
specimens were examined and the majority (71%) showed only mild
inflammatory changes. Fibrosis was mild or absent in 88% and bridging
fibrosis seen in 12% by 13 years (51). Although ALT appeared to be an
excellent correlate to liver histology based on this study, discrepancies
still existed attesting to the continued value of liver biopsy (51). The
need for a liver transplantation for complications of chronic HCV infec-
tion is fortunately rare during childhood since the outcome is rather
poor, due to the strong possibility of recurrence of HCV in the allo-
graft, according to the Study of Pediatric Liver Transplantation (SPLIT)
Registry (53).

9. SCREENING AND PREVENTION

The American Academy of Pediatrics (AAP) recommends screening
of infants born to HCV-infected mothers and persons with risk factors
for HCV infection as shown below; at this time routine screening of
pregnant women is not recommended (4, 44). Among infants born to
anti-HCV positive mothers, passively acquired antibodies may persist
up to 18 months. In these cases assays for HCV RNA detection (HCV
PCR) may be used to detect active infection in the infant rather than
passive transfer of antibodies from the mother (16).
Current recommendations for screening for HCV infection (16):

- Children born to HCV-positive mother
- Transfusion/solid organ transplantation before 1992
- Recipient of clotting factors before 1992
- Chronic hemodialysis
- After known parenteral exposure
- Persistently high ALT
- History of drug use
- Adoptees from endemic areas

HCV exhibits substantial heterogeneity as a result of mutations occurring during viral replication. This rapid mutation appears to be the mechanism by which the virus escapes immune surveillance and the largest stumbling block in the creation of an effective vaccine. In addition, since antibodies created against one HCV genotype do not recognize other viral genotypes, previous infection does not protect against re-infection with the same or different genotype of the virus (44). Currently, there is no evidence of clinical efficacy from the use of immune globulin for post-exposure HCV prophylaxis of HCV. Due to the lack of a vaccine and immuno-prophylaxis, general precautions and prevention techniques such as blood product screening have a critical role in prevention of propagation of HCV infection. Patients should be advised to abstain from IV drug use, alcohol, and risky sexual behaviors. Current guidelines of the CDC and AAP do not consider maternal HCV infection a contraindication to breastfeeding (16). Also, routine serologic testing of adoptees is not recommended though advised, unless the biological mother has an increased risk for HCV.

10. TREATMENT

The decision to treat a child with HCV infection is complex because of the lack of predictable success, the presence of significant toxicity related to currently available therapies, and the relatively benign nature of the disease in childhood and adolescence. Children infected with HCV tend to have a milder degree of liver disease with minimal fibrosis. This presumably can be attributed to the shorter duration of infection and the absence of co-morbid conditions such as HIV-infection, alcohol abuse, or autoimmune disorders that would complicate treatment (17). In contrast, by virtue of their longer life expectancies, there is a likelihood of development of complications from HCV later in life. The
decision to treat may have to be based on several factors such as clinical assessment, laboratory parameters especially synthetic functions of the liver, viral genotype, evidence of portal hypertension, liver biopsy findings, and any contraindication to treatment (1).

Before considering therapy:

1. Infection should be confirmed via HCV RNA PCR and laboratory tests determining HCV genotype.
2. Liver function tests should be done to assess inflammation and to rule out other types of liver diseases, such as autoimmune hepatitis and Wilson’s disease.
3. Patients should be monitored for complications such as portal hypertension and hepatic decompensation.
4. Prior to a decision to treat, a liver biopsy to assess the extent of histological disease is suggested, except in those infected with genotype 2 or 3 due to their excellent response rate to treatment and associated mild liver disease (1).
5. Additionally, the presence of other co-morbidities such as obesity and alcohol use in adolescents should be investigated to determine their effect on treatment outcomes.
6. Optimal timing of therapy should also take into consideration age-related limitations, growth, social, and psychological factors.

Treatment of HCV infection may be considered in the following settings:

1. Children with genotypes 2, 3 because of excellent response and shorter duration of treatment (1).
2. Children with evidence for advancing disease, compensated cirrhosis, or moderate to severe fibrosis on biopsy require close monitoring for sudden hepatic decompensation.
3. Treatment in the setting of clinical trials involving therapeutic options not currently approved for use in children.
4. Availability of newer therapies.

10.1. Interferon Alpha

Interferon alpha (IFN) monotherapy has been extensively studied in adults and was shown to result in sustained virologic response (SVR) 6 months after completion of therapy in approximately 8% of patients (54). To date, no large multi-center trials have studied sustained response rates, predictors of response to therapy, or safety of and tolerance of IFN monotherapy in children with chronic hepatitis C. One of the earliest studies involving children came from Japan in 1997 in which 22 children with transfusion acquired HCV infection
were treated with IFN and 11 children (50%) reached sustained viral response with 1–2 rounds of treatment (55). Furthermore, Jacobson et al. attempted to answer these questions by doing a meta-analysis of 19 trials involving 366 IFN treated and 105 untreated children aged 2–21 (56). Initial treatment trials using a dose of 3 MU/ m² of IFN three times weekly showed that children had a much better response rate of about 35–40% compared to adults; genotypes other than 1 responded better (70% vs. 27%) (54, 56, 57). No differences were noted among the studies with regard to the duration of treatment or dosage. The most commonly reported adverse events in children were influenza-like symptoms including fever, weight loss during treatment (with subsequent resolution), neutropenia, and alopecia (56). High-dose IFN alpha (10 MU/m²) was studied by Marcellini et al. and was not found to be superior to the standard dosage (58).

10.2. Interferon-Alpha and Ribavirin

Ribavirin (RBV) is a synthetic nucleoside which has some in vitro antiviral activities providing synergy when used in combination with IFN, but has no direct antiviral effect if used as a single agent. However, superiority of combination therapy with standard interferon over interferon monotherapy was established in adults by several studies in 1998 (59). In 2000, a cohort study consisting of 12 children and adolescents with chronic hepatitis C and previous malignancy were treated with recombinant IFN alpha-2a (6 MU/m² body surface area, three times a week, subcutaneously) combined with RBV (15 mg/kg body weight/day, orally) for 12 months (60). All of the patients had HCV subtype 1 and none had previously been treated with IFN. Sustained response as defined by normalization of aminotransferases and negativity of HCV RNA at the end of treatment and >6 months after withdrawal of therapy was seen in 50% of participants 12 months after withdrawal of therapy, the majority of which had achieved HCV RNA negativity within 1–3 months of treatment (60). A second study published in 2000 consisted of 11 children with chronic hepatitis C and malignancy in remission being treated with IFN and RBV for 48 weeks with a 12-month follow-up period (61). At the 12-month follow-up, 64% had sustained virologic and biochemical responses with undetectable HCV RNA and normal ALT levels; all those with genotype 3 sustained SVR (61). In a larger study involving 40 HCV-infected children, patients were treated with IFN-alpha at a dose of 3 or 5 MU/m² 3 times weekly in combination with oral RBV 15 mg/kg/d for 12 months. Twenty-five patients (61%) were HCV RNA negative at the end of treatment and remained negative at the 6-month follow-up
with normalization of ALT levels. Sustained viral response (SVR) was achieved in 53% of patients with HCV subtype 1 and 100% of patients with either subtype 2 or 3 (62).

The most notable study to date was published in 2005 by the International Pediatric Hepatitis C therapy group (63). This phase 3 study encompassed 48 weeks of treatment followed by 24 weeks of observation, conducted in 29 centers in the United States and Europe, and enrolled 118 children between 3 and 17 years. They were treated with IFN-alpha-2b 3 million IU/m² subcutaneously three times a week in combination with RBV at 15 mg/kg PO. The eligibility criteria included serum positivity for HCV by quantitative PCR and a liver biopsy consistent with chronic HCV. The study excluded children with anemia, thrombocytopenia, positive ANA ≥160 and abnormal synthetic liver functions, other viral infection and systemic illnesses. In all, 46% attained SVR at follow-up, and SVR was higher in those infected with genotype 2 or 3 (84%) vs. genotype 1 (36%). This study concluded that HCV genotype 2 or 3 and a viral load below 2 million copies/mL for HCV genotype 1 were favorable factors for SVR. Side effects included anemia, neutropenia leading to dose modifications in 31% and discontinuation in 7%. A notable aspect of this trial was the impact of therapy on growth. The linear growth and weight gain were temporarily inhibited during the study which was partially compensated during the observation period (63). Additionally, the teratogenic properties of RBV was considered as a serious side effect and RBV was used with extreme caution in both adolescent males and females of childbearing potential (63, 64). Overall, baseline ALT, mode of acquisition of HCV, ethnicity, gender, and the duration of infection did not seem to affect the SVR in any of these studies.

In 2003, the Food and Drug Administration approved the combination of standard IFN and RBV for the treatment of chronic hepatitis C in children between 3 and 17 years of age and this remains as the only FDA approved treatment to date.

10.3. Pegylated Interferon (PEG-IFN) Monotherapy

The limited attainment of SVR, the need for frequent injections and the side effect profile were recognized as the major obstacles or disadvantages in the use of standard interferons. The use of PEG-IFN once a week seemed to improve response rate and was more acceptable to patients. Pegylation of IFN, i.e., covalent linking of a protein molecule to polyethylene glycol was found to provide a longer half-life and reduced clearance allowing for less frequent dosing. Three large trials in adults addressing the end of treatment response (ETR) and sustained
virologic response (SVR) using a dose of 180 μg/kg or a randomized weight-based dosing of 0.5, 1, 1.5 μg/kg of body weight once weekly showed an ETR (end of treatment response) of 33–69 % and SVR of 23–39% (65). Subsequently, the safety and efficacy of PEG-IFN monotherapy was evaluated in a pharmacokinetic study in 14 children infected with HCV 1 genotype from 2 to 8 years (66). PEG-IFN was given subcutaneously at a dose of 180 μg/1.73 m² once a week for 48 weeks (66). The primary efficacy end point, defined as HCV RNA level of <50 IU/mL at 24 weeks after the end of therapy, was attained in 43% (6/14) patients (46% for genotype 1). Side effects were milder than reported in adults and included fever, headache, fatigue, vomiting, and rarely irritability and need for dose adjustment, but weight loss was not reported (66).

### 10.4. Peginterferon and Ribavirin Combination Therapy

Peginterferon (PEG-IFN) and RBV combination is currently being used as standard therapy for chronic HCV infection in adults, but has not yet been approved by FDA for use in children. Studies have shown that this combination therapy has significantly improved rates of SVR when compared to thrice-weekly IFN and RBV therapy (54–56% vs. 44–47%) (67, 68). Additionally, the combination therapy in adults has also been associated with a decrease in hepatic inflammation and a decrease in relapse rates especially in patients with genotype 1 (42% vs. 29–33%) (68). To date, there are very few reports of PEG-IFN and RBV combination therapy in children. In 2005, an open-label uncontrolled pilot study evaluated this therapy in 62 children and adolescents aged 2–17 years (69). The patients were treated with subcutaneous PEG-IFN-alpha-2a at a dose of 1.5 micrograms/kg body weight once per week plus oral RBV at 15 mg/kg/day for 48 weeks. SVR was documented in 59% at the 6 month follow-up after therapy cessation. All 13 patients with genotype 2 or 3 achieved SVR, while 22 of 46 (47.8%) of those with genotype 1 achieved SVR, a rate similar to that of the thrice-weekly IFN and RBV therapy. The likelihood of SVR was independent of baseline ALT and mode of acquisition. Overall, treatment was well-tolerated with leukopenia (83%) being the most reported side effect (69). A pivotal multi-center, placebo-controlled study has been underway since 2004 involving 11 centers and 112 patients from the United States evaluating the safety and efficacy of the combination therapy in children between 5 and 18 years (70). Patients received PEG-2a injection (180 micrograms/1.73 m²) once a week and either placebo or RBV tablets orally twice daily (15 mg/kg/day with a maximum dose of 1200 mg/day if >75 kg and 1000 mg/day if <75 kg). The purpose
of the study was to assess the safety and efficacy of PEG-2a plus RBV, and to evaluate additional clinical features impacting treatment such as health-related quality of life and growth and body composition before, during, and after chronic HCV treatment. The final results of this crucial study will be presented and published shortly. From Europe, Jara et al. have published a similar study on 30 children between 3 and 16 years treated with PEG-IFN 1.0 μg/kg/week and RBV 15 mg/kg/day for 24–48 weeks (71). Overall, a 50% SVR was attained in patients with all genotypes and virologic response at week 12 was predictive of SVR at 24 weeks after cessation of therapy.

10.5. Newer Therapies

Several emerging therapies are being considered for treatment of HCV infection, but none has been approved for use by the FDA, particularly in children. Besides alternate forms of interferons such as omega IFN or gamma IFN, RBV substitutes such as viramidine and levovirin have been considered for trials in adults with HCV because of lesser hematologic side effects (1). VX-497 or merimepidoib combined with PEG-IFN and RBV has been evaluated in adults as a more effective drug for achieving durable SVR (72). Hepatitis C virus (HCV) NS3/4A protease inhibitors also have potential for treating chronic HCV disease and are being considered for future trials (73).

10.6. Considerations and Recommendations

Based on these studies, the following recommendations may be beneficial when considering treatment of HCV in children:

1. All children to be monitored closely for side effects during therapy and dose reduction instituted for serious effects, such as neutropenia, depression, and growth.
2. Those infected with genotypes 2, 3 should be treated for 24 weeks and the others (genotype 1) for 48 weeks.
3. If patients with genotype 1 did not achieve early virologic response or clear HCV by 24 weeks, treatment should be discontinued.
4. Those who complete and clear HCV should be monitored for SVR 6 months after completion (1).
5. Decision to be individualized on a case-by-case basis.

Children who acquire HCV early in life manifest few symptoms and have relatively mild liver involvement, but have the potential to develop more serious sequelae (51). Benefits of early treatment include a better outcome in the absence of co-morbidity such as alcohol use, and avoidance of social stigma. Studies as discussed above have shown that children tolerate treatment with IFN well although they also do not
appear to achieve such optimal sustained viral response rates that would prompt treatment of all infected children. The slow progression and lack of symptoms affecting activity and lifestyle, on the other hand, favor deferment of therapy, given the spectrum of side effects from the therapy especially involving growth. Currently, the combination of PEG-IFN and RBV holds the best promise in terms of efficacy and safety and awaits FDA approval. It can be hoped that newer trials involving drugs with less toxicity and a more attractive response profile will be extended to involve not only adults with chronic HCV but children as well.

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**Hepatitis B**


Hepatitis C


Viral Hepatitis and Hepatocellular Carcinoma

Jorge A. Marrero, MD, MS

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Key Principles

- Hepatocellular carcinoma (HCC) usually occurs in patients with chronic liver disease, commonly due to viral hepatitis.
- Surveillance of patients with cirrhosis of the liver can lead to the detection of HCC at early stages.
- The diagnosis of HCC is made by histopathology or by MRI/CT scan showing a hypervascular lesion with washout in the delayed phases.
- The Barcelona staging system is the preferred staging system as it combines hepatic function, performance status, and tumor burden, and it is linked to an evidence-based treatment strategy.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third cause of cancer-related death worldwide (1). Approximately 560,000 people develop liver cancer each year with an almost equal number of mortalities. Thus, it is a tumor with a significant burden worldwide. We herein will discuss the epidemiology, risk factors, surveillance, diagnosis, and prevention of HCC as it pertains to individuals with viral hepatitis.

2. EPIDEMIOLOGY

2.1. General

The largest concentration of cases of liver cancer in the world is in Asia, followed by Africa, Europe, and North and South America (2). The incidence of HCC varies among ethnic groups, with increasing incidence rates found in Japanese (5.5/100,000 in men and 4.3/100,000 in women), African American (7.1/100,000 in men and 2.1/100,000 in women), Hispanics (9.8/100,000 in men and 3.5/100,000 in women), and Chinese (16.2/100,000 in men and 5/100,000 in women) populations. Even though the incidence rates are greater in men compared to women, there is a two- to fivefold higher incidence rate among women of various ethnicities compared to non-Hispanic white females. During the last two decades, increasing trends in the incidence of HCC have been noted in Australia, Central Europe, United Kingdom, Japan, and North America (3). In addition, during the same two decades, there has been an increase in mortality due to liver cancer in all countries. In the United States, as the incidence of HCC has increased in recent years, the distribution of patients with HCC has shifted toward younger patients, with the greatest increase in those between 45 and 60 years of age, likely due to the fact that the hepatitis C virus (HCV) epidemic was in the 1960s and 1970s and the aging of this cohort (4). The recent review of the Surveillance, Epidemiology and End-Result (SEER) program in the United States, which is a population-based study of cancer epidemiology, has shown that over the last 10 years liver cancer has been the tumor with the highest change in increase compared to other solid tumors.

2.2. Viral Factors

The etiological agents leading to HCC have been largely established. In Japan, Europe, and America, about 60% of the patients with HCC are attributed to chronic HCV infection, whereas 20% are attributed to chronic hepatitis B (HBV) infection and about 20%
between cryptogenic and alcoholic liver disease. The broad traits of the epidemiology of HCC can be traced to the prevalence of hepatotrophic viral infections.

Chronic HBV infection is the most common underlying etiology of HCC in the world (5). In high-prevalence areas such as Eastern Asia, China, and Africa, about 8% of the population is chronically infected as a result of vertical (mother-to-child) or horizontal (child-to-child) transmission. The pattern of transmission is different in areas with lower prevalence of HBV such as North America, Western Europe, and Australia where infection mostly occurs in adulthood through sexual and parenteral routes. The higher prevalence of chronic HBV, as well as the longer period of exposure to infection, largely explains the higher HBV-related HCC risk in endemic areas.

Chronic HCV infection is found in a variable proportion of HCC cases in different populations, accounting for 75–90% of cases in Japan, 31–47% in the United States, 44–76% in Italy, and 60–75% of HCC cases in Spain (6). HCC is the cancer with the highest increase in incidence over the last 10 years in the United States and this increase is mostly due to chronic hepatitis C (HCV) (7).

Most of the cases of HBV- and HCV-related HCC occur in the setting of cirrhosis as shown in Table 1(6). The risk of HCC in persons with chronic HBV is from 2.2 to 4.3 per 100 person-years in compensated cirrhotics, while it is less than 1 per 100 person-years in non-cirrhotic patients. About 20% of patients with HBV-related HCC present without evidence of cirrhosis, indicating that other factors are important in hepatocarcinogenesis.

The risk of HCC among patients with chronic HCV infection also occurs in the setting of patients with cirrhosis as shown in Table 1 (6). In Japanese studies, the summary HCC incidence rate for patients with chronic hepatitis was 1.8 per 100 person-years and 7.1 per 100 person-years in those with compensated cirrhosis. In the United States and Europe, the summary incidence rate was 3.7 per 100 person-years, which is lower than that in Japan (8–11). The natural history of cirrhosis in patients with chronic HCV infection was assessed in 136 patients and followed for a mean of 6.8 years (12). The 5-year cumulative risk for HCC was 10%, the mean interval between diagnosis of cirrhosis and HCC development was 5 years (range, 0.5–10 years), and the median age for diagnosis of HCC was 63 (range, 50–74). Interestingly, more than half of the patients that developed HCC did not experience hepatic decompensation at the time of HCC diagnosis, indicating that HCC arising in cirrhosis can be clinically silent.

In addition to the presence of cirrhosis, host viral factors are important in hepatocarcinogenesis. Important host factors are male gender
and age >50 years of age, which increase the risk for HCC significantly in both HCV- and HBV-related HCC (13, 14). With regard to HBV-derived HCC, evidence of viral replication measured by e antigen status and high serum HBV DNA levels (>10^5 copies/mL) (15, 16), and HBV genotypes, specifically B, increases the risk of HCC (17). Therefore, host and hepatitis B viral factors play an important part in the development of HCC. To the contrary, viral factors do not appear to increase the risk of HCC in HCV-related HCC.

The burden of HCV-related HCC in the United States is expected to continue to increase during the next decades. A recent study using molecular evolutionary analysis based on the coalescent theory (molecular clock) investigated the time origin of HCV infection in Japan and the United States (18). The authors showed an earlier onset of the HCV epidemic in Japan and, therefore, a longer duration of infection in affected individuals, which increases the likelihood for HCC

<table>
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*Carcinoma in Prospective Studies in Patients with Hepatitis B and Hepatitis C Infection

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*Incidence is per 100 person-years.
development compared to the United States. The authors postulate that the incidence of HCC in the United States will continue to increase over the next two to three decades. It has been estimated that the number of cases of HCC will continue to increase by 81% (from a baseline of 13,000/year) by the year 2020, primarily due to the hepatitis C (HCV) epidemic (19). Because of the poor overall survival, the projected increase in the number of patients with HCC may lead to a significant health-care burden in North America.

2.3. Non-Viral Factors

Other important risk factors in the development of HCC in patients with chronic viral hepatitis are alcohol and tobacco. Synergism between alcohol and viral hepatitis has been found to increase the risk of HCC (20). Obesity is another important risk factor that increases the risk of HCC (21). Aflatoxin B$_1$ (AFB$_1$) is a mycotoxin that grows on food stored in humid conditions and is a carcinogen predisposing to human HCC. AFB$_1$ ingestion has been associated with mutations in the coding regions of p53 tumor suppressor gene (22). Diabetes has also been shown in prospective studies to increase the risk of HCC (23). A recent study showed that there is synergy between alcohol exposure >60 g of ethanol a day, >20 pack years of tobacco smoking, and obesity (BMI >30) in a predominant population of patients with HCV infection (24). Therefore, multiple risk factors are important in the process of carcinogenesis in individuals with viral hepatitis.

3. DIAGNOSTIC EVALUATION

3.1. Surveillance

The decision to screen an at-risk population for cancer is based on well-established criteria (25). The objective of screening is the use of a relatively simple, inexpensive test in a large number of individuals to determine whether they are likely or unlikely to have the cancer for which they are being screened. Screening is the one-time application of a test that allows detection of a disease at a stage where curative intervention may improve the goal of reducing morbidity and mortality. Surveillance is the continuous monitoring of disease occurrence (using the screening test) within a population to achieve the same goals of screening. HCC meets these criteria for performing surveillance (26).

Despite advances in medical technology, the 1-year survival of patients with HCC improved minimally from 2 to 5% between 1977 and 1996 (27). This may be largely due to diagnosis at late stages of the disease. According to US vital statistics, the age-adjusted mortality rates
of primary liver cancer increased from 2.8/100,000 persons in 1997 to 4.7/100,000 persons in 2001. In the report to the nation on cancer, the change in mortality rates for liver cancer was the highest among all solid tumors from 1996 to 2005 (www.seer.gov). Surveillance of the high-risk group, i.e., patients with cirrhosis, may improve overall outcomes for patients with HCC.

Since the goal of surveillance is to reduce mortality by detecting patients with occult disease prior to developing symptoms, the performance characteristics of a test utilized for diagnosis and/or staging (e.g., CT or MRI) cannot be assumed to be the same when utilized in a surveillance/screening situation. The most commonly used screening test for HCC is α-fetoprotein (AFP). It has been shown that the optimal balance of sensitivity and specificity is achieved by a cutoff level of 20 ng/mL (28). However, this cutoff leads to sensitivities between 41 and 60% and specificities between 80 and 94% (29, 30). The other surveillance test that is commonly used is ultrasonography of the liver (US). The sensitivity and specificity of ultrasound (US) have been shown to be between 58 and 78% and 93 and 98%, respectively (31–32). However, the performance characteristics of US as a surveillance test for HCC have been extrapolated mostly from studies that evaluated US as a diagnostic test; therefore, its performance as a surveillance test has not been properly identified. A recent randomized controlled study of screening for HCC has been performed in China (33). This study compared US and AFP vs. no screening and achieved a compliance rate of <60%. It showed that screening led to a reduction of 37% in mortality compared to no screening. One problem with this study is that it enrolled patients with exposure to HBV, without mention of the degree of hepatic fibrosis or viral replication; therefore, not all patients were at the same risk level for developing HCC. This is the first evidence that the strategy of surveillance for HCC with AFP and US improves mortality in a population of HBV carriers. Surveillance is recommended in patients with cirrhosis with US and AFP at a frequency of 6–12 months (29). Screening is also recommended in non-cirrhotic HBV patients that are older, >20 years of age, born in Africa, have family history of HCC, and evidence of significant inflammation and fibrosis with viral replication (29). New biomarkers are needed to improve the efficacy of the current surveillance tests.

3.2. Diagnosis

Once an abnormal screening test is obtained in a cirrhotic patient, there is a need for a recall test to perform a diagnostic evaluation to assess for the presence of HCC. For the diagnosis of HCC, radiological
imaging such as a triple-phase spiral CT or a dynamic MRI and biopsy are the diagnostic tests of choice. There have been several studies that have compared CT scan to MRI for the diagnosis of HCC as shown in Table 2 (34–37). It appears that MRI is superior to CT scan for the diagnosis of HCC. However, recent studies suggest that not only arterial enhancement of a hepatic nodule but also “washout” of contrast in the delayed phases of enhancement (38) are important findings in making this diagnosis. “Washout” is defined as hypointensity of a nodule in delayed phases of CT or MRI examination compared to surrounding

<table>
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<td>136</td>
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Sens, sensitivity (%); Sp, specificity (%)

Fig. 1. An MRI showing a hepatocellular carcinoma. (A) shows the arterial phase indicating an enhancing mass in the right lobe (white arrow). (B) shows the same lesion in the delayed venous phase (3 min after contrast injection) showing hypointensity compared to the rest of the liver parenchyma indicating washout (black arrow).
liver parenchyma. It is likely due to arterial neovascularization that is greater in HCC nodules than it is in the surrounding non-neoplastic hepatic parenchyma, while in the delayed phases there is early venous drainage. Figure 1 shows an example of washout in a patient with an arterially enhancing mass in the presence of cirrhosis. The diagnostic work-up of a patient with abnormal surveillance tests for HCC is shown in Fig. 2. In the case of using AFP, a level above 20 ng/mL is the trigger to obtain a CT scan or an MRI for the diagnosis of HCC.

Fig. 2. Recommendations on the evaluation of an abnormal ultrasound performed during surveillance for hepatocellular carcinoma (Ref. (29), obtained by permission).

4. TREATMENT

4.1. Staging

The degree of tumor burden, performance status, and liver dysfunction have repeatedly been shown to be independent factors of prognosis (29). At least seven staging systems for HCC have been proposed over the years. The system that provides the most prognostic information is the Barcelona Clinic Liver Cancer (BCLC) and it is the only staging system that has been validated in various countries (39). As shown in Fig. 3, it is the only staging/prognostic system that combines tumor...
burden, hepatic function, and performance status linked to an evidence-based treatment algorithm. It is divided into very early, early, intermediate, advanced, and terminal stages.

4.2. Treatment

4.2.1. Very Early Stage

This stage includes single tumors <2 cm with excellent hepatic synthetic function and no portal hypertension, which should be the goal of a surveillance program. For patients at this stage, it is important to determine if there is underlying cirrhosis or not. For those without cirrhosis, surgical resection is the treatment of choice. However, this represents a minority of patients with HCC in North America and Europe. In patients with underlying cirrhosis, the risks and benefits of resection must be carefully weighed against the available alternatives. For patients with cirrhosis at this stage with normal bilirubin, no portal hypertension (portal pressure <10 mm Hg, no varices, and no ascites), and at least 40% of the liver volume remaining after resection, resection leads to 5-year survivals of about 75% (40–42). The efficacy of resection is related to tumor diameter with better outcomes in those with tumors <3 cm. Another treatment alternative for these patients is radiofrequency ablation (RFA). A randomized trial compared
surgical resection vs. RFA for patients with HCC <3 cm without portal hypertension and normal liver function (43). There was no difference in 5-year survivals among the two groups and the group treated with RFA, but the latter had less morbidity. The main limitation of resection and RFA is the significant recurrence rates: more than 50% at 5 years (29). The choice between resection and RFA should be determined by tumor location, hepatic function, degree of portal hypertension, and local expertise.

4.2.2. Early Stage HCC

This stage includes a single tumor between 2 and 5 cm in size in diameter or three tumors each ≤3 cm in size, without gross vascular invasion or metastatic disease. These criteria are termed the “Milan” criteria (44). Liver transplantation is considered the best treatment for patients at this stage because it removes not only the malignancy but also the diseased liver, thus preventing complications of end-stage liver disease as well as de novo hepatocarcinogenesis in the future. When these criteria are applied, the 5-year survival after transplantation is approximately 70% (45), which approaches the survival of patients transplanted for non-malignant liver disease.

The main limitation of transplant is the overall organ shortage. In areas of the country with the most severe organ shortage, patient dropout while waiting for a transplant can approach 50% (46). This has led some centers to perform neoadjuvant ablation or chemoembolization as a bridge to transplantation, especially in patients with larger tumors. While there is no evidence that this is beneficial, there is also no evidence of a lack of benefit. Higher quality trials are needed. Another option to address the high dropout rates while awaiting deceased donor liver transplantation (DDLT) is living donor liver transplantation (LDLT). Recently, the enthusiasm for LDLT has been dampened by evidence of increased HCC recurrence after transplantation when compared with DDLT (47). Furthermore, the risk to the donors raises a number of ethical concerns (48). LDLT should be performed only at experienced centers, ideally in the setting of a clinical trial.

Patients with HCC exceeding Milan criteria may still benefit from transplantation, but transplanting these patients has been controversial because of the shortage of organs. A recent study showed that transplanting such patients increases the risk of another patient dying on the waiting list by 44%, with the risk being the highest among those without HCC and MELD scores >20 (49).

Percutaneous ablation is the best treatment option for patients with early stage HCC who are not suitable candidates for resection or OLT.
In three randomized trials comparing percutaneous ethanol injection (PEI) with radiofrequency ablation (RFA), patients treated with RFA required fewer treatment sessions, higher rates of complete tumor ablation, had fewer recurrences, equal rates of complications, and better overall survival (50, 51, 52). The 5-year survival rate of RFA has been about 50% in these randomized trials and in large case series (53). Therefore, RFA is the method of choice for local ablation for tumors within this tumor range.

4.3. Intermediate Stage

The tumors at this stage exceed Milan criteria and may be either single tumors >5 cm or multinodular tumors. Unlike the liver parenchyma which derives most of its blood supply from portal venous flow, HCC is almost entirely supplied by branches of the hepatic artery. Chemoembolization takes advantage of this vascular anatomy, combining particle embolization with local delivery of a chemotherapeutic agent. A meta-analysis of 14 randomized controlled trials showed that transarterial chemoembolization (TACE) increases survival from 27% to 41% at 2 years compared to best supportive care in patients with unresectable HCC (54). No significant benefit was seen in patients receiving embolization without chemotherapy (TAE), though the chemotherapeutic agent chosen does not seem to matter. It is important to note that the majority of patients in the clinical trials of chemoembolization had Child A cirrhosis, only half had multinodular tumors, and the median tumor size have been between 5 and 7 cm. Intraarterial radioembolization with Yttrium-90 is another form of intraarterial therapy that has been performed delivering higher doses of radiation locally in the liver, thereby potentially allowing the treatment of diffuse multifocal therapy. A recent single-center study showed some efficacy in patients with tumors at this stage but was no different than TACE (55). Further trials are needed to assess the efficacy of this therapy and its place in the treatment armamentarium.

4.4. Advanced Stage

Despite surveillance efforts for early detection of HCC, more than 70% of patients are currently diagnosed at late stages of the disease and are thus ineligible for potential curative therapies discussed above. The main characteristic that separates intermediate from advanced tumors is the presence of portal vein invasion (29). Patients with HCC and vascular invasion have median survivals between 6 and 8 months (56). Systemic chemotherapy and hormonal therapy have been shown to be ineffective. Sorafenib has been studied in a large placebo-controlled
randomized trial. Sorafenib is a multikinase inhibitor that has antiproliferative and antiangiogenic properties. In this randomized study of 602 patients, median overall survival was 7.9 months in the patients receiving placebo and 10.7 months in the patients receiving sorafenib (hazard ratio 0.69; 95% confidence interval, 0.55–0.87; P<0.001) (57). Importantly, it delayed the time to progression from 2.8 months to 5.5 months (p<0.001). The medication was very well tolerated. This is the first large study that showed that systemic therapy does improve survival in advanced HCC. It should be the first-line therapy for patients with advanced HCC.

4.5. Terminal Stage

This stage include patients that have poor liver function and significant tumor burden and that are not transplant candidates. The median survival is 3 months for these patients. Supportive care should be recommended to these patients.

5. PREVENTION

5.1. Hepatitis B

Regardless of the mechanisms for the occurrence of hepatocarcinogenesis in patients with chronic HBV infection, eliminating the virus is of utmost importance in decreasing the risk of HCC. HBV vaccine is the first vaccine shown to prevent cancer. A study of Taiwanese children found that the average annual incidence of liver cancer decreased from 0.70/100,000 between 1981 and 1986 to 0.57/100,000 between 1986 and 1990 and to 0.36/100,000 between 1990 and 1994 (p < 0.0001) (58). It is anticipated that the implementation of global vaccination of all newborns will ultimately lead to a worldwide reduction in the incidence of HBV-related HCC, although it may take a few decades for the impact to be observed among adults.

For the 350 million persons estimated to have chronic HBV infection worldwide, HBV vaccination would not be effective in preventing HCC. The best strategy for prevention is eliminating modifiable risk factors such as alcohol and tobacco and to treat with agents aimed at eliminating viral replication. A meta-analysis of seven studies that evaluated the use of interferon-α showed that treatment had reduction in the risk of HCC of 6.4% (59). With the recent development of newer antivirals such as lamivudine, adefovir, and entecavir, the potential of antiviral therapy to prevent HCC may be further enhanced. There has been one randomized controlled trial that aimed to determine whether treatment with lamivudine can prevent HCC (60). The
authors randomly assigned 651 patients who were HBe antigen-positive and/or had detectable HBV DNA (98% Asian and 85% male) to receive lamivudine or placebo. HCC occurred in 3.9% (n = 17) of those in the lamivudine group and 7.4% (n = 17) of those in the placebo group (hazard ratio, 0.49; p = 0.047). Even though this trial was very well done and provided some insights, there are still questions remaining about the appropriate length of treatment, target HBV DNA level, endpoints of therapy, and management of viral resistance. The development of newer and more powerful antivirals raises the possibility for an improved prevention rate for HCC among those with chronic HBV infection.

5.2. Hepatitis C

Cirrhosis is by far the single most important risk factor for the development of HCC. It is also known that male gender, older age, coinfection with HBV, alcohol, tobacco, obesity, and diabetes are important risk factors for HCC. As with HBV, only alcohol and tobacco are modifiable. There are three randomized controlled trials that focused on prevention of HCC as an outcome (61–63). The largest trial from Japan randomized 90 patients to treatment or no treatment. HCC developed in 33 of the untreated patients and in only 12 of the treated patients after a follow-up of 8.2 years (p<0.001). The other studies suffer from lack of an adequate sample size, long-term follow-up and also the fact that the overall effect was small. The largest trial to date for secondary prevention of HCC is a multicenter trial in the United States that randomized non-responders with advanced fibrosis to chronic pegylated interferon-α for 3 years vs. no treatment (64, 65). The results showed that chronic interferon therapy did not reduce the risk of HCC or hepatic decompensation when compared to best supportive care.

REFERENCES


Hepatitis B Vaccines

Oren Shibolet, MD and Daniel Shouval, MD

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Key Principles

- Initial immunization strategies against hepatitis B virus (HBV) infection concentrated on high-risk groups. This strategy, which was successful in individual situations, failed to reduce the incidence and prevalence of HBV in the general population.
- As a result, in 1991 the World Health Organization (WHO) has recommended that HBV universal immunization should be integrated into national immunization programs in all countries with an HBsAg prevalence of >8%.
- The results of this impressive global effort have already been translated into a reduction in the incidence of HBV infection, and its complications including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) in individual countries who were among the pioneers of universal infant immunization.
- Yeast (and plasma)-derived HBV vaccines are highly efficacious in preventing HBV infection, and in reducing the incidence of persistent infection as well as of hepatocellular carcinoma.
- Hepatitis B vaccines have had an excellent safety profile, and are generally well tolerated. Adverse reactions which are usually mild and resolve quickly tend to decrease with successive doses of the vaccine.
- Despite the excellent efficacy of HBV vaccines, immunization failure may occur, and can sometimes be explained by variables such as improper storage or administration, advanced age, obesity, renal failure, chronic liver disease, and especially immunosuppression.
- Another important factor that may affect non-responsiveness to HBsAg immunization seems to be genetically determined resistance.
- Bypass of non-response to conventional vaccination has recently been achieved using Pre-S1/Pre-S2/S third-generation HBV vaccines.
- Contraindications for HBV vaccines include hypersensitivity to yeast or any component of the vaccine. Patients who develop hypersensitivity after vaccination should not receive further injections of the vaccine.
- Actively pursued research avenues intended to stimulate TH1 immune responses, include the development of more potent adjuvants as compared to the currently used aluminum hydroxide as well as development of DNA vaccines.

1. INTRODUCTION

Persistent hepatitis B virus (HBV) infection is a common public health problem, worldwide (1). Following acute HBV infection, between 30 and 90% of babies born to HBV-carrier mothers and 5–10% of infected adults will become chronic HBV carriers, positive for HBV surface antigen (HBsAg). Such HBsAg+ individuals are at increased risk for developing chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) (2). The global burden of HBV infection, as determined by prevalence of HBsAg positivity, can be divided into three major areas of high (>8%), intermediate (>2–<8%) and low (<2%) endemicity. According to the World Health Organization (WHO) estimates, at least 2 billion people of the world population have been infected by HBV during their lifetime of whom about 380 million (~6% of the world population) are still HBV carriers with persistent infection. Annually, approximately 4.5 million patients develop acute HBV infection worldwide and about 600,000 die of complications of chronic HBV infection including cirrhosis and HCC (3).

Efficacious and safe hepatitis B vaccines are now available for more than 25 years. This chapter will review the history of development, the immunogenicity, safety and impact of this technological marvel on the global epidemiology of HBV infection.

2. STRUCTURE AND GENOMIC ORGANIZATION OF THE HEPATITIS B VIRUS ENVELOPE PROTEINS RELEVANT TO VACCINE DEVELOPMENT

HBV is a 3,200 base pair (bp) DNA virus. It has a genome of relaxed, circular, partially double-stranded configuration (see Chapter 1 for a detailed discussion of HBV structure). The genome has four reading
frames encoding several overlapping viral proteins including Pre-S₁, S₂, S, core, HBe, X, and the polymerase proteins. The virus requires an RNA intermediate for replication and undergoes reverse transcription lacking proof reading that leads to a high mutation rate. To date, eight genotypes have been identified with an 8% divergence in the complete nucleotide sequence (4–8).

All available HBV vaccines contain one or more of the hepatitis B envelope proteins or surface antigens HBsAg. The HBsAg is composed of three related envelope proteins, synthesized by an alternate use of three translational start codons and a common stop codon. The HBsAg proteins include a major polypeptide of 226 amino acids (aa) designated small HBs (SHBs) in a non-glycosylated (p24) and glycosylated (gp27) forms. The middle-sized protein (MHBs), which shares the 226 aa of the p24 region at the C-terminus and has an additional 55 aa residue at the N-terminus, is termed Pre-S₂ corresponding to p33 and gp36. The large HBs protein (LHBs) contains, in addition to the S and Pre-S₂ domains, the Pre-S₁ domain of 119 aa (p39, gp42). In the native HBV envelope, all proteins SHBs, MHBs, and LHBs are covalently linked by intermolecular disulfide bonds between the S domains, and partially embedded in membrane lipids (9).

3. ROLE OF THE IMMUNE RESPONSE AGAINST HBV ENVELOPE PROTEINS IN LONG-TERM PROTECTION AGAINST HBV

Induction of immune memory against HBsAg and generation of anti-HBs antibodies are essential for long-term protection against HBV. In addition, Pre-S antigen(s) induce neutralizing antibodies, which block attachment, endocytosis, and possibly membrane penetration of HBV into the hepatocyte (10).

In patients with acute HBV infection who clear HBV from their blood, detection of protective anti-HBs antibodies in serum may be delayed for weeks up to 6 months. In contrast, Pre-S₁ and Pre-S₂ antigens, which have a role in inducing T-cell help for anti-HBs production, induce generation of anti-Pre-S antibodies which appear and disappear early after acute infection, sometimes in a biphasic pattern. T-cell recognition of HBV proteins requires presentation to T cells of HBV antigenic determinants, which must be processed by antigen presenting cells prior to expression on the surface of T cells in association with HLA antigens (11–14). Milich and coworkers have characterized the T-cell-mediated immune response to Pre-S antigens and have shown
that immunity to HBsAg can be enhanced using Pre-S antigens in non-responder mice resistant to SHBs antigen (15, 16).

Antibody responses to HBV envelope proteins after exposure to wild-type HBV or after immunization are well characterized. Anti-HBs antibodies can be either subtype-specific or common to all genotypes of HBsAg, for example, against the “a” determinant, which is an epitope present in all HBV vaccines in use (14). By convention, seroconversion to anti-HBs is defined as detection of anti-HBs in an immunological assay at ≥2.1 standard deviations from the reading of the negative control, which is usually 1 mIU/ml (2.1–9 mIU/ml). Seroprotection against infection is present when anti-HBs levels are ≥10 mIU/ml. Numerous studies have shown that most children and young adults will develop hundreds to several thousand mIU/ml of anti-HBs following three doses of a conventional HBV vaccine (17,18). Vaccinee that develop an anti-HBs level between 10 and 100 mIU/ml after three doses are referred to as low responders. In the United Kingdom, seroprotection against HBV infection was re-defined at anti-HBs levels ≥100 mIU/ml (19). This approach has public health implications and may require redefinition of non-responsiveness to routine immunization. Anti-HBs levels tend to fall with time, but primed immune memory will usually respond to challenge with wild-type virus as well as to booster inoculation with HBsAg by an anamnestic anti-HBs response. Currently, there is no reason to offer booster doses to vaccinées who developed anti-HBs titers >100 mIU/ml following immunization with three vaccine doses (20–23).

4. TRANSMISSION OF HBV INFECTION

The virus is transmitted from patients with acute or chronic infection to HBV naive persons via parenteral or mucosal exposure to body fluids. Infective virions are present in all body fluids with a decreasing concentration in blood, serous fluids, saliva, semen, breast milk, tears, sweat, urine, stool, and respiratory secretions, respectively (24).

Vertical and perinatal transmission of HBV from HBsAg-positive mothers with a high viral load to their babies, as well as sexual contact of HBV patients are the most common routes of transmission in Africa and Asia. In the western hemisphere, the most important routes of infection include sexual contact (both heterosexual and Men who have sex with Men – MSM), via sharing of needles in I.V drug abusers, and occupational exposure among health-care workers (25). Other less common routes include tattooing, body piercing, acupuncture, eye splashes, and other forms of direct contact between infected material and mucosal surfaces (26).
5. HISTORY OF HBV VACCINATION

Following the detection of the hepatitis B surface antigen in 1967 by Dr. Baruch Blumberg, Dr. Saul Krugman has made the first, at that time controversial, attempt to develop a hepatitis B vaccine. He used crude, heat-inactivated serum obtained from children with HBV as an immunogen for protection of individuals at risk (27). The first HBV vaccines were developed simultaneously between 1976 and 1980 in France and in the United States using plasma obtained from HBsAg carriers (28, 29). These first-generation plasma-derived vaccines contained mainly HBsAg, subjected to various combinations of urea, pepsin, formaldehyde, and heat, which led to partial disruption of the HBV envelope proteins. Similar vaccines were then produced in Korea and China. Plasma-derived vaccines had an excellent record of safety and immunogenicity. Yet, the advance in recombinant DNA technology and concerns regarding the safety of the plasma used for purification of HBsAg, led in 1986 to the development of the second-generation recombinant yeast-derived vaccines which are currently used in the majority of countries worldwide (30, 31). These new HBV vaccines were the first genetically engineered vaccines produced from yeasts (i.e., *Saccharomyces cerevisiae* and later also *Hansenula polymorpha*) transfected with HBV–DNA sequences coding for the small HBV envelope protein.

All second-generation hepatitis B vaccines contain purified nonglycosylated HBsAg (small HBs) absorbed to aluminum hydroxide as an adjuvant for stimulation of anti-HBs producing B cells. Currently, there are several available yeast-derived vaccines with variable amounts of the envelope protein, but with comparable immunogenicity (Table 1).

In the past decade, several bivalent and pentavalent combination vaccines were developed in an attempt to reduce the number of vaccine injections, especially in young babies. These vaccines, mainly available in the western hemisphere, include DTP, HBV, Hemophilus influenza, polio, and hepatitis A antigens in various combinations, which in general do not significantly reduce the immunogenicity of the individual components (32–34).

A “third-generation” mammalian cell-derived vaccine was first developed in the early 1980s at the Pasteur Institute in transfected Chinese hamster ovary (CHO) cells, expressing S and Pre-S₂ antigens (35). Subsequently, two mammalian cell-derived vaccines containing three HBV envelope proteins, namely Pre-S₁, Pre-S₂ and small S, were developed in mouse and CHO cell lines in Germany and Israel, respectively (36, 37). Such vaccines were shown to induce a rapid and augmented anti-HBs response with anti-HBs seroconversion appearing earlier as
<table>
<thead>
<tr>
<th>Name of Vaccine</th>
<th>Dose</th>
<th>Components</th>
<th>Vaccine Schedule and Route of Administration</th>
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| **Engerix-B®** | 1. Pediatric formulation – 20 mcg/ml–0.5 ml  
2. Adult formulation – 20 mcg/ml–1 ml | HBsAg (recombinant yeast) | Infants/children/adolescents (0–19 years) 10 mcg/0.5 ml × 3 (0, 1, 6 months)  
Adults 20 mcg/1 ml (0, 1, 6 months)  
Adults dialysis 40 mcg/2 ml (0, 1, 2, 6 months) |
| **Recombivax-HB®** | 1. Pediatric/adolescent formulation – 10 mcg/ml–0.5 ml  
2. Adult formulation – 10 mcg/ml–1 ml  
3. Dialysis formulation – 40 mcg/ml–1 ml | HBsAg (recombinant yeast) | Infants/children/adolescents (0–19 years) 5 mcg × 3 (0, 1, 6 months)  
Adolescent (11–15 years of age) 5 mcg × 3 or 10 mcg × 2  
(0 and 4–6 months later)  
Adults 10 mcg × 3 (0, 1, 6 months)  
Predialysis/dialysis 40 mcg × 3 (0, 1, 6 months) |
| **Sci B Vac®** | 10–20 mcg/0.5–1.0 ml | HBsAg (incl. recombinant S, Pre-S1, and Pre-S2 in CHO cells) | Infants and children 5 mcg × 3 (0.1, 6 months)  
Adults 10 mcg × 3 (0.1, 6 months)  
Non-responders (adults) to yeast-derived vaccines 20 mcg × 2 (0 and 2–3 month) |
| **Fendrix®** | 20 mcg/0.5 ml–0.5 ml | HBsAg (recombinant, yeast) | Predialysis and dialysis patients age 15 years and older 20 mcg/0.5 ml × 4 (0, 1, 2, 6 months) |
| **Twinrix®** | 720 IU/ml inactivated HAV and 20 mcg/m HBsAg–1 ml | Hepatitis A inactivated and HBsAg (recombinant yeast) | Adults 1 ml × 3 (0, 1, 6 months) or 1 ml × 4 (0, 7, 21–30 days, followed by booster at month 12) |
| **Convax®** | 7.5 mcg of HIB and 125 mcg meningococcal protein conjugate with 0.5 mcg of HBsAg | Hemophilus b conjugate (HIB) and hepatitis B recombinant yeast | 0.5 ml × 3 (0, 1, 12–15 months of age) should not be given before 6 weeks of age |
| **Pediarix®** | 0.5 ml | Diphtheria and tetanus toxoids and acellular pertussis, hepatitis B recombinant yeast, and inactivated poliovirus | 0.5 ml × 3 at 6–8-week intervals (preferably 8 weeks), starting at 2 months of age (may be given starting at 6 weeks of age) |

*Examples of licensed vaccines. Not all vaccines are available globally.
compared to yeast-derived vaccines and with higher anti-HBs titers (38, 39). Furthermore, these vaccines were also more immunogenic in immune suppressed patients, in patients on hemodialysis and in non-responders to conventional SHBs-containing vaccines as compared to yeast-derived vaccines (36, 40, 41).

6. IMMUNOGENICITY AND EFFICACY OF HEPATITIS B VACCINES

Following the successful clinical trial by Szmuness et al. reported in 1980 on efficacy of a plasma-derived HBV vaccine in MSM (29), and the development of yeast-derived vaccines, hundreds of million doses were administered worldwide with excellent immunogenicity. After administration of three HBV vaccine doses, usually given at 0, 1, and 6 months, seroprotection rates, as measured by anti-HBs serum levels >10 mIU/ml, reach almost 100% in children and ~95% in young healthy adults (17, 41, 42, 43). HBV vaccines are also highly immunogenic when started at birth. Risk factors associated with a suboptimal response to immunization include overweight, age, heavy smoking, immune suppression, hemodialysis, systemic diseases, and genetically determined non-response (see later). Anti-HBs levels following the third yeast-derived vaccine dose usually reach several hundreds to thousands mIU/ml, dropping rapidly within 12–24 months after complete immunization. Frequently, anti-HBs antibodies become undetectable within several years. However, immune memory providing protection against challenge with HBV remains intact for at least two decades in the majority of healthy vaccinees (43–45). The recently developed third-generation Pre-S/S HBV vaccines containing Pre-S/S envelope proteins have been shown to induce a higher and faster anti-HBs response as compared to yeast-derived vaccines (36, 38–40, 46). A mathematical model has been designed to assess the changes, through time after vaccination, in circulating vaccine antigen, immune memory, and antibody titer. The model parameters were estimated from a database of 10,815 post-vaccination antibody titers obtained from 1923 adult individuals from hepatitis B vaccine trials in Belgium, Cuba, Israel, Italy, Sweden, and the United States (47). Results of this assessment suggest that a Pre-S/S vaccine induced an immune memory against HBsAg, already after the first vaccine dose and a faster and higher anti-HBs response as compared to yeast-derived vaccines. Furthermore, the models predicted that such third-generation HBV vaccines may induce a long-lasting immune memory for protection against HBV even after two instead of the three conventional vaccine doses.
7. HEPATITIS B VIRUS MUTANTS FOLLOWING IMMUNIZATION AGAINST HBV

HBV envelope protein escape mutants have been described in Italy, Singapore, Gambia, and the United States, in recipients of plasma-derived, as well as recombinant, HBV vaccines (8, 48–50). Such mutants have emerged in children born to HBsAg-carrier mothers who were immunized against HBV receiving active (plasma or recombinant vaccines) as well as passive immunization with hepatitis B immune globulin (HBIG). Breakthrough infection occurred in these children despite an adequate anti-HBs response to active immunization. Similar mutants have also been described in liver transplant patients receiving polyclonal or monoclonal HBIG for protection against reinfection with HBV (48–52). The common reason for generation of such mutants is a single point mutation in one of the amino acids coding for the determinant of HBsAg, often as a result of amino acid substitution from glycine to arginine at position 145 (G145R). Yet, despite the fact that such mutants were shown to be infective in chimpanzees, a conventional yeast-derived vaccine was able to protect these animals against an HBV envelope mutant (53). At present, emergence of such mutants is a relatively rare event, and does not seem to pose a serious threat to public health.

8. MODE OF ADMINISTRATION

All commercially available HBV vaccines should be administered intramuscularly preferably in the deltoid region or the lateral aspect of the thigh. Infants and neonates have a low muscle mass in the deltoid and in this population it may be preferable to inject in the thigh.

Injections in the gluteal region are not recommended as it was shown that they are frequently given into fatty tissue instead of muscle and result in lower seroconversion rates. For patients with a risk of hemorrhage following intramuscular injection, HBV vaccines can be given subcutaneously. However, the effect of this route of administration on seroconversion efficacy is unknown (54).

9. INTERCHANGEABILITY OF VACCINES

Yeast-derived HBV vaccines containing aluminum hydroxide are usually interchangeable regardless of manufacturer (55). Furthermore, the HBsAg used in combination vaccines such as in Pediarix® and
Twinrix® is the same as that used in Engerix-B® and is, therefore, interchangeable. For example, a yeast-derived monovalent HBV vaccine may be given within 48 h after birth followed by combination pentavalent vaccine containing among others also HBsAg given 2–6 or 12 months later.

10. STRATEGIES OF IMMUNIZATION AND WORLDWIDE IMPACT

Following licensure of HBV vaccines in the early 1980s, immunization strategies concentrated on high-risk groups including newborns to HBsAg-positive mothers (with simultaneous HBIG administration), health-care workers (HCW), family members and partners with close contact to an HBV carrier, illicit drug users, MSM, and patients at sexually transmitted disease clinics (STD). This strategy, which was successful in individual situations, failed to reduce the incidence and prevalence of the general population. As a result, in 1991 the World Health Organization (WHO) has recommended that HBV universal immunization should be integrated into national immunization programs in all countries with an HBsAg prevalence of >8% by 1995 (56). This recommendation was extended to countries with an HBsAg prevalence between 2 and 8% in 1997. In countries with high and intermediate prevalence, it was recommended to start immunization within 48 h of birth (preferably within 24 h) followed by booster doses at the age of 1 and 6 months (54). In countries with low HBV endemicity, starting immunization may be postponed to the second month of life or even later, provided that pregnant women are screened for HBsAg (57). Catch-up vaccination against HBV has been introduced in many European countries as well. By 2005, 168 countries (85% of the world) introduced routine immunization (usually universal) with an estimated three-dose coverage over 60% (Fig. 1). Cost–benefit analyses have strongly supported the introduction of universal immunization against HBV to newborns in countries with high and intermediate endemicity of HBV infection (58–63).

The results of this impressive global effort has already been translated into a reduction in the incidence of HBV infection, and its complications including chronic hepatitis, cirrhosis, and hepaticcellular carcinoma (HCC) in individual countries who were among the pioneers of universal infant immunization.

In Taiwan, which was the first country to introduce universal vaccination in newborns in 1984, the prevalence of HBsAg in children up to the age of 15 years, decreased from 9.8% in 1984 to 0.7% in 1999

(51, 64). Furthermore, the average annual incidence of HCC in children 6–14 years of age measured between 1981 and 1986 dropped by 50% (measured between 1990 and 1994) (65). In Gambia, childhood prevalence of HBsAg dropped from 10.0 to 0.6% in an immunized population of children followed since 1984 (66). A similar and gradual drop in incidence of HBV is now being observed in several regions and countries worldwide which were the pioneers of massive vaccination efforts, including Alaska, Italy, and Hawaii (67–69).
11. SAFETY AND TOLERABILITY OF HBV VACCINES

During the past 25 years, hepatitis B vaccines have had an excellent safety profile, and are generally well tolerated. Adverse reactions which are usually mild and resolve quickly tend to decrease with successive doses of the vaccine.

The most common reactions for the two most frequently used HBV vaccines, namely Engerix-B® and Recombivax-HB®, include injection site soreness (17–22%) and fatigue (13–15%). Other reactions included local reaction at the injection site such as swelling, induration, and erythema (1–10%). Systemic adverse events following immunization include fatigue, fever, headache, tingling, sweating, nausea, abdominal pain, vomiting, and weakness may occur (≤1%). Rarely serious adverse reactions were reported, including anaphylaxis, serum sickness-like syndrome, erythema multiforme, Steven-Johnson syndrome, Guillain-Barre syndrome, transverse myelitis, and optic neuritis.

In 1998, French investigators reported a putative association between immunization against HBV, and relapse or induction of multiple sclerosis (MS) (70). In 2004, Hernán and coworkers suggested, in a prospective study, a possible relation between hepatitis B vaccination and an increased risk of MS (71). However, the WHO Global Advisory Committee on Vaccine Safety concluded that the evidence reported in this study was insufficient to support such a link (72). Yet, in patients with MS the benefit of immunization should be weighed against the putative risk of exacerbation of the disease.

Hepatitis B vaccination has been anecdotally linked to rheumatoid arthritis, lupus erythematosus, diabetes mellitus, acute lymphoblastic leukemia, chronic fatigue syndrome, and even hair loss. Reviews of these studies by WHO experts do not support such a link (72–74).

Despite the absence of controlled trials, individual experience suggests that vaccination against hepatitis B is not contraindicated in pregnant or lactating women (75). The only absolute contraindications are known hypersensitivity to any component of the vaccine or a history of anaphylaxis to a previous dose.

12. RATIONALE FOR IMMUNIZATION OF SPECIAL POPULATIONS AT RISK

Heterosexuals with multiple sex partners. Sexual transmission of HBV is the most common route of HBV transmission in North America, accounting for approximately 40% of all new HBV cases in the United States. Sexual transmission among homosexuals accounts for another 25%.
**Intravenous drug users (IVDU).** These account for approximately 15% of all new HBV infections. The risk for HBV transmission is associated with frequency of injection and sharing of drug paraphernalia.

**Household contacts of chronic HBV infected persons.** The risk of acquiring HBV infection is high in household contacts of persons with chronic HBV infection, and high viral load and can reach 60%. Populations at highest risk include sex partner, where seroprevalence ranges from 25 to 60%, and in children.

**Occupational exposure to HBV.** Occupational exposure was previously considered a significant risk for contracting HBV (25). Following implementation of vaccination programs, the incidence of HBV infection among health-care workers in the western hemisphere is dropping rapidly. The prevalence of HBV infection among public service workers with exposure to blood (police and correctional facilities officers and fire fighters) is similar to the general population.

**Hemodialysis patients.** Following implementation of HBV vaccination and infection control programs in dialysis patients, the rates of new infection considerably declined.

**HIV-positive persons.** Because of similar routes of transmission, the prevalence of HIV/HBV coinfection is relatively high ranging from 6 to 75% in certain groups. Optimal response to immunization against HBV is achieved when HIV is controlled and in remission.

**Persons in long-term care and correctional facilities.** The rates of new HBV infections in these facilities have been declining since the implementation of vaccination programs. However, because of a higher prevalence of chronic HBV-infected patients in these facilities; unvaccinated persons are at an increased risk for acquiring HBV infection.

**Patients with chronic non-HBV liver disease.** Such patients should be immunized against hepatitis B (and A).

**Persons traveling to endemic regions with HBV.** These persons are not necessarily at a higher risk for acquiring HBV infection. However, immunization against HBV is advised especially for those individuals who intend to stay in such areas for a prolonged time. Acceleration of vaccine administration (i.e., at 0, 1, 2, and then 6 or 12 months) may be considered in individuals requiring rapid protection.

## 13. NON-RESPONDERS TO CONVENTIONAL VACCINATION

Yeast (and plasma)-derived HBV vaccines are highly efficacious in preventing HBV infection, and in reducing the incidence of persistent infection as well as of hepatocellular carcinoma. In 2001, a review of 181 clinical studies evaluating 24,277 individuals who were immunized
with Engerix-B® and 8627 with Recombivax HB® provided evidence for the extraordinary immunogenicity of yeast-derived HBV vaccines (42). The recommended pediatric dose per injection was 10 and 5 µg for both vaccines, and the adult dose was 20 and 10 µg, respectively. Seroprotection (>10 mIU/ml) was achieved in 95.8 and 94.3%, respectively, using the three-dose schedule at 0, 1, and 6 months. Children and adolescents (1–19 years) achieved the highest seroprotection rates, namely 98.6 and 98.8%, respectively. Thus, the degree of non-response in children and young adults is low. Yet, despite the excellent efficacy of second-generation HBV vaccines, immunization failure may occur, and can sometimes be explained by variables such as improper storage or administration, advanced age, obesity, renal failure, chronic liver disease, and especially immunosuppression. Another important factor that may affect non-responsiveness to SHBs immunization seems to be genetically determined resistance (76, 77). The ability to produce antibodies in response to immunization with HBsAg is controlled by autosomal dominantly expressed HLA class II molecules (76–79). Hohler and coworkers have reported enhanced expression of DRB 1*3, DRB 1*7, and DRB 1*14 in non-responders to an HBV vaccine (78). In contrast, response to vaccination is related to DRB 1*13, which seems to have a promoting effect on anti-HBs seroconversion. Milich and others have shown that non-responsiveness to SHBs immunization in mice can be circumvented through immunization with Pre-S proteins (79). Furthermore, immunization with synthetic MHBs (Pre-S2 or Pre-S2/S) peptides, as well as with yeast and mammalian CHO cell-derived Pre-S2/S vaccines, may induce neutralizing antibodies and protect chimpanzees against HBV (80). A major determinant of immunological response to vaccination is age. Response rates decrease by about 15 and 25% in persons aged 40 and 60, respectively, compared to younger persons. Other determinants that cause decreased response to vaccination include smoking, obesity, and immunosuppression. Although the HBV vaccines are highly immunogenic, 5–15% of vaccinated persons do not respond to the primary vaccine series (i.e., anti-HBs <10 IU/ml). These persons should complete a second three-dose vaccine series, and be evaluated for HBV-carrier status. Following the second vaccine series, there is a 30–50% chance of developing a protective anti-HBs titer. A person who does not develop protective antibodies (and is not an HBsAg carrier) is considered a HBV “vaccine non-responder”. Non-responders belonging to risk groups for contracting HBV infection are susceptible to HBV infection, and should be protected with H BIG after exposure as soon as possible, preferably within 24 h and no later than 1 week post exposure.
Bypass of non-response to conventional vaccination has recently been achieved using Pre-S$_1$/Pre-S$_2$/S third-generation HBV vaccines (41). In one such study, the primary study population of 330 HBV vaccine non-responders after $\geq$4 previous injections was randomized 2:1 to receive one or two injections of Hepimmune$^\text{TM}$ (Bio-Hep-B$^\text{®}$, Sci B Vac$^\text{®}$) 20 mcg/dose or Engerix-B$^\text{®}$, 20 mcg/dose given at 1-month interval. An intention to treat analysis revealed that 81.7% of the Pre-S/S CHO-derived vaccine recipients compared to 49.1% recipients of the SHBs yeast-derived vaccine developed anti-HBs titers $>10$ mIU/ml at 1 month after the first or second dose ($P<0.001$). Similar results were obtained with another mammalian Pre-S/S HBV vaccine (36). Finally, in a preliminary study performed in Hong Kong in a small cohort of 20 HBV liver transplant recipients, $\sim$50% of stable patients after transplantation seroconverted to anti-HBs+ following immunization with a Pre-S/S HBV vaccine (81). Thus, non-response to conventional HBV vaccination can be partially resolved using Pre-S/S HBV vaccines.

14. RECIPIENTS OF BONE MARROW AND PERIPHERAL STEM-CELL TRANSPLANTATION

Bone marrow transplantation (BMT) recipients are immunosuppressed, respond poorly to conventional vaccination post-BMT, and are at risk of acquiring severe infections including HBV. Non-response to HBV immunization in such patients may be partially overcome through immunization of bone marrow and peripheral stem-cell donors against HBV. Such a maneuver may induce anti-HBs seroconversion in $>50\%$ of BMT recipients through adoptive transfer of immunity from the healthy immune-competent donor to the BMT recipient (82, 83).

15. CONTRAINDICATIONS

Contraindications for HBV vaccines include hypersensitivity to yeast or any component of the vaccine. Patients who develop hypersensitivity after vaccination should not receive further injections of the vaccine. Patients who develop serious hypersensitivity after receiving a multivalent vaccine should not receive further vaccination with the same vaccine or any of its components.

16. THE FUTURE OF HBV VACCINES

Despite the excellent efficacy and safety of currently used HBV vaccines, attempts are still being made to improve vaccine efficacy in special non-responder risk groups. Actively pursued research
avenues intended to stimulate TH1 immune responses, include the development of more potent adjuvants as compared to the currently used aluminum hydroxide as well as development of DNA vaccines. New adjuvants include MPL (3-deacetylated monophosphoryl lipid A) (84), MF59 (85), and synthetic oligodeoxynucleotides containing immunostimulatory CpG motifs that specifically target Toll-like receptor 9 with a corresponding innate immune response (86). All three adjuvants have been shown to induce excellent anti-HBs seroconversion rates and titers when administered to non-responders of conventional HBV vaccines or immunosuppressed patients. An MPL-containing HBV vaccine intended for patients in renal failure or on dialysis (Fendix®) is already licensed in several countries (87). In the past decade, several immune modulatory and immunotherapeutic modalities intended to augment an anti-HBV response have been tested. These include among others, development of experimental T cell (88) and DNA vaccines for protection against HBV infection as well as intervention in persistent HBV infection (89); stimulation of the innate immune responses through administration of cytokines and granulocyte-macrophage colony-stimulating factors (90, 91); and injection of HBV vaccines intradermally (92). So far, except for Fendix®, none of these compounds has reached clinical application.

17. SUMMARY

HBV is a major human pathogen causing morbidity and mortality. HBV-associated disease can be almost totally prevented by universal vaccination with the highly effective HBV vaccine. Already there have been major therapeutic benefits from HBV vaccination, including a reduction in the rates of HCC and cirrhosis. Now, efforts are being directed at further improving response rates, shortening dosing regimens and targeting non-responsive, or hard to vaccinate populations.

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T-Cell-Mediated Immunity and Immunotherapy of Chronic Hepatitis C

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Key Principles

- Numerous reports suggest that viral replication, the level of viremia, and progression to the chronic state in hepatitis

C-infected individuals are influenced directly and indirectly by HCV-specific cellular immunity mediated by CD4⁺ helper (Th) and CD8⁺ cytotoxic T lymphocytes (CTLs).

- A method to increase the ability of dendritic cells to adequately present antigens should lead to an improved T-cell-mediated cellular response.
- More recently, it has been recognized that in addition to the immunoregulation afforded by differences in the balance between Th1 vs. Th2 cells, some T cells can also exhibit distinct inhibitory properties and directly prevent the activation, proliferation, and function of helper and cytotoxic effector T cells.
- As described above, successful control of HCV is associated with strong and broadly directed HCV-specific CD4⁺ and CD8⁺ T-cell responses, while chronic HCV infection is characterized by attenuated and functionally impaired T-cells. Therefore, an immunotherapeutic approach that stimulates potent cellular immunity against one or more HCV antigens could be beneficial in chronic HCV disease.
- In addition to being able to interact directly with dendritic cells, yeast have a variety of other characteristics that make them an ideal platform for immunotherapy.
- The proposed action of an immunotherapy, for example, a yeast-based Tarmogen engineered to induce adaptive cellular immune responses to multiple HCV antigens (GI-5005) (61) would favor hepatic clearance (the rate-limiting portion of the viral dynamics profile) and should complement the direct antiviral effects of IFN-based therapy.

1. INTRODUCTION

Hepatitis C virus (HCV) is a major causative agent of acute and chronic hepatitis worldwide. HCV infection affects more than 200 million people worldwide and represents a significant health problem in many countries (1, 2). Approximately 20–40% of individuals infected with HCV clear the virus during the acute phase, whereas the remaining 60–80% develop chronic disease which may result in hepatic failure and liver cancer (3–5). There is at present no preventative vaccine, and therapeutic options are limited to interferon/ribavirin therapy which is often poorly tolerated, is contraindicated in many subjects, and is expensive. In addition, the efficacy of the current standard treatment with interferon and ribavirin is limited, especially in genotype 1, the most prevalent genotype in the United States and most industrialized countries
(6). Thus, only a proportion of HCV-infected persons can be success-fully treated at this time and alternative treatment modalities, including immunotherapies and prophylactic vaccines, are clearly needed.

2. T-CELL-MEDIATED IMMUNE RESPONSES TO HCV INFECTION

HCV is a non-cytopathic virus that induces both acute and chronic hepatitis and interacts in a highly complex manner with the immune system (7). Likewise, the immune system has a unique role in the pathogenesis of HCV infection as it contributes both to control of viral infection and liver repair, and also to the development of chronic infection and liver cirrhosis. T-cell-mediated immune responses in acute and chronic HCV infection will now be discussed in brief; however, it should be appreciated that humoral (antibody)-mediated immune responses as well as cells and effectors of innate immunity (e.g., natural killer (NK) cells, macrophages, and type 1 interferons) also impact the outcome of HCV infection, and the reader is referred to excellent reviews that discuss these responses in greater detail (8–12). It is equally important to understand that T-cell responses both influence and are influenced by innate and humoral immunity.

Numerous reports suggest that viral replication, the level of viremia, and progression to the chronic state in hepatitis C-infected individuals are influenced directly and indirectly by HCV-specific cellular immunity mediated by CD4+ helper (Th) and CD8+ cytotoxic T lymphocytes (CTLs) (13–17). Studies of humans and chimpanzees have revealed that HCV can replicate for weeks before the onset of CD4+ and CD8+ T-cell responses can be detected in the liver and in the blood. Moreover, there may be a delay in the acquisition of function by CD8+ (and perhaps CD4+ ) T cells even after their expansion in blood (17). The appearance of functional CD8+ T cells is kinetically associated with control of viremia and, at least in some cases, with an elevation in serum amino-transferases, suggesting that liver damage during acute hepatitis C is immunopathological. At highest risk of persistent HCV infection are those individuals who fail to generate a detectable virus-specific T lymphocyte response in the blood, liver, or both. Perhaps most importantly, generation of a cellular immune response does not necessarily ensure that the infection will be permanently controlled. CD4+ and CD8+ T-cell responses must be sustained for weeks or months beyond the point of apparent control of virus replication to prevent relapse and establishment of a persistent infection.

CD4+ T cells play an essential role in anti-HCV immunity by providing help for activating and sustaining CD8+ T-cell responses. Protective
CD4⁺ T cells appear to predominantly recognize epitopes in Core, NS3, NS4, and NS5 proteins although responses against the other HCV gene products have also been reported (1, 18). In addition to the help that CD4⁺ T cells provide to CD8⁺ T cells, it also appears critical that they produce gamma interferon and other lymphokines that are pro-inflammatory/pro-cell-mediated immunity, as opposed to pro-humoral immunity (Th1- vs. Th2-type lymphokines as described below). Equally important for control of chronic infection is the establishment of HCV-specific memory CD4⁺ T cells (18, 19).

The finding that CD4⁺ and CD8⁺ T-cell responses are common to self-limited HCV infections suggests that they cooperate to bring about control of viremia. Memory CD4⁺ and CD8⁺ T cells primed during acute resolving hepatitis C infection provide long-term protection from virus persistence in chimpanzees and probably humans. Through antibody-mediated depletion of each memory T-cell subset, the chimpanzee model has provided direct proof of the importance of CD8⁺ T cells in the control of acute hepatitis C and their dependence on CD4⁺ T-cell help (20). In contrast to CD4⁺ T cells, both acute and memory CD8⁺ T cells appear to recognize all of the HCV proteins equally and, as with CD4⁺ T cells, it may be critical that they be capable of producing pro-inflammatory cytokines including gamma interferon (17).

The transition from acute to chronic HCV infection is associated with substantial loss of HCV-specific CD4⁺ T cells that do not appear to recover during the life of the host. CD8⁺ T-cell activity is also impaired, as it is insufficient for resolution of infection. The conclusion from the clinical observations is that control and clearance of HCV requires both CD4⁺ and CD8⁺ T cells and that the lack and/or loss of adequate cellular immunity is associated with development of chronic infection. It is appealing, therefore, to hypothesize that stimulation of existing, but insufficient HCV-specific CD4⁺ and CD8⁺ T cells in chronically HCV-infected individuals may have a therapeutic benefit.

3. GENERAL OVERVIEW OF T-CELL-MEDIATED IMMUNITY

It is the aim of this chapter to provide the reader with an understanding of T-cell-mediated immune responses in HCV infection, and how immunotherapy can be approached and utilized in the chronic setting. In this regard, a general overview of T-cell-mediated immune responses is warranted. In brief, the immune system consists of a collection of bone marrow-derived cells and several plasma proteins that work together to fight threats to an organism’s integrity including organ and tissue damage, infection, and cancer. The immune system uses a variety of
receptor-based recognition mechanisms to discriminate between “self” (i.e., the body’s own constituents) and “non-self” (i.e., any foreign material including toxins, bacteria, viruses, and parasites that penetrates the body’s external surfaces).

The immune system has two major mechanisms that allow the body to combat microbial diseases: innate and adaptive immunity. Innate immunity is evolutionarily conserved and refers to the first line of defense to protect against invading organisms and consists of the preexisting defense mechanisms of skin, macrophages, neutrophils, stomach acid, and anti-microbial proteins in the bloodstream. It also refers to the innate response of cells upon viral infection and would, therefore, include type 1 interferons. Adaptive immunity, which is present in vertebrates and is comprised of humoral and cellular immunity, relies on receptors expressed on T and B lymphocytes which allow these cells to specifically recognize “antigens.” An antigen is any substance, but most typically a protein, that can be bound by antigen receptors present on T and B cells. Humoral immunity results from production of antibodies by B cells that, in regard to viral infections, bind specifically to viruses that have entered the body, but not yet infected cells, while cellular immunity induces death of cells that have already been infected. Understanding how these types of immunity are controlled is essential to the design of effective vaccines and immunotherapy.

3.1. Cells of the Immune System

All of the cells of the immune system arise from precursors in the bone marrow. They circulate freely throughout the bloodstream, are able to leave the vasculature, and enter tissues. They recirculate into the blood via the lymphatics. Cells of the immune system congregate in lymph nodes and in the spleen. In these “peripheral” lymphoid organs, the necessary interactions between antigen-presenting cells and antigen-specific T and B cells can occur leading to a productive immune response.

The first cells that respond during an immune response are dendritic cells and macrophages which play a central role in both innate and adaptive immunity. These cells are actively recruited to the site of tissue damage, and are intimately involved in repair processes. Until recently, innate immunity was considered as being a largely non-specific response mediated by neutrophils and macrophages that engulf and digest microbial pathogens. However, it is now clear that both cell types, and especially macrophages and the related dendritic cells, recognize “not-self” to some degree in that they preferentially phagocytose damaged proteins and cells as well as bacteria, yeast, and other
substances that enter the body. Macrophages and dendritic cells do so because they express receptors that are collectively referred to as pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs). PAMPs represent organism-specific differences in glycosylation patterns, lipoproteins, and nucleic acid composition. Hence, APCs have receptors for microbial mannoproteins, peptidoglycans, glucans, lipoproteins, double-stranded RNA, and CpG island-containing DNA (21–23). Engagement of these receptors results in what has been termed a “danger” signal leading to dendritic cell maturation, activation, enhanced phagocytosis, and efficient presentation of antigens that were associated with the engaging material (24). Examples of PRRs include Toll-like receptors (TLRs) that will be described in detail later (25).

Dendritic cell and macrophage activation is further amplified by pro-inflammatory lymphokines, soluble stimulatory factors secreted by T cells during an immune response, and by cytokines secreted by cells at the site of tissue damage. Activated macrophages and dendritic cells play a central role in induction of inflammation and fever due to the release of arachidonic acid metabolites (e.g., prostaglandins) and endogenous pyrogen (also called interleukin-1; IL-1). Inflammation is beneficial in the sense that it recruits additional white blood cells to the tissue or organ where an immune response may be needed. Dendritic cells and macrophages are intimately involved in the activation of T lymphocytes by acting as “professional antigen-presenting cells” (APC). Macrophages are also capable of killing and causing damage to normal, infected, or tumor cells by releasing toxic substances such as tumor necrosis factor (TNF) and nitric oxide (NO).

While macrophages and dendritic cells are absolutely critical in initiating immune responses, T cells, or T lymphocytes, are the central control cells of the adaptive immune system. T lymphocytes do not respond to foreign materials directly but, rather, recognize peptide fragments of foreign proteins that are presented in association with major histocompatibility (MHC) molecules (see Figs. 1 and 2). Each T cell has an antigen receptor (TCR) of only one specificity. It has been estimated that at least 100 million different receptors can be made. This diversity allows the immune system to react against any foreign substance, natural or man-made. T cells come in two functional types: helper/inducer and cytotoxic/killer T cells.

Helper T cells (Th) express the CD4 cell surface molecule and respond to antigens presented in the context of MHC class II molecules. CD4+ helper T cells produce and secrete soluble factors (lymphokines) that activate macrophages (to induce inflammation), cytotoxic T cells (to kill virally infected or tumor cells), and B cells (to secrete antibody).
Fig. 1. Dendritic cell presentation of exogenous antigens via MHC class II to CD4⁺ helper T cells.

Fig. 2. Recognition of endogenous antigens by CD8⁺ cytotoxic T cells via MHC class I.

Cytotoxic T cells (CTL) express the CD8 cell surface molecule and recognize antigens in the context of MHC class I molecules. (Note: MHC class I molecules are expressed by all cell types in the body whereas MHC class II expression is limited, for the sake of this discussion, to professional antigen-presenting cells including macrophages, dendritic cells, and B cells.) CTL are effective at killing cells infected with viruses, as well as intracellular bacteria and parasites (e.g., mycobacterium tuberculosis, toxoplasma, and leishmania). They hold promise for tumor immunotherapy as well because of their ability to recognize cells making mutant proteins.

Finally, B lymphocytes use antibody molecules as their antigen receptors and, in contrast to T cells, recognize foreign materials like
bacteria, viruses, and toxins directly. Each B cell expresses antibody of only one specificity and, as for T cells, at least 100 million antibody specificities are possible.

3.2. Humoral Immunity in Response to Exogenous (Extracellular) Antigens

Immune responses are initiated when dendritic cells and macrophages endocytose foreign particles (e.g., bacteria and viruses), protein antigens (e.g., toxins and allergens), or cellular debris (e.g., from cells killed by viruses) that are present in extracellular fluids. Foreign proteins are digested into polypeptides (10–20 amino acids long) which are bound to MHC class II molecules in specialized vesicles, endosomes, dendritic cells, and macrophages. The peptide and MHC class II complex is then expressed on the surface of the dendritic cell or macrophage (Fig. 1). An antigen-specific CD4⁺ helper T cell binds to the combination of MHC class II and peptide, becomes activated and produces lymphokines, including IL-2, IL-4, and IL-5 (not all produced by the same helper T cell – see below). Type 2 helper T cells (Th2), which produce IL-4 and IL-5, but not IL-2, seem to dominate over type 1 helper T cells (Th1) cells in responses to exogenous antigens and typically lead to an antibody-mediated (humoral) immune response. CTL are not normally activated in response to exogenously introduced antigens.

Antibody is effective in coating bacteria and making them more likely to be phagocytosed by macrophages and granulocytes (IgG subclass). Antibody can “neutralize” viruses and prevent them from getting into the body (IgA) or infecting cells (IgG) and, with the help of complement factors, can directly kill certain bacteria (IgM and IgG). However, it is important to understand that antibody generated in an individual, as opposed to therapeutic monoclonal antibodies, generally has little direct effect on cells expressing foreign antigens, including tumor or viral antigens expressed on the surface of a cell.

3.3. Cellular Immunity in Response to Endogenous (Intracellular) Antigens

In contrast to extracellular antigens, foreign or mutant proteins being synthesized by, or merely present in the cytoplasm of infected cells, are digested into polypeptides (8–10 amino acids long) in the ER/Golgi/proteasome complex. The polypeptides are bound to MHC class I and expressed on the surface of the infected or tumor cell (Fig. 2). CD8⁺ T cells respond to the combination of MHC class I and peptide,
and produce lymphokines which, in general, lead to a cell-mediated immune response. They can also kill infected cells directly. CTL appear to require IL-2 and IL-12 in order to be effectively activated. While CTL can produce some IL-2, it is generally accepted that CD4+ Th1 cells are the major source of IL-2, and dendritic cells are the major source of IL-12 for CTL-mediated responses. In addition, it is also clear that in order to obtain maximal CTL activation, presentation of antigens by dendritic cells is required. Thus, as for CD4+ Th1 cells, CTL require interaction with an APC in order to become maximally activated and then respond to virally infected cells.

It was initially unclear how antigens being synthesized by a virally infected cell could find their way into the MHC class I pathway in dendritic cells, unless the dendritic cell itself became infected. However, recent data indicates that dendritic cells can recognize infected cells that become apoptotic as a result of infection and that “cross-priming” (delivery of exogenous antigens into the endogenous antigen presentation pathway) can occur such that some of the proteins associated with cells/particles engulfed by dendritic cells and macrophages find their way into the MHC class I pathway (26). In addition, certain “danger” signals mediated by PRRs including TLRs (described below) can enhance this process (27).

As with presentation to CTL, it was also unclear how virus-specific CD4+ Th1 cells would become activated in response to endogenously synthesized antigens (particularly in cells which do not express MHC class II); however, it is possible that cells that die as a result of the initial infection are ingested by dendritic cells or macrophages leading to helper T-cell activation, production, and secretion of the appropriate lymphokines to initiate cellular immunity.

3.4. Th1 vs. Th2 Cells: Cell-Mediated vs. Antibody-Mediated Immunity

As discussed above, there are two potential positive outcomes in response to antigens: cell-mediated and antibody-mediated immunity. In the past, it was thought that both occurred with any antigen. However, today it is recognized that these arise by diametrically opposed mechanisms, and in fact one or the other type of response may dominate against many antigens. Understanding how these types of immunity and dominance are controlled is essential to the design of effective vaccines and immunotherapy.

At the present time, it can be shown that Th1 cells are preferentially involved in cell-mediated responses. These cells lead to the activation of CTL and macrophages by producing IL-2, and the pro-inflammatory
cytokines gamma interferon (IFN-γ), granulocyte/macrophage colony-stimulating factor (GM-CSF), and lymphokinin (TNF-β). IL-12 produced by activated dendritic cells and macrophages is also necessary for development of cell-mediated immunity, and in combination with IL-2 may act to suppress Th2 cells and, hence, antibody-mediated responses. Th2 cells produce IL-4 and IL-5 that act on B cells to stimulate antibody production. IL-10 produced by Th2 cells is also necessary for development of antibody-mediated immunity, and in combination with IL-4 appears to suppress Th1 cells and, hence, cell-mediated responses.

In brief, the body usually initiates a cellular response against infectious agents first, and then works its way toward a humoral response. As noted above, immune responses are initiated by dendritic cells and macrophages that take up foreign material from extracellular fluids. A method to increase the ability of these cells to adequately present antigens should lead to an improved T-cell-mediated cellular response. Exogenous antigens are preferentially expressed via MHC class II leading to activation of CD4+ T cells that initially differentiate into predominantly Th1 cells and stimulate cell-mediated immunity including activation of CTL and pro-inflammatory macrophages. Over time, the immune response matures and CD4+ T cells increasingly differentiate into Th2 cells. The lymphokines produced by Th2 activate B cells to make antibody while at the same time suppressing Th1 cells. This shift from Th1 to Th2 allows production of protective antibody that prevents reinfection, but unfortunately may not lead to eradication of cells that are already infected with viruses or other intracellular pathogens. In addition, if the body should become reintroduced to that antigen, it will generally respond with a much stronger secondary humoral response than cellular response.

3.5. Regulatory T Cells

More recently, it has been recognized that in addition to the immunoregulation afforded by differences in the balance between Th1 vs. Th2 cells, some T cells can also exhibit distinct inhibitory properties and directly prevent the activation, proliferation, and function of helper and cytotoxic effector T cells (28–32). These regulatory T cells (Tregs) most typically express the forkhead P3 transcriptional factor that is involved in T-cell differentiation, the CD4 molecule and the CD25 IL-2 receptor, and are thus referred to as Foxp3+/CD4+/CD25+ Tregs. An increased frequency of T cells with regulatory phenotypes and functions have been described by numerous groups in HCV-infected humans and non-human primates. In humans, it has been reported that Tregs are expanded during acute HCV infection, maintained during the
chronic stage, and lowered to the frequency of control individuals upon immune-mediated clearance (33, 34). Collectively, these findings suggest an important role for Tregs in the pathogenesis of HCV infection, but also imply that a certain balance between the frequency and the function of Tregs is necessary to achieve an immune response that provides a reasonable chance for HCV clearance.

It is not clear how Tregs mediate their suppressive activity, but likely involves both cell-to-cell contact and induction of inhibitory cytokines. Cell surface molecules that appear to be involved include CTLA-4, GITR, and PD-1; whereas IL-10 and TGF-β have been implicated as soluble suppressor molecules (35–38). It remains largely unknown how Tregs are induced, how they function, and how they contribute to acute and chronic HCV infection.

4. IMMUNOTHERAPEUTIC VACCINES AND CONCEPTS

As described above, successful control of HCV is associated with strong and broadly directed HCV-specific CD4+ and CD8+ T-cell responses, while chronic HCV infection is characterized by attenuated and functionally impaired T-cells (6, 7). Therefore, an immunotherapeutic approach that stimulates potent cellular immunity against one or more HCV antigens could be beneficial in chronic HCV disease.

Prophylactic vaccines (i.e., vaccines that protect an individual against subsequent infections) have had a major impact worldwide in reducing both morbidity and mortality for a variety of bacterial and viral diseases. The majority of these are pediatric vaccines, and they are effective because the pathogens and toxins they target are readily inactivated by “neutralizing” antibody. These vaccines typically lead to strong humoral immune responses at the expense of cellular immunity because they tend to induce Th2 rather than Th1 cells. Thus, it is clear that approaches that stimulate cellular immunity may be needed for many unmet targets including HCV. Prophylactic vaccines consist of three main types: purified, pathogen-derived antigenic proteins (subunit vaccines); heat- or chemically killed viruses; and live attenuated viruses. The following is a description of these conventional vaccine approaches and some of their shortcomings.

4.1. Conventional Vaccine Approaches

Purified, pathogen-derived, antigenic proteins or killed pathogens (e.g., tetanus, diphtheria, Salk polio, hepatitis B subunit, whole and a cellular pertussis, rabies, yellow fever, influenza) are injected along with acceptable adjuvants (cofactors that stimulate the immune
response; e.g., aluminum salts). Antigenic proteins can be difficult to produce and/or purify and viruses can be costly to make and production can be unpredictable. The adjuvants that are currently approved for use in humans do not induce a significant inflammatory response, meaning that the vaccines typically induce weak humoral, but not cellular immune responses and require multiple “booster” administrations.

Conventional vaccine approaches to neutralize HCV by inducing antibodies that target the surface glycoproteins E1 and E2 have so far shown only limited success. In chimpanzees, immunization with recombinant E1 and E2 glycoproteins was able to prevent experimental infection after challenge with homologous virus, but not heterologous virus (39). A study evaluating a recombinant E1 protein as a therapeutic HCV vaccine clearly showed that this strategy was not sufficient to achieve virus clearance. A small phase 1 study of truncated recombinant HCV E1 protein intramuscularly showed good tolerability in chronic hepatitis C patients, a limited increase in E1-specific CD4⁺ T-cell responses and E1 antibody levels, but failed to decrease viral RNA titers. (40). Overall, approaches using vaccines delivering HCV surface glycopolypeptides seem to be of limited promise, especially as immunotherapy for chronic disease. In the setting of chronic infection, where HCV-harboring cells need to be eliminated, immunotherapeutic approaches inducing a cellular immune response are a logical approach to improved control of HCV. In addition, if sterilizing antibody-mediated immunity cannot be achieved for HCV, as is indicated by many studies, agents inducing CD4⁺ and CD8⁺ T-cell responses specific for HCV might also be the best option for prophylactic vaccines, as they might help to prevent the establishment of chronic infection.

Live attenuated vaccines (weakened versions of pathogenic viruses or bacteria that closely resemble the actual disease; e.g., smallpox, Sabin polio, measles, mumps, rubella, chicken pox, and BCG tuberculosis) are injected or ingested and cause a mild form of the natural infection. The tissue damage that occurs as a result of the initial infection causes inflammation and leads to potent cellular and humoral immunity. This class of vaccine has been extremely useful for some diseases, but the potential to revert to a more pathogenic form and the difficulty of generating attenuated versions of many pathogenic viruses and bacteria limit its potential use for unmet indications. For example, it has not proven possible to make clinically useful, attenuated vaccine versions of HIV, RSV, HCV, HBV, herpes, salmonella, shigella, etc.

To overcome the shortcomings of existing vaccine technologies, a variety of new technologies are under development in academic laboratories and industrial settings. In general, these approaches tend to take advantage of recombinant DNA technology to create vaccines that do
not require purification of an antigenic protein or the pathogen itself. A brief description of these approaches follows.

4.2. Recombinant DNA and Virus-based Vaccine Approaches

Live, attenuated recombinant viruses and bacteria that express pathogen-derived, antigen-encoding genes (e.g., recombinant vaccinia (smallpox), canary pox, Venezuelan equine encephalitis virus, adenovirus, and salmonella) that are injected and cause a mild form of the natural infection are being developed. These types of vaccines can induce potent cellular and humoral immunity. However, as with live, attenuated naturally occurring organisms, the potential pathogenicity of the recombinant vaccine vectors themselves may limit application (none are currently approved) and they are costly and difficult to make. In addition, recombinant viruses are subject to neutralization which limits their usefulness in immunotherapy settings where repeated immunization may be required.

DNA vaccines encoding pathogen-derived antigen genes employ naked DNA that is injected directly into muscle tissue where it is taken up by cells that then produce the pathogen-derived antigens encoded by the DNA “vaccine”. DNA vaccines are easy to prepare and are relatively inexpensive. However, long-lived protection may require booster shots composed of “subunit” proteins and/or recombinant viruses. In addition, it has proven difficult to predict the degree of immunity that results with new applications and individuals.

Despite the shortcomings described above, promising results with both recombinant viral vectors and DNA vaccine approaches as well as with dendritic cell-based vaccines in the setting of HCV prophylaxis have been reported (41–46). Nonetheless, the considerations described above suggest that the ideal HCV immunotherapy might consist of a non-pathogenic vector that can deliver multiple HCV antigens into the MHC class I and class II antigen presentation pathways to stimulate potent CD4+ and CD8+ T-cell responses. If possible, this vector would also be capable of repeated administration similar to that for therapeutic drugs.

4.3. Vaccine Approaches that Target Pattern Recognition Receptors

As discussed above, the role of pattern recognition receptors (PRRs), including Toll-like receptors (TLR), in the activation of innate and adaptive immunity has recently been elucidated (Table 1) (21–24, 47, 48). Recognition of the importance of PRRs has led to the testing of agents that target specific TLRs. These agents include isatoribine
Table 1
Pattern Recognition Receptors, Their Ligands, and Cellular Expression Patterns

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Pathogen</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR-1</td>
<td>Triacyl lipopeptides</td>
<td>Bacteria</td>
<td>Cell surface</td>
</tr>
<tr>
<td>TLR-2</td>
<td>Glycolipids, lipopeptides, lipoproteins, lipoteichoic acid, peptidoglycans, zymosan, Hsp70</td>
<td>Bacteria, fungi, tissue damage</td>
<td>Cell surface</td>
</tr>
<tr>
<td>TLR-3</td>
<td>Double-stranded RNA</td>
<td>Viruses</td>
<td>Intracellular</td>
</tr>
<tr>
<td>TLR-4</td>
<td>Lipopolysaccharide, heat shock proteins, fibrinogen</td>
<td>Bacteria, tissue damage</td>
<td>Cell surface</td>
</tr>
<tr>
<td>TLR-5</td>
<td>Flagellin</td>
<td>Bacteria</td>
<td>Cell surface</td>
</tr>
<tr>
<td>TLR-6</td>
<td>Diacyl lipopeptides</td>
<td>Mycoplasma</td>
<td>Cell surface</td>
</tr>
<tr>
<td>TLR-7/8</td>
<td>Single-stranded RNA, imidazoquinolone, loxoribine, bropirimine</td>
<td>Viruses</td>
<td>Intracellular</td>
</tr>
<tr>
<td>TLR-9</td>
<td>CpG-containing DNA</td>
<td>Bacteria</td>
<td>Intracellular</td>
</tr>
<tr>
<td>CD14</td>
<td>Lipopolysaccharide</td>
<td>Bacteria</td>
<td>Cell surface</td>
</tr>
<tr>
<td>Dectin-1/2</td>
<td>Beta-glucan</td>
<td>Fungi</td>
<td>Cell surface</td>
</tr>
<tr>
<td>Mannose receptors</td>
<td>Mannans</td>
<td>Fungi</td>
<td>Cell surface</td>
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</tbody>
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and imiquimod that target TLR-7 and CpG containing double-stranded DNA that targets TLR-9 (49–54). Additional agents are in preclinical development that target TLR-2, TLR-4, and TLR-5. In practice, these agents act as adjuvants to stimulate innate immunity in infected individuals, and will hopefully induce a productive adaptive immune response.

Another approach that is currently being employed in human phase II clinical trials and that also targets PRRs involves the use of recombinant, non-pathogenic *Saccharomyces cerevisiae* (Baker’s yeast), termed Tarmogens™, as a vaccine and immunotherapy vector. Yeast cell wall components appear to interact with more pattern recognition receptors than perhaps any other microbe and are especially effective at stimulating antigen presentation. These receptors include TLR-2, TLR-4, TLR-6, CD14, Dectin-1, Dectin-2, DEC-205, and the mannose receptor.
family (21, 47). Uptake of zymosan, a crude S. cerevisiae yeast cell wall preparation, results in upregulation of a multitude of pro-inflammatory genes (47, 50–57). Data indicate that uptake of whole yeast by mouse and human dendritic cells and macrophages results in upregulation of a variety of cell surface molecules including adhesion molecules (ICAM-1, CD54), co-stimulatory molecules (B7-1, B7-2, CD80, CD86), and MHC class I and class II molecules, as well as promoting the secretion of pro-inflammatory cytokines including IL-12 (58). Yeast-associated proteins are efficiently presented via MHC class I and class II and Targogens have been shown to induce antigen-specific, protective, and therapeutic immune responses in preclinical studies (58–63).

In addition to being able to interact directly with dendritic cells, yeast have a variety of other characteristics that make them an ideal platform for immunotherapy. First, multiple antigens may be engineered for expression within a single yeast strain, and these formulations share many advantages with DNA vaccines, including ease of construction and the ability to target multiple antigens. Unlike DNA vaccines, yeast-based immunotherapeutic formulations do not require extensive purification to remove potentially toxic contaminants. Finally, and perhaps most surprising and important, yeast can be administered multiple times without apparent neutralization of their ability to boost antigen-specific T-cell responses (61) making them an ideal candidate for immunotherapy which may require repeated administration.

5. GOALS AND EVALUATION OF IMMUNOTHERAPY IN CHRONIC HCV INFECTION

The behavior of the serum HCV RNA levels in chronic HCV has been predicted in various settings using a three compartment model of viral kinetics which includes uninfected liver cells, infected liver cells, and free virus in the serum. Viral levels in the peripheral blood early during the course of interferon (IFN) therapy have served as an early predictor of response to therapy due to the fact that they can be measured easily, and have been correlated to other more meaningful endpoints in the setting of long-term IFN treatment such as sustained virologic response (SVR, defined as negative peripheral viral levels for at least 6 months after the completion of IFN-based therapy). Viral clearance in the setting of interferon therapy is biphasic; a rapid early phase of peripheral viral load reduction which occurs in the first week(s) (phase 1), followed by the rate limiting, gradual second phase of peripheral viral load reduction which occurs over many months (phase 2, see Fig. 3) (64, 65). While phase 1 kinetics reflect the efficiency of inhibition of viral replication (driven by rapid peripheral
viral clearance), phase 2 kinetics represent direct clearance of infected liver cells. Clearance of infected hepatocytes is the rate-limiting step in achieving complete eradication of hepatic infection and SVR. The use of an agent that stimulates an adaptive cellular immune response would be predicted to have a favorable impact on second phase viral clearance and, therefore, improve virologic endpoints and ultimately improve SVR rates.

The proposed action of an immunotherapy, for example, a yeast-based Tarmogen engineered to induce adaptive cellular immune responses to multiple HCV antigens (GI-5005) (61), would favor hepatic clearance (the rate-limiting portion of the viral dynamics profile) and should complement the direct antiviral effects of IFN-based therapy. The potential of this combination of immune and antiviral effects to substantially improve response rates in chronic genotype 1 HCV infection support development of GI-5005 and analogous agents in combination with IFN-based standard of care (pegylated IFN plus ribavirin). Furthermore, type 1 interferons have been associated with other immune effects that are potentially synergistic with cell-mediated immunity, including upregulation of major histocompatibility genes in antigen-presenting cells and target cells, as well as upregulation of dendritic cells, natural killer cells, and CD8+ cytotoxic T lymphocytes (66).
6. SUMMARY

There is an unmet medical need for treatment of chronic HCV infection that is either more effective, better tolerated, or both. Combination of an immunotherapeutic vaccine which elicits adaptive cellular immunity with the standard of care (PegIFN/ribavirin) should be studied to evaluate the complementarity of the two approaches with a goal to improve remission rates in the form of sustained virologic responses for patients with chronic genotype 1 HCV infection. Long-term development goals should also include interferon and/or ribavirin sparing approaches to mitigate the safety and tolerability issues inherent in interferon-based therapies.

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