REVIEW

Management of Hepatitis B: Summary of a Clinical Research Workshop

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Chronic hepatitis B is caused by persistent infection with the hepatitis B virus (HBV), a unique DNA virus that replicates through an RNA intermediate produced from a stable covalently closed circular DNA molecule. Viral persistence appears to be due to inadequate innate and adaptive immune responses. Chronic infection has a variable course after several decades resulting in cirrhosis in up to one-third of patients and liver cancer in a proportion of those with cirrhosis. Sensitive assays for HBV DNA levels in serum have been developed that provide important insights into pathogenesis and natural history. Therapy of hepatitis B is evolving. Peginterferon induces long-term remissions in disease in one-third of patients with typical hepatitis B e antigen (HBeAg) positive chronic hepatitis B, but a lesser proportion of those without HBeAg. Several oral nucleoside analogues with activity against HBV have been shown to be effective in suppressing viral levels and improving biochemical and histological features of disease in a high proportion of patients with and without HBeAg, at least in the short term. What is uncertain is which agent or combination of agents is most effective, how long therapy should last, and which criteria should be used to start, continue, switch or stop therapy. Long-term therapy with nucleoside analogues may be the most appropriate approach to treatment, but the expense and lack of data on long-term safety and efficacy make recommendations difficult. Clearly, many basic and clinical research challenges remain in defining optimal means of management of chronic hepatitis B. (HEPATOLOGY 2007;45:1056-1075.)

In the last decade, important advances have been made in the understanding of the hepatitis B virus (HBV), the disease that it causes, and its treatment. Insights from the viral life cycle and pathogenesis of hepatitis B have provided the basis for developing therapies, and knowledge of the natural history has provided the basis for indications for treatment. Six therapies are now licensed for chronic hepatitis B and several more are likely to be available in the near future. Despite these advances, the optimal approach to management of hepatitis B remains unclear. What are the important elements in evaluation of patients? Which patients should be treated? With which agent or combination of agents? For how long? Using what factors to decide whether to continue, discontinue, or switch antiviral therapy? These questions provided the basis for a clinical research workshop sponsored by the Liver Disease Research Branch of the National Institutes of Health (NIH) with support and collaboration from the Food and Drug Administration (FDA), the American Association for the Study of Liver Diseases (AASLD), the Hepatitis B Foundation, the Hepatitis Foundation International, and the American Liver Foundation. The meeting included 436 participants and 42 speakers and moderators who met for 3 days to survey the current understanding of the pathogenesis, natural history, complications, virological and histological manifestations, and means of treatment of hepatitis B. This manuscript summarizes the presentations and final recommendations from that meeting.

Abbreviations: HBV, hepatitis B virus; NIH, National Institutes of Health; FDA, Food and Drug Administration; AASLD, American Association for the Study of Liver Disease; HBsAg, hepatitis B surface antigen; anti-HBs, antibody to HBsAg; HBcAg, hepatitis B core antigen; anti-HBc, antibody to HBcAg; HBeAg, hepatitis B e antigen; anti-HBe, antibody to HBeAg; ccc DNA, covalently closed circular DNA; DHBV, duck hepatitis B virus; TNFα, tumor necrosis factor alpha; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; HR, hazards ratio; HAI, histology activity index; PCR, polymerase chain reaction;

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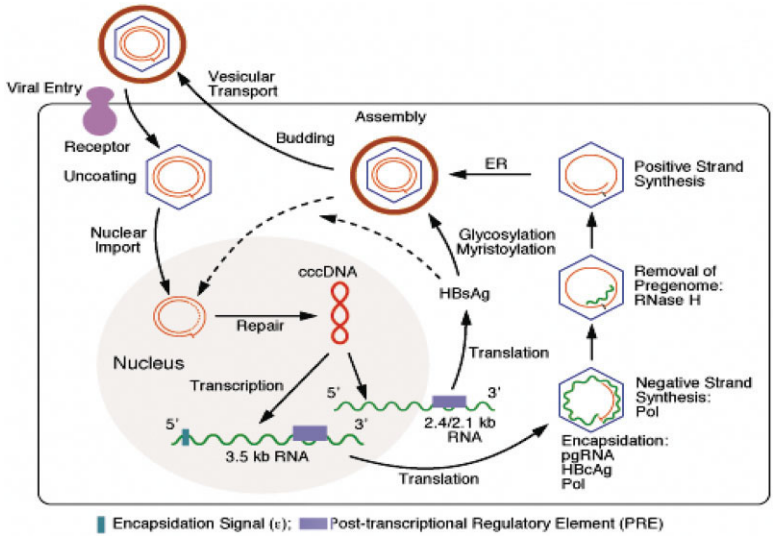

Fig. 1. HBV Life Cycle: HBV enters the hepatocyte mediated by receptor binding, followed by internalization, uncoating of virion DNA and delivery to the nucleus. The virion DNA is repaired and converted to circular covalently closed (ccc) HBV DNA from which HBV RNA is transcribed in several forms for translation of HBV antigens (HBcAg, HBsAg and polymerase [pol]) and for viral replication through encapsidation of the RNA pregenome (pg RNA) mediated by the encapsidation signal (ϵ). Within core particles, negative followed by positive strand viral DNA is synthesized directed by the HBV polymerase while pregenome RNA is degraded by the polymerase RNase H activity. The core particle with doubled stranded HBV DNA is transferred to the endoplasmic reticulum (ER) where it is coated with glycosylated and myristoylated HBsAg producing intact virions that exit the cell by budding and vesicular transport. (Reprinted from Gastroenterology, Volume 120, Doo E and Liang TJ, Molecular anatomy and pathophysiologic implications of drug resistance in hepatitis B virus infection, pages 1000-1008, Copyright 2001, with permission from the American Gastroenterological Association.)

Hepatitis B Virus

HBV is a member of the family *hepadnaviridae,* viruses with a double-stranded circular DNA genome that replicates through an RNA intermediate.[1] HBV infects only humans and higher apes, but other similar hepadnaviruses are endemic in rodent and bird species. The virus is detectable in serum in high levels and on electron microscopy is a 50 nm double-shelled particle with an outer envelop (HBsAg) and an inner nucleocapsid (HBcAg) protein.[2] Within the nucleocapsid is a partially double-stranded, circular molecule of HBV DNA. The HBV genome is ≈3.2 kb in length and replicates in a unique manner through an RNA intermediate. The genome encodes four open reading frames for surface, core, polymerase, and X genes. The core gene can also produce a soluble small molecular weight protein called hepatitis B e antigen (HBeAg) by an alternate start codon and post-translational modification. The virus gains entry into hepatocytes, the primary site of infection, through yet undefined pathways and receptors (Fig. 1).[3] After entry, HBV DNA is transported to the nucleus and converted to covalently closed circular DNA (cccDNA), which serves as the stable template for transcription of both messenger RNA (for translation of viral proteins) and pre-genomic RNA (for reverse transcription into genomic DNA). HBV cccDNA is present in the range of 5-50 copies per hepatocyte and is the stable form of viral DNA that is the most resistant to antiviral therapy and host immunological response.[4]

Viral replication occurs in the cytoplasm where the pre-genomic RNA associates with HBcAg and the HBV polymerase to form nucleocapsid core particles.[3] HBV DNA is produced within the core particle by synthesis of the negative DNA strand from HBV RNA and then the positive DNA strand from the newly synthesized negative strand. The nucleocapsid then assembles with HBsAg molecules in the endoplasmic reticulum to form the virion and is secreted from the cell, probably by vesicular transport and budding from the plasma membrane.

HBV is non-cytopathic, and the cellular injury of hepatitis B appears immune-mediated.[5] Most persons exposed to HBV have a transient infection which may or may not be accompanied by symptoms and jaundice. In a proportion, however, chronic infection ensues as a result of failure of the host immune response to eliminate virus. Viral clearance is mediated by both cytopathic and non-cytopathic mechanisms, the existence of both pathways being supported by extensive evidence in cell culture and animal models.[5] Cytopathic clearance is based on direct killing of virus-harboring hepatocytes by virus-specific T cells, followed by compensatory proliferation of hepatocytes. This proliferation may also result in gradual loss of cccDNA. Uninfected hepatocytes are protected from re-infection through induction of neutralizing antibodies and innate immune mechanisms. Studies in the duck hepatitis B virus (DHBV) model support a cytopathic mechanism for viral clearance, as shown by the turn-over of large number of hepatocytes during viral clearance.[6] Studies in the HBV chimpanzee model and in transgenic animals, on the other hand, support the importance of non-cytopathic clearance as mediated by antiviral cytokines such as type I interferon and tumor necrosis factor alpha (TNFα).[7-9] Substantial reduction in viral replication during transient infection precedes infiltration of the liver by virus-specific T cells and elevation of serum aminotransferase levels.[7] The early decrease in virus correlates with the appearance of antiviral cytokines such as interferon and TNFα which have direct inhibitory effects on

Table 1. Phases in Natural History of Chronic Hepatitis B

Phase	ALT	Liver Histology	HBV DNA	HBeAg	HBsAg
Immune Tolerance	Normal or minimally elevated	Minimal Activity, Scant Fibrosis	High levels (10^8 to 10^{11} copies/ml)	Present	Present
HBeAg +ve Chronic Hepatitis B	Elevated usually persistently	Active with variable amounts of fibrosis	High levels (10^6 to 10^{10} copies/ml)	Present	Present
HBeAg −ve Chronic Hepatitis B	Elevated, often fluctuating	Active with variable amounts of fibrosis	Moderate levels, often fluctuating (10^3 to 10^8 copies/ml)	Absent	Present
Inactive Carrier State	Normal	Inactive with variable, usually minimal amounts of fibrosis	Low or no detectable levels ($<10^4$ copies/ml)	Absent	Present
Recovery	Normal	Inactive with scant amounts of fibrosis	No detectable levels in serum (low levels may be present in liver)	Absent	Absent

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

HBV replication.[10] Thus, both cytopathic and non-cytopathic mechanisms are important in clearance of HBV during transient infection.

In addition to the humoral response, cell-mediated immunity is crucial for the control of HBV infection.[5] Vigorous virus-specific CD4+ and CD8+ T cell responses which target multiple viral antigens with broad specificity are induced during transient infection, and these responses can persist for decades after viral clearance.[11] The continued presence of T cell responses suggest that HBV persists at low levels despite absence of detectable HBV DNA in serum and presence of antibodies to HBsAg (anti-HBs).[12] The presence of low-levels of virus in the liver after apparent recovery has been demonstrated by transmission studies in chimpanzees and results of organ transplantation in humans.[13,14] Reactivation of hepatitis B in previously recovered people during immunosuppressive therapy has been amply documented.[15,16] Thus, recovery from hepatitis B may not indicate virus eradication so much as firm immunological control.

Acute evolves into chronic infection if T cell responses to HBV are not induced or are not capable of controlling the virus.[17] T cell responses to HBV antigens disappear gradually with the onset of chronic infection, probably because of exhaustion and/or tolerance induction. Most patients with chronic hepatitis B have no or few HBV-specific T cells in the circulation. An increase in the HBV-specific T cell response can occur during acute exacerbations of chronic hepatitis B or during seroconversion from HBeAg to anti-HBe.[18] Nucleoside analogue-induced treatment responses have been shown to result in a transient restoration of the T cell response.[19,20] With long-term therapy, however, these T cell responses appear to be lost.

Clinical Features and Natural History

The natural history of hepatitis B is variable, complex and still not completely well defined.[21] At the onset of HBV infection, only one-third of adults experience symptoms of acute hepatitis, while the majority (≈65%) have subclinical disease.[22] Importantly, about 5% of adults (≈ 2% of women and ≈7% of men) develop chronic hepatitis B. Rates of chronicity are higher in newborns (≈90%) and children (≈30%) and in immune deficient individuals. Some of the variation in outcome of HBV infection may relate to the genetic heterogeneity of the virus. Eight genotypes of HBV have been described, genotype A being most common in the United States and Northern Europe, B and C in Asia, and D in Mediterranean counties and the Middle East.[23,24] Chronic infection with HBV genotype C appears to have a poorer prognosis and greater likelihood of cirrhosis and hepatocellular carcinoma (HCC) than genotype B.[25]

The transition from acute to chronic infection appears to represent a failure of immune clearance of virus-infected cells and is marked by persistence of high levels of HBV DNA and HBeAg in serum.[5] The accompanying acute hepatitis is typically mild and subclinical with only modest serum alanine aminotransferase (ALT) elevations and no jaundice. Importantly, the subsequent course of chronic hepatitis B is highly variable.

The variability in chronic hepatitis B has led to its classification into phases of disease based upon ALT elevations, the presence of HBeAg, HBV DNA levels, and suspected immune status (Table 1).[21,26,27] Typical chronic hepatitis B is marked by the presence of HBeAg and high levels of HBV DNA with variable elevations in ALT and histological activity. A proportion of HBeAg-positive persons, however, have no ALT elevations and scant histological activity and are referred to as "immune tolerant."[26,27] The immune tolerant phase of hepatitis B is most common in children, adolescents, and young adults with perinatally acquired infection and may represent the earliest phase of chronic infection. The prognosis and natural history of the immune tolerant phase of chronic hepatitis B are not well defined, although most studies

suggest that very little liver injury occurs during this phase. Transition to typical chronic hepatitis B with activation of disease can be abrupt and resemble acute hepatitis.[28]

The duration of typical HBeAg-positive chronic hepatitis B can be prolonged and severe and may result in cirrhosis, but for many persons it does not cause clinical symptoms and eventually transitions to an inactive phase with loss of HBeAg, seroconversion to antibody (anti-HBe) and fall of HBV DNA to low or undetectable levels.[29-31] This transition is referred to as HBeAg seroconversion and often results in the disappearance of disease activity despite persistence of HBsAg and low levels of HBV DNA in serum, a phase of disease referred to as the "inactive carrier state."[32] This transition can be preceded by a transient flare of disease with marked elevations in serum ALT levels, decreasing concentrations of HBV DNA in serum and appearance of HBcAg-specific CD4+ and CD8+ T cells in the circulation.[18]

The inactive carrier state generally has a benign course, but can be reactivated either spontaneously or by immune suppression.[32-34] Indeed, up to one-third of patients undergo another transition, with increases in HBV DNA and ALT elevations and disease activity without reappearance of HBeAg.[35] This phase is referred to as HBeAg-negative chronic hepatitis B and its consequences can be as severe (if not more so) than HBeAg-positive disease.[36] The molecular basis for this form of disease appears to be the development of a variant HBV which is incapable or only poorly able to produce HBeAg.[36-38] The HBeAg-negative form of disease is more frequent with specific HBV genotypes B, C and D than with genotype A[38] and is characterized by marked fluctuations in serum HBV DNA and ALT levels.[27,36,37] The underlying pathogenesis for the transition from one phase of disease to another is not known, and some patients clearly do not progress or may transition backwards (reactivation) either spontaneously or due to change or manipulation of the immune system.[15,16,39]

Large, long-term natural history studies of HBsAg-positive persons have been conducted in Asia[40-42] and Europe.[33,34,43] While these studies provided excellent data on outcome, most lacked information on results of regular testing for molecular HBV markers, immunological assays and liver histology. Nevertheless, these studies showed that development of cirrhosis and HCC was frequent and correlated with several pre-existing host and viral factors. In the largest and most thorough evaluation, HCC developed in 4.5% of 3,653 HBsAg-positive persons identified in a population-based survey who were followed for an average of 11.4 years (0.4% per year).[42] Risk factors for HCC included male sex (hazard ratio [HR] = 3.0), more advanced age (HR = 3.6 to 8.3), cigarette smoking (HR = 1.7) and alcohol consumption (HR = 2.6). Viral and disease factors predictive of HCC development included elevations in serum ALT levels (HR = 4.1), presence of HBeAg (HR = 4.2) and higher levels of HBV DNA. Importantly, levels of HBV DNA at the time of initial evaluation were most closely linked with eventual development of HCC, levels above 10^5 copies per ml being strongly linked (HR = 8.9 to 10.7) and above 10^4 significantly linked (HR = 2.7). These relationships between HBV DNA levels and HCC held true, even for patients with normal ALT levels at the time of initial evaluation.

Therapy of Hepatitis B: Background and Definitions

The course of chronic hepatitis B is typically silent and associated with few signs or symptoms of disease until cirrhosis and/or HCC arise. As a consequence, the major goals of therapy are not immediate amelioration of symptoms, but rather long-term prevention of progression, development of cirrhosis and HCC.[21] Because these endpoints arise only after decades of infection, studies of therapy (other than in patients with cirrhosis) have used short-term, surrogate outcomes to assess benefit, many of which have yet to be shown to be durable or reliably predict lack of further progression.

Standardization of terminology and definitions for endpoints of therapy of hepatitis B are important in assessing antiviral agents and in guiding management.[21,44] Therapeutic endpoints can be categorized as *biochemical*, *virological* and *histological*, and as *initial, maintained* (on-treatment) or *sustained* (off-treatment).

Biochemical responses are defined by changes in serum ALT levels; a *biochemical response* by a decrease into the normal range. There are shortcomings in using biochemical response as an end-point for successful therapy. This endpoint requires that patients have elevated ALT levels initially. Furthermore, minor ALT elevations may persist after successful therapy due to presence of another liver disease or injury. Finally, there is no widely accepted definition for the normal range of ALT values, average values being lower in women than men, in children than adults, and in normal-weight than overweight or obese persons.[45]

Histological responses are usually defined based upon scoring systems for the grade and stage of chronic hepatitis.[46-48] In most trials, histological improvement has been defined as a two-point decrease in the histological activity index (HAI: which ranges from 0 to 18) with no worsening of fibrosis between pre-treatment and end-of-treatment liver biopsies. The clinical significance of this degree

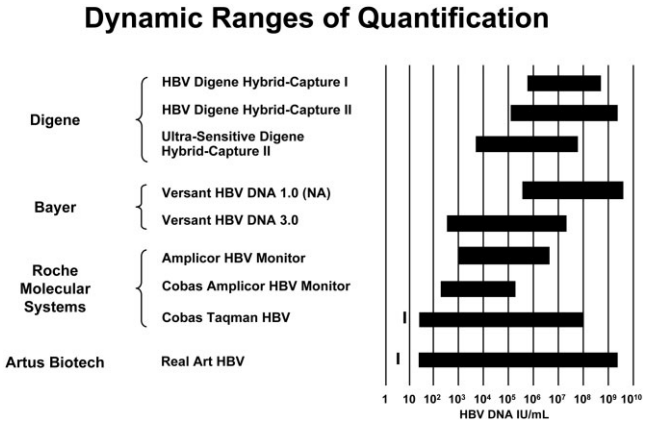

Fig. 2. Relative sensitivity and range of quantification of commercial tests for HBV DNA. Assays from the Digene Corporation (Gaithersburg, Maryland) and Bayer Heathcare LLC (Tarrytown, NJ) are hybridization based; those from Roche Molecular Systems (Pleasanton, CA) & Artus (Artus GMbH, Hamburg, Germany) are PCR-based; Cobas Taqman™and Real Art HBV™ being real-time PCR assays. (Figure courtesy of Dr. Jean-Michel Pawlotsky).

of histological improvement has never been shown; furthermore, the variation in scores due to sampling error can be great, particularly with small liver biopsy samples.[49] Thus, while a two-point improvement in HAI scores may be useful in assessing histological responses in clinical trials where large cohorts of patients are compared, it is not reliable for assessing benefits to individual patients. A more appropriate histological endpoint would be resolution of the chronic hepatitis with minimal or mild HAI scores (<3 points). Because liver biopsy is invasive, potentially harmful and expensive, histological endpoints cannot be applied repeatedly and are of limited use in clinical practice. Nevertheless, histological response has been the gold standard against which other surrogate endpoints are measured.

Virological responses based upon testing for levels of HBV DNA in serum are probably the most appropriate criteria of assessing beneficial outcome of antiviral therapy. Commercial assays for HBV DNA have been evolving. Currently most assays are based on polymerase chain reaction (PCR), and there is an increasing shift toward real-time PCR assays (Fig. 2).[50] An important issue is the ability of these assays to quantify HBV DNA levels over a wide range of concentrations with acceptable accuracy and reproducibility. Most hybridization assays provide excellent and reliable quantification but are restricted by lack of sensitivity below 10^4 to 10^5 IU per ml. Polymerase chain reaction based assays[51] are more sensitive and detect HBV DNA to levels of 10^2 to 10^3 IU per ml, but are not as reliable in quantification, particularly when viral levels are high (above 10^6 IU per ml). The recently described real-time PCR assays provide increased sensitivity and a greater dynamic range of quantification (from 10^1 to 10^9 IU per ml).[52] Nevertheless, viral responses usually have been defined by results of PCR-based assays, with a *virological response* defined as the lack of detectable HBV DNA in serum using an assay that is sensitive to 20 to 100 IU/ml, which is roughly equivalent to 100 to 500 copies/ml. The major difficulty with this endpoint is its durability, in that it can be rapidly lost once therapy is stopped.

Loss of HBeAg or seroconversion to anti-HBe has often been used as a virological endpoint in assessing therapy of hepatitis B and has the advantage of being more sustained than suppressed HBV DNA levels. This endpoint, however, is not always durable and relapse rates of 10% to 30% have been reported after interferon[53-56] and as high as 60% after nucleoside analogue therapy, particularly if treatment is stopped soon after HBeAg becomes undetectable.[57] Furthermore, some patients do not improve clinically despite loss of HBeAg, evolving into HBeAg-negative hepatitis B.[35] Finally, loss of HBeAg cannot be used as an endpoint in HBeAg-negative chronic hepatitis B.

Loss of HBsAg and seroconversion to anti-HBs is clearly the most desired endpoint and can be considered a *complete response* indicating resolution of hepatitis B and recovery. Loss of HBsAg is durable in all but rare instances. The difficulty is that loss of HBsAg is not frequent after antiviral therapy of hepatitis B, occurring in 3% to 8% of patients receiving interferon or peginterferon[58-62] and less than 2% of patients receiving a one-year course of nucleoside analogue therapy.[63-71] Nevertheless, in studies of long-term therapy, loss of HBsAg becomes increasingly common and should be the ultimate endpoint sought in long-term treatment trials.

Responses to therapy can also be categorized as initial, maintained, and sustained. An *initial response* can be measured at 6 or 12 months and these responses have been used in most clinical trials of antiviral therapy. More appropriate for trials of long-term therapy, however, would be a *maintained response*, indicating that the response was still present when the patient was last seen on therapy. In a similar manner, a *sustained response* is defined as being present 6 months after stopping therapy. Importantly, sustained responses in hepatitis B still need to be assessed for durability during long-term follow-up and shown to be present when the patient was last seen more than 6 months after stopping therapy.

Definitions are also needed to describe and assess viral resistance and breakthrough. Resistance is typically categorized as genotypic, viral, and clinical. *Genotypic resistance* is based upon detection of HBV mutations that are associated with *in vitro* and *in vivo* resistance to antiviral agents.[3,72-74] Thus, during treatment with nucleoside an-

alogues, mutations in the polymerase gene of HBV often can be detected before there is a rise in HBV DNA or ALT levels.[75,76] The difficulty with this definition is that it requires molecular testing which is expensive and may not be warranted clinically if there are no other signs of antiviral resistance. *Viral resistance or virological breakthrough* indicates that HBV DNA levels have increased, the usual criteria being greater than a one $\log_{10}$ increase from a previous nadir in a patient who is compliant and still on treatment. A difficulty with this definition is that it requires frequent determinations of HBV DNA levels to detect the rise. *Clinical resistance or biochemical breakthrough* is defined by a rise in serum ALT levels. For patients whose serum ALT levels fall into the normal range during therapy, clinical resistance can be defined as a rise to above twice the upper limit of the normal range in conjunction with a rise in HBV DNA levels and/or genotypic resistance. These criteria become difficult to apply in the situation in which ALT levels never fall into the normal range, or were normal before therapy, or fluctuate spontaneously.

Antiviral Resistance in Hepatitis B

Treatment of chronic hepatitis B using nucleoside analogues can result in development of antiviral resistance, marked by appearance of circulating HBV with reduced sensitivity to the antiviral agent.[3,72-74] The pattern of development of HBV resistant mutants varies by chemical class of nucleoside analogues which can be categorized as:

1. L-nucleosides, such as lamivudine, emtricitabine, telbuvidine and clevudine.
2. Acyclic phosphonates such as adefovir and tenofovir.
3. Cyclopente(a)nes such as entecavir.

Nomenclature in discussing HBV resistance uses an abbreviation for the gene region in lower case (rt for reverse transcriptase, c for HBcAg, s for HBsAg) followed by the wild-type amino acid symbol, its position in the gene region, and finally the mutant or variant amino acid symbol.[77] The typical lamivudine resistant mutations involve the conserved "YMDD" motif of the polymerase gene, changing it to YVDD or YIDD, the standardized nomenclature being rtM204V and rtM204I. The rtM204V/I mutation is usually accompanied by a compensatory mutation upstream of the YMDD motif at rtL180M and/or rtV173L. The rtM204V/I mutations are considered primary resistant mutations that lower the susceptibility of HBV to lamivudine, while the rtL180M and rtV173L mutations are considered secondary or compensatory, allowing for the resistant mutant to replicate at a higher rate. Generally, development of the lamivudine resistant HBV effectively makes other L-nucleosides ineffective. However, rates of development and proportions of various mutants may vary with different L-nucleosides. Typical antiviral resistance mutations are shown in Table 2.[74] These mutations that have been associated with a decrease in activity of the antiviral agent are found in domains A, B, C and D of the polymerase (rt) gene at the amino acid positions listed. A more thorough discussion of antiviral resistance has been provided in recent reviews.[74,77]

Table 2. Location and Terminology of Antiviral Resistant Mutations

	Domains Within Polymerase Region of HBV P gene and Their Amino Acid Locations			
Agent	**A**	**B**	**C**	**D**
Lamivudine and Emtricitabine	rtL80V/I*	rtA181T, rtV173L*, rtL180M*	rtM204V/I/S	
Telbivudine			rtM204I	
Entecavir	rtI169T	rtT184S/A/I/L/F/G, rtL180M**	rtS202G/I, rtM204V/I**	rtM250V
Adefovir		rtA181V/T		rtN236T

*Secondary, compensatory mutation. ** Mutations selected during the first steps toward entecavir resistance. Abbreviations: rt, reverse transcriptase.

Adefovir and tenofovir have potent activity against lamivudine-resistant strains *in vitro* and *in vivo*[78,79] whereas entecavir has reduced efficacy against rtM204V/I mutants.[80] The most common resistant mutations associated with adefovir therapy have been rtA181V/T and rtN236T, but several other single or multiple mutations have been described.[81-83] Resistance to entecavir has been encountered mainly in patients with pre-existing lamivudine-resistance and include multiple changes, typically rtI169T, rtT184S/A/I/LG/C/M, rtS202G/C/I, or rtM250I/V and one or more lamivudine-resistant mutation sites, typically rtL180M and rtM204V.[80,84] Detection of resistant mutations usually requires sequencing of the polymerase gene, but various assays including reverse hybridization and restriction fragment length polymorphism have been developed that detect the more common mutations.[85]

Monotherapy for Hepatitis B

Six antiviral agents (standard interferon, peginterferon, lamivudine, telbivudine, adefovir dipivoxil, and entecavir) have been approved for use in chronic hepatitis B in the United States and at least three others (emtricitabine, clevudine, tenofovir disoproxil fumarate) are being evaluated and may be approved in the near future. Selected results of recently published randomized controlled trials of one-year courses of monotherapy with these

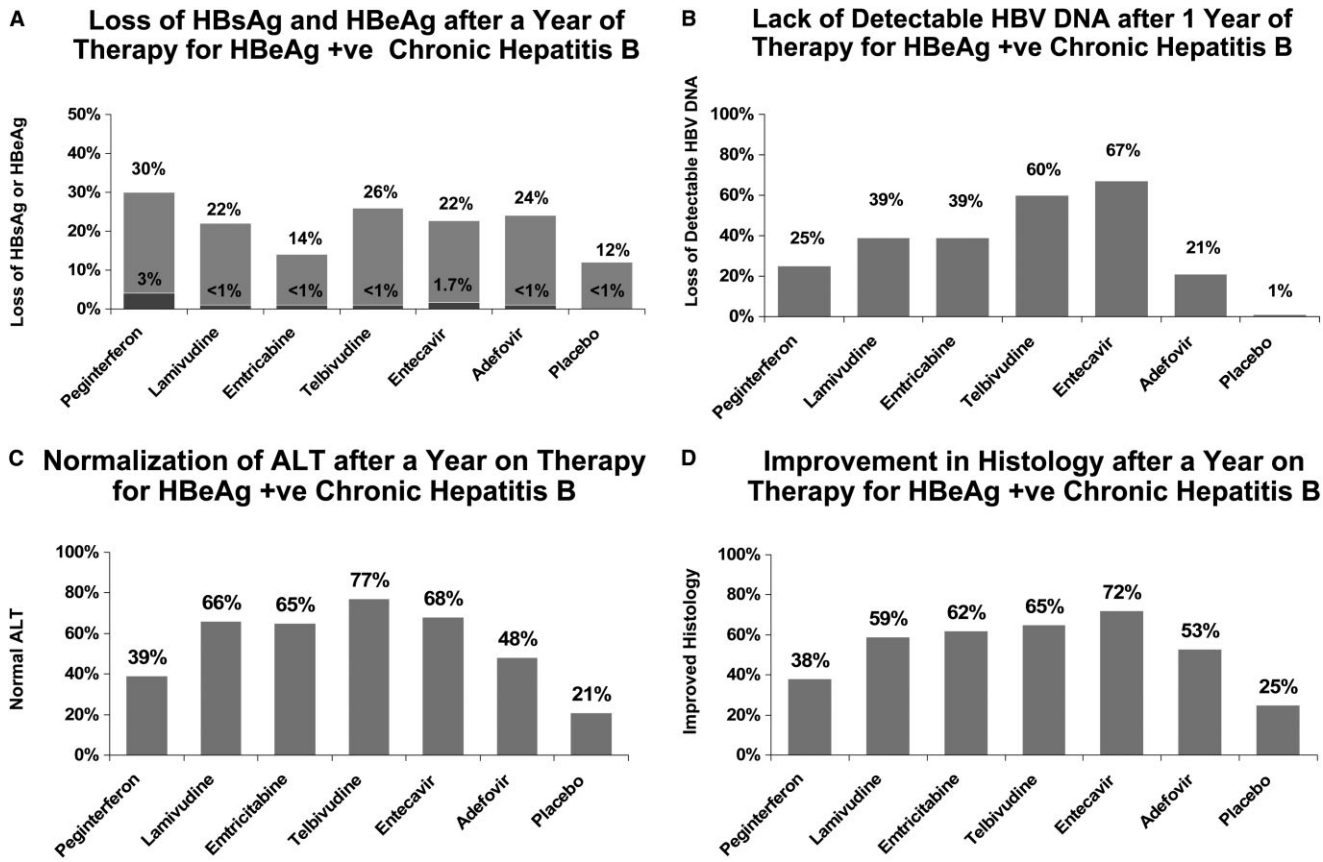

Fig. 3. Results of one-year courses of therapy with single agents in treating HBeAg-positive chronic hepatitis B using different endpoints at 48 weeks: (A) loss of HBeAg and HBsAg; (B) lack of detectable HBV DNA by PCR based assays, (C) fall of alanine aminotransferase levels into the normal range (among those with elevations before therapy), and (D) two point improvement in hepatic histology using the hepatic activity index. Data on lamivudine from comparative arm in recent trials of peginterferon[60] telbivudine[65] and entecavir[70] Data on placebo from control arms of studies of adefovir[67] and emtricitabine.[66]

agents are shown in Fig. 3 (HBeAg-positive) and Fig. 4 (HBeAg-negative). The relative rates of antiviral resistance with each agent are shown in Fig. 5.

All agents showed evidence of benefit using biochemical, virological, and histological endpoints.[58-71] Loss of HBeAg occurred in 14% to 30% of patients treated with antiviral agents compared to ~12% of placebo recipients. Loss of HBsAg occurred in 0.3% to 4% of treated patients but rarely in placebo recipients. Most strikingly, HBV DNA became undetectable by PCR based assays after one year of therapy in 21% to 67% of HBeAg-positive and 51% to 90% of HBeAg-negative patients but in almost no control patient. Finally, biochemical and histological improvements occurred more frequently with antiviral therapy than with placebo treatment, but a proportion of control subjects (averaging 25%) had improvements in these features.

Peginterferon alfa-2a (Pegasys™, Roche Pharmaceuticals, Nutley, NJ) has replaced use of standard interferon in both hepatitis B and C, largely because it is more convenient to administer and is more effective.[59] Tolerance and side effects of peginterferon appear to be similar to standard interferon. In two large, randomized controlled trials, a one-year course of peginterferon yielded higher 6-month post-treatment sustained rates of loss of HBsAg, HBeAg and HBV DNA and higher rates of improvements in ALT and histology than did a one-year course of lamivudine.[60,61] However, in clinical practice lamivudine therapy is not routinely discontinued after one year unless there is loss of HBeAg or HBsAg. Indeed, in these trials withdrawal of lamivudine was associated with a transient exacerbation of disease in a proportion of patients which was fatal in some instances. Thus, these studies evaluated an approach to therapy that cannot be recommended for nucleoside analogues.

Basically, there are two approaches to therapy of hepatitis B: one using a defined, self-limited course of treatment and the other using long-term, continuous therapy. Interferon and peginterferon are typically used in a limited course of 4 to 12 months, stopped and the outcome assessed 6 months afterwards. When evaluated in this manner, peginterferon appears to be su-

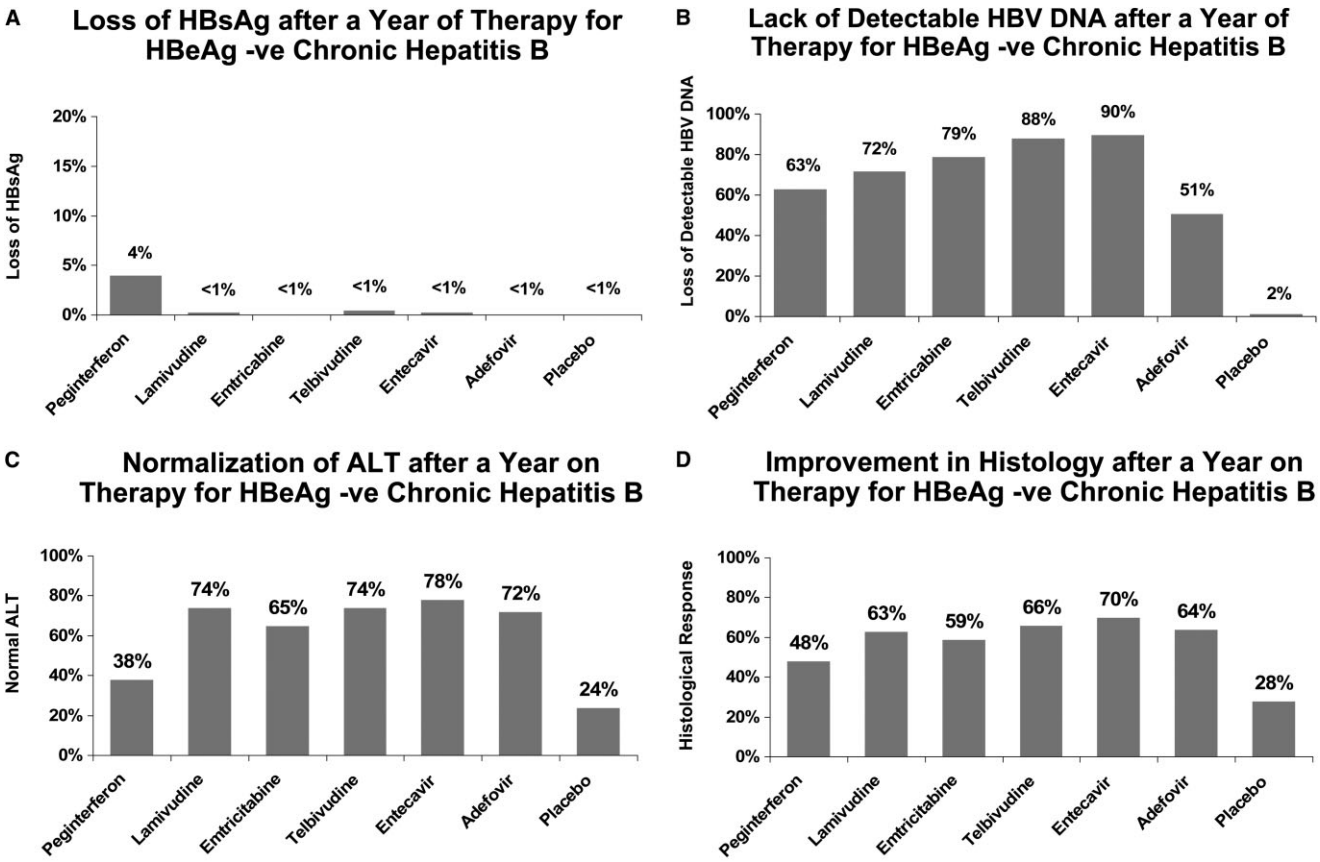

Fig. 4. Results of monotherapy in treating HBeAg-negative chronic hepatitis B using different endpoints: (A) loss of HBsAg; (B) lack of detectable HBV DNA by PCR based assays, (C) fall of alanine aminotransferase levels into the normal range (among those with elevations before therapy), and (D) two point improvement in hepatic histology using the hepatic activity index. Data on lamivudine from comparative arm in recent trials of peginterferon,[61] telbivudine,[65] and entecavir.[71] Data on placebo from control arms of studies of adefovir[68] and emtricitabine.[66]

perior to current nucleoside analogues,[59-62] largely because rates of loss of HBsAg and HBeAg after one year are more common. In contrast, continuous, long-term therapy with nucleoside analogues yields rates of response somewhat lower than interferon at one year, but promises response rates that are ultimately higher, although they may not be sustained when therapy is stopped.

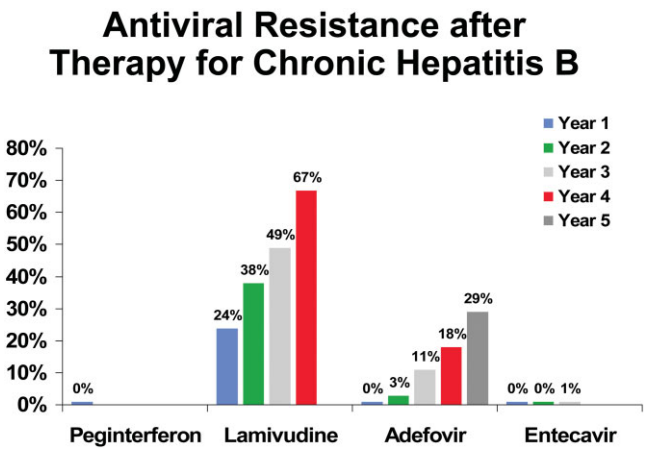

Fig. 5. Rates of genotypic antiviral resistance for four therapies of chronic hepatitis B.[58-72]

Post-hoc analyses of trials of peginterferon therapies have shown several pre-treatment factors that were predictive of loss of HBeAg, the major ones being higher ALT levels, lower HBV DNA levels, and greater disease activity on liver biopsy. These factors also predicted responses to nucleoside analogue therapy as well as spontaneous improvement in hepatitis B. The influence of genotype on outcome of antiviral therapy is controversial.[62,86,87] Among patients with typical HBeAg-positive chronic hepatitis B, response rates to peginterferon are higher among those infected with genotype A and B than genotypes C and D, but the differences have not been statistically significant in all studies. Relapse after loss of HBeAg may also be less common in patients with genotypes A and B than C and D. Importantly, success of antiviral therapy and degree of viral suppression by nucleoside analogues appears independent of HBV genotype.[88]

Lamivudine (3-thiacytidine: Epivir-HBV™, GlaxoSmithKline, Research Triangle Park, NC) was the first L-nucleoside analogue licensed for use in chronic hepatitis B and has been considered the standard of therapy for this disease. While initially evaluated as a 52-week course of treatment,[63,64] its major use has been as a continuous long-term therapy. Long-term therapy can be very effective and cases of resolution of disease and even loss of HBsAg after long-term lamivudine have been reported.[89,90] Unfortunately, lamivudine has a high rate of antiviral resistance, averaging 15% to 20% per year, so that 70% to 80% of patients have resistance after 4 to 5 years of treatment (Fig. 5).[74] Development of resistance is usually followed by loss of the clinical response, rise of ALT levels and worsening of hepatic histology. Instances of continued improvement despite lamivudine resistance have been reported,[90] but the long-term consequences of viral resistance are generally poor.[91] Indeed, severe exacerbation of disease and decompensation have been reported at varying intervals after development of resistance. For these reasons, long-term results of lamivudine therapy are poor, particularly in HBeAg-positive chronic hepatitis B.

Emtricitabine (5 Fluorocytidine: Emtriva™, Gilead Sciences, Foster City, CA) is an L-nucleoside that has a level of potency and pattern of resistance similar to lamivudine. In a randomized controlled trial, emtricitabine given in a dose of 200 mg once daily for 52 weeks was associated with suppression of HBV DNA to undetectable levels in 39% of HBeAg-positive and 79% of HBeAg-negative patients (compared to 2% in placebo recipients).[66] Rates of resistance were 17% among HBeAg-positive but only 3% of HBeAg-negative patients, the resistance patterns being the same as those with lamivudine. Thus, emtricitabine and lamivudine are similar both in rates of response and patterns of resistance.

Telbivudine (L-deoxythymidine: Tyzeka™, Idenix Pharmaceuticals, Cambridge, MA) is an L-nucleoside with potent activity against HBV in cell culture and in animal models. In phase II trials, telbivudine resulted in 5.8 to 6.5 $\log_{10}$ reduction in HBV DNA levels.[92,93] In a large, ongoing multicenter trial, telbivudine therapy was associated with similar rates of loss of HBeAg at one year (26%) compared to lamivudine (23%), but with higher rates of loss of detectable HBV DNA (60% versus 40%) ($P < 0.05$).[65] At 2 years, rates of loss of HBeAg were still similar but virological breakthrough with confirmed genotypic resistance was found in 22% versus 35% of HBeAg-positive and 9% versus 22% of HBeAg-negative patients receiving telbivudine versus lamivudine.[65] Rates of resistance with telbivudine were less than with lamivudine and only rtM204I mutants without rtM204V/rtL180M were detected. Thus, telbivudine may have enhanced potency and lower rates of resistance than lamivudine, but resistance rates are substantially higher than with other approved therapies.

Adefovir dipivoxil (Hepsera™, Gilead Sciences, Foster City, CA) is a pro-drug of adefovir and was the second nucleoside analogue to be licensed for use in this disease. Rates of clearance of HBeAg and lack of detection of HBV DNA were lower during adefovir than lamivudine therapy (Fig. 3), but biochemical and histological responses were similar. Importantly, adefovir was associated with a low rate of antiviral resistance, generally less than 1% at one year although rising to 29% after 5 years (Fig. 5).[67-69,81,82] Adefovir is also associated with primary non-response as shown by only a modest decrease of HBV DNA levels and little improvements in ALT in as many as one-third of patients with HBeAg-positive hepatitis B, although a lower rate in HBeAg-negative hepatitis B. Primary non-response correlates closely with high initial HBV DNA levels and may relate to an only moderate antiviral activity of adefovir. The presence of HBV variants with primary resistance to adefovir may also be important.[94] Higher doses of adefovir have greater potency against HBV, but are associated with an unacceptably high rate of renal toxicity. Long-term trials of adefovir are ongoing and have demonstrated a high rate of maintained response particularly in HBeAg-negative chronic hepatitis B.[69] Adefovir also has activity against lamivudine-resistant HBV and significant improvements in biochemical, virological, and histological features of disease occur in at least half of patients with lamivudine-resistance treated with adefovir for one year.[95-97] Unfortunately, adefovir resistance may be more common in patients with pre-existing lamivudine resistance, and instances of combined resistance to both agents have now been published.[82,98,99]

Tenofovir disoproxil fumarate (Viread™, Gilead Sciences, Foster City, CA) is an acyclic adenine nucleotide with potent activity against both HBV and HIV *in vitro* and *in vivo*.[100] Tenofovir is licensed for use in HIV infection and has been evaluated extensively in patients with HIV/HBV co-infection.[101-103] Tenofovir appears to be more potent than adefovir and is effective against lamivudine-resistant strains of HBV DNA. Small comparative studies have been conducted in cohorts of patients with HBeAg-positive chronic hepatitis B and lamivudine-resistance without HIV co-infection. In a study with greater than 48 weeks of follow up, all 35 patients treated with 300 mg of tenofovir daily were HBV DNA negative compared to only 44% (7 of 15 patients) treated with 10 mg of adefovir daily.[104] Tenofovir could also rescue patients with lamivudine resistance who had an inadequate re-

sponse to adefovir.[105] Side effects and renal toxicity were comparable. These results suggest that tenofovir may be the agent of choice for lamivudine-resistant HBV and may ultimately replace adefovir in treatment of typical hepatitis B. Phase III trials of tenofovir for chronic hepatitis B are now underway.

Entecavir (Baraclude™ Bristol-Myers Squibb Company, Princeton, NJ) is an acyclic guanosine derivative with marked activity against HBV. In preliminary studies and in randomized controlled trials, entecavir showed excellent potency, high rates of suppression of HBV DNA levels and improvements in biochemical and histological features of disease.[70,71] Rates of clearance of HBeAg and HBsAg at one year were similar to those of other nucleoside analogues (Figs. 3 and 4). Importantly, antiviral resistance to entecavir occurred in less than 1% of nucleoside-naïve patients after 1 and 2-year courses of therapy.[84] In contrast, entecavir resistance and only modest response rates were reported in cohorts of patients with pre-existing lamivudine-resistance.[80] The entecavir-resistant strains of HBV appear to be sensitive to adefovir. Preliminary reports on patients receiving entecavir for 2 years indicate maintained suppression of HBV DNA to undetectable levels in more than 85% of patients with both HBeAg-positive as well as HBeAg-negative disease. These results are extremely heartening, but the ultimate long-term safety and efficacy of entecavir therapy remain to be shown.

Combination Therapy of Hepatitis B

The potential advantages of combination versus mono-therapy of hepatitis B include greater antiviral activity and lower rates of resistance to the individual agents used. Unfortunately, there have been few properly controlled trials comparing combination therapy with each of the agents alone. In the few studies that have been done, combination therapy was no more effective than monotherapy.

In a pilot study in HBeAg-positive chronic hepatitis B, 115 patients were treated with either lamivudine alone or lamivudine and adefovir.[106] At 52 weeks, the degree of HBV DNA suppression (4.8 versus 5.4 $\log_{10}$) and HBeAg loss (20% versus 19%) were similar, while normalization of ALT (70% versus 48%) was more frequent with monotherapy than combination therapy. A group receiving adefovir alone was not included. In another multicenter study, 38 patients received either adefovir alone or adefovir and emtricitabine.[107] The combination yielded a more rapid and more marked end-of-treatment decrease in HBV DNA, but similar rates of loss of HBeAg. Importantly, a group receiving emtricitabine alone was not included. A third study has evaluated telbivudine alone (in several doses) compared to lamivudine alone and the combination.[93] Telbivudine alone was found to be more potent in suppressing HBV DNA levels than lamivudine alone (median reductions of 6.2 versus 4.7 $\log_{10}$) but the combination was no more potent than telbivudine alone. These three studies suggest that the combination of two nucleoside analogues is no more effective than the more potent of the two agents alone. Combination therapy was associated with a lower rate of antiviral resistance, but the duration of the studies did not allow for meaningful analysis of long-term resistance rates.

Combination therapy has been more thoroughly evaluated in the treatment of lamivudine-resistant chronic hepatitis B. In a prospective, randomized controlled trial in 59 patients with HBeAg-positive chronic hepatitis B and lamivudine resistance, continuing lamivudine alone was associated with little change in HBV DNA levels and no improvements in ALT levels.[95] Switching to adefovir (monotherapy) was associated with similar average decreases in HBV DNA levels compared to adding adefovir to lamivudine (combination therapy) (4.0 versus 3.5 $\log_{10}$ declines) and similar rates of normalization in ALT levels (47% versus 53%). These results suggested that patients with lamivudine-resistance should be switched to adefovir and lamivudine stopped. However, the possible effects of continuing lamivudine in preventing adefovir-resistance could not be assessed, because adefovir resistance usually arises only after one year of treatment.

Support for combination therapy in treating lamivudine resistance in chronic hepatitis B has come from several more recent studies. In a retrospective analysis of a large number of patients with chronic hepatitis B and lamivudine-resistance from Italy, 303 patients were switched to adefovir (monotherapy) and 285 had adefovir added (combination therapy).[108] At 2 years, the rates of suppression of HBV DNA to undetectable were similar in the two groups (67% versus 69%) but rates of virological breakthrough (9% versus 2%) and adefovir-genotypic resistance (5% versus 0.8%) were higher with monotherapy. Preliminary results from a prospective trial from Greece among 46 patients with HBeAg-negative chronic hepatitis B showed similar results of adefovir monotherapy to the combination of adefovir and lamivudine, with lack of detectable HBV DNA and normal ALT levels in 80% of patients in both groups at month 34.[109] However, adefovir resistance developed only among patients treated with adefovir alone (22%), suggesting that the role of combination therapy is in prevention of resistance. Neither of these studies, however, demonstrated whether pre-emptive combination therapy was more effective than adding lamivudine to adefovir once resistance appears.

Furthermore, adefovir therapy may be more effective in suppressing HBV DNA levels if begun soon after emergence of lamivudine resistance, before HBV DNA rises to high levels or disease activity worsens, as shown by uncontrolled but sizeable studies in patients with advanced fibrosis and chronic hepatitis B.[110,111]

Theoretically, combinations of nucleoside analogues to treat chronic hepatitis B may be limited because they act upon the same viral target, the HBV polymerase, which may explain the lack of additive or synergistic activity. In this regard, the combination of interferon or peginterferon with nucleoside analogues may be more appropriate as the antiviral effects of interferon are directed more at viral RNA stability and virus assembly rather than the HBV polymerase enzyme. The combination of peginterferon and lamivudine has been assessed in three large, multicenter trials.[60-62] All three studies failed to show an increase in sustained response rate of the combination of peginterferon and lamivudine over peginterferon alone assessed 24 weeks after discontinuation of treatment. On the other hand, the on-treatment suppression of HBV DNA levels was superior in patients who received combination therapy: by the end-of-treatment, HBV DNA levels decreased by 5.2 $\log_{10}$ in lamivudine-, 5.1 $\log_{10}$ in peginterferon-, and 7.2 $\log_{10}$ in combination-treated HBeAg-positive patients.[60] These results suggest that combinations of agents that have different targets against HBV may provide additive antiviral activity and higher potency.

At present, however, medical evidence does not support the use of combination therapy for chronic hepatitis B with the possible exception in lamivudine-resistant disease where the combination of adefovir and lamivudine appears preferable to adefovir alone. Because antiviral resistance is slow to develop in chronic hepatitis B, longer term studies are needed before one can assume that the prevention of resistance and maintenance of a response may be more common with combination therapy. Finally, studies of combination of nucleoside analogues have used agents (lamivudine, emtricitabine, adefovir) with lower potency than more recently developed antivirals (telbivudine, tenofovir, entecavir), and the advantages of combination versus monotherapy using the more potent agents awaits further study.

Special Populations

Most trials of therapy of hepatitis B have been in selected patients without comorbidities or complications of chronic liver disease, frequently excluding special populations such as in children, or adult patients with acute hepatitis B, chronic hepatitis B and cirrhosis, liver decompensation, pregnancy, organ transplantation or co-infection with human immunodeficiency virus (HIV).

Children. The few studies of antiviral therapy of chronic hepatitis B in children suggest that response rates are similar if not higher than in adults.[112] Both standard interferon alfa and lamivudine have been found to be beneficial and are now approved for use in children above the age of 2 years.[112-114] The efficacies of peginterferon, adefovir, tenofovir, telbivudine, entecavir and combination therapies await further study.[115] While these drugs are likely to be as effective in children as they are in adults, concerns about the long-term safety and consequences of drug resistance are greater in the pediatric age groups. Furthermore, the long-term efficacy of antiviral therapy of hepatitis B in children remains unclear, and many children with this disease have benign outcomes without therapy.[116]

Acute Hepatitis B. Most patients with symptomatic, acute hepatitis B recover, and antiviral therapy is usually neither recommended nor needed.[21] Probably as a result of wide-scale use of HBV vaccine and routine blood donor screening, acute hepatitis B has been decreasing in frequency in the United States and is currently at a historic low level, an estimated 20,000 to 25,000 cases occurring yearly, greatest decreases having occurred in children.[117] Nevertheless, cases of acute hepatitis B still occur in adult populations and can result in severe and even fatal outcomes.

Use of standard interferon for typical acute hepatitis B was evaluated in a small controlled study and was without benefit.[118] There have been no prospective studies of nucleoside analogues in typical acute hepatitis B, but case reports and small series in severe or prolonged acute hepatitis and HBV-related liver failure suggest some benefit of early therapy.[119-122] The serious nature of acute liver failure and the safety of nucleoside analogue therapy support its use in patients at the first sign of severe injury or impending liver failure (prolongation of prothrombin time or hepatic encephalopathy), particularly since a proportion of patients will be referred for emergency liver transplantation and require prophylaxis against recurrence, which usually includes nucleoside analogue therapy.[123,124]

Cirrhosis and Decompensated Liver Disease. Recommendations for therapy of patients with HBV-related cirrhosis and decompensation are clearly supported by current medical evidence.[125] Interferon and peginterferon are contraindicated in patients with decompensated liver disease, largely because of the high rate of serious, sometimes life-threatening adverse events.[126,127] In contrast, the nucleoside analogues can be administered safely in patients with advanced cirrhosis, and recent studies indi-

cate that they are effective.[128-130] In a landmark, randomized controlled trial from Asia, long-term lamivudine therapy was associated with improved survival and lower rates of HCC in patients with cirrhosis or advanced liver disease due to hepatitis B.[131] The benefits were most clear among patients who maintained a virological response and did not develop lamivudine resistance. These results indicate that patients with advanced fibrosis and cirrhosis and high levels of HBV DNA should receive nucleoside analogue therapy. At issue is which agent to use and how to avoid antiviral resistance with long-term therapy. HCC can develop despite successful suppression of HBV DNA, so that further studies are needed to identify the optimal long-term management of patients with advanced chronic hepatitis B.

Liver Transplant Recipients. With application of high doses of hepatitis B immune globulin usually in combination with nucleoside analogue therapy, hepatitis B can be prevented in most patients after liver transplantation for HBV-related end-stage liver disease or HCC.[123,124] For these reasons, recurrence of hepatitis B after liver transplantation is now uncommon.[132-134] Nevertheless, breakthrough HBV infections do occur, usually related to development of resistance to the antiviral agents used to prevent re-infection.[135-137] While such breakthrough infections can be treated with other nucleoside analogues with different patterns of resistance, serial therapy with nucleoside analogues can lead to complex HBV strains with resistance to multiple agents.[82] Combination nucleoside analogue therapy is likely to be most beneficial in post-transplant patients, but the combination has to be chosen based upon previous exposure and patterns of resistance.[123]

HIV Co-infection. Patients with HBV/HIV co-infection present a particular challenge to management. Hepatitis B can be severe in patients with HIV infection,[138,139] and, as therapies for HIV infection improve, an increasing number of patients are dying from liver disease.[140-142] Fortunately, many of the agents used to treat HIV infection have activity against HBV, in particular lamivudine, emtricitabine, and tenofovir. Lamivudine resistance is frequent in patients with HIV/HBV infection who have been treated for prolonged periods.[143] In patients with lamivudine-resistance, tenofovir has proven to have excellent activity.[101-103,144-147] The combination of tenofovir and emtricabine (Truvada™, Gilead Sciences, Foster City, CA) has been approved as therapy for HIV infection and is currently recommended by several expert international panels as the optimal therapy for patients with HIV/HBV co-infection who require treatment of both diseases.[148,149] Indeed, recent findings indicate an improved prognosis for patients with HIV-HBV co-infection possibly as a result of better use of anti-retroviral and anti-HBV antiviral therapy.[150]

Prevention of Reactivation. An important but often overlooked role of antiviral therapy is prevention of HBV reactivation during immune suppression. Patients who are inactive HBsAg carriers and a proportion of those who have recovered from hepatitis B (who have anti-HBc without HBsAg in serum) can suffer from reactivation in response to immune suppression either as a result of cancer chemotherapy,[15,16,151-155] short courses of corticosteroids, immuno-modulatory agents (anti-cytokines),[156-158] heart, kidney and liver transplantation[159] and even as a result of advancing immune deficiency due to HIV infection.[160,161] Reactivation is reported to occur in 29% to 56% of inactive HBsAg carriers treated with chemotherapy[162] and can also occur in patients who have apparently recovered from hepatitis B and have anti-HBc without HBsAg in serum (regardless of anti-HBs status).[15,163] This pattern of reactivation is particularly common in bone marrow transplant recipients.[164-167] Reactivation of hepatitis B in an immunosuppressed host can be severe resulting in acute liver failure or an unremitting and progressive chronic hepatitis. Several studies have shown that pretreatment with lamivudine can prevent or at least ameliorate the course of reactivation.[154,155,162,163,167] Delaying therapy until HBV DNA levels rise is ineffective.[16,163] Thus, patients who are to undergo chemotherapy for cancer, intensive immune suppression for autoimmune disease, or bone marrow transplantation should be screened for HBsAg and anti-HBc, and given prophylaxis with lamivudine or another nucleoside analogue for the duration of immune suppressive therapy. Some patients will experience a flare of hepatitis B when the antiviral therapy is stopped and require reinstitution of treatment.[168] Interferon and peginterferon are ineffective and likely to be poorly tolerated in this situation.

Safety of Antiviral Therapy

In contrast to interferon and peginterferon therapy, nucleoside analogue therapies for hepatitis B have had few and only minor side effects. Despite the concerns about pancreatitis, neuropathy, acute fatty liver and lactic acidosis associated with nucleoside analogue therapies, these side effects have been rarely reported and not definitely linked with lamivudine, adefovir, and entecavir monotherapy. In many clinical trials, common adverse events (headache, pharyngitis, diarrhea) have occurred as frequently among placebo as lamivudine or adefovir recipients,[64-68] and most cited serious side effects have not been definitely linked to these therapies.

An important exception is the occurrence of renal dysfunction with long-term adefovir and tenofovir thera-

py.[169-172] Renal tubular acidosis, phosphate wasting and renal impairment occurred in as many as one-quarter of patients treated with higher doses of adefovir (30 to 60 mg daily), appearing after 5 to 6 months of treatment and resolving rapidly if identified and the medication stopped. This side effect is rare in patients receiving 10 mg of adefovir and standard doses of tenofovir.[173] Because of this side effect, patients on adefovir or tenofovir are advised to be monitored for creatinine levels and the dose rapidly modified or discontinued if toxicity appears.[125] Prolonged tenofovir therapy may also be associated with bone loss and decreases in bone density.[174,175] All of the nucleoside analogues have major renal excretion, so that dose adjustments are needed for renal insufficiency, and adefovir and tenofovir should be used with caution in patients with pre-existing kidney disease.

The side effects of interferon and peginterferon are well described particularly in studies of therapy of hepatitis C.[176] Interestingly, the recommended doses of peginterferon are similar in chronic hepatitis B than C, but side effects appear less.[60,61] The better tolerance of interferon in hepatitis B may be due to the fact that patients with HBV infection are more likely to be younger and less affected by other medical co-morbidities than patients with hepatitis C.

Pregnancy

The safety of antiviral therapy for hepatitis B during pregnancy and during breast feeding is not well defined.[44,125] Interferon and peginterferon are contraindicated during pregnancy largely because of their known anti-proliferative effects. In the event of pregnancy, peginterferon should be discontinued.

Medications are classified by the FDA in regard to safety during pregnancy into five categories: Category A (controlled studies show no risk and the possibility of fetal harm appears remote); B (animal studies show no risk, but human studies have not been done or *vice versa*); C (animal data show teratogenic effects but no controlled studies done in humans); D (human fetal risk exists but benefit outweighs risks); or X (fetal risk outweighs benefit). Currently, lamivudine, telbivudine, emtricitabine, and tenofovir are classified as category B, indicating that they demonstrate no evidence of teratogenicity in animal studies but have not been adequately evaluated in humans and ongoing registries include too few instances of pregnancy during therapy to provide reliable guidance. These agents could be used if the potential benefit of treating during pregnancy is believed to outweigh potential risks to mother or fetus; although the possible effects of tenofovir on bone density argue against its use during pregnancy and while mothers are breast feeding.[174,175] Entecavir and adefovir are classified as Category C, in that embryo and fetal toxicities have been observed in animals, but these reproduction studies are not always predictive of human response.

A central issue regarding safety of therapy during pregnancy is whether nucleoside analogue therapy should be stopped in young women who are attempting pregnancy or who become pregnant during treatment. Currently, lamivudine and zidovudine are recommended for HIV-1 infected women during pregnancy. Thus, in women being treated for hepatitis B who become pregnant, switching to lamivudine for the duration of pregnancy is a reasonable recommendation.[125,149] The problem of antiviral resistance, however, makes use of lamivudine monotherapy problematic, even for a period of 9 to 12 months.

There have been two small trials of lamivudine therapy during pregnancy in women with chronic hepatitis B and high levels of HBV DNA that focused the prevention of transmission of hepatitis B to the infant.[177,178] HBV transmission was less among women treated with lamivudine, but none of these studies was adequately powered or controlled to prove the efficacy or advisability of this approach. Therapy appeared to be safe, at least to the infant. A potential complication for the mother is that flares of hepatitis B can occur if nucleoside analogue therapy is stopped after childbirth.

Table 3. Costs of Therapy for Chronic Hepatitis B

Agent	Dose	Cost per dose*	Cost per year**
Lamivudine	100 mg daily	$6.80	$2,482
Emtricitabine	200 mg daily	$10.61	$3,872
Telbivudine	600 mg daily	$16.23	$5,924
Adefovir	10 mg daily	$18.11	$6,647
Tenofovir	300 mg daily	$15.92	$5,811
Entecavir	0.5 mg daily	$23.82	$8,694
Entecavir	1.0 mg daily*	$23.82	$8,694
Peginterferon alfa-2a	180 μg weekly	$385.00	$18,480

*A 1.0 mg dose is recommended for treatment of patients with lamivudine resistance. **Data from references 179 and 180 for average wholesale prices in the United States, except for telbivudine for which the wholesale acquisition cost is provided. Data from peginterferon are for 48 weeks; all others for 365 days. (Modified with permission from John Wong, Tufts University, New England Medical Center, Boston, MA.)

Costs of Therapy

The costs of therapy of hepatitis B are considerable (Table 3) and must be considered when initiating treatment. A one-year course of peginterferon costs $18,000 to $20,000, considerably more than a one-year course of nucleoside analogues.[179,180] However, nucleoside analogue therapy may be continued indefinitely, and cumulative costs rapidly can exceed those of peginterferon.

Furthermore, insurance coverage may be limited because the majority of randomized controlled trials typically focused on the benefits of only a one-year course of treatment. Additional costs of monitoring disease activity and HBV DNA levels must also be considered. In clinical trials, a full panel of liver tests, blood counts and HBV DNA levels were done every 4 weeks during and after therapy, a level of monitoring that is not necessary in clinical practice. Serum testing at 3 to 6 month intervals may be adequate for routine treatment of compensated chronic hepatitis B using nucleoside analogues.[125]

Future Therapy

Much of the effort on antiviral drug development in hepatitis B has been focused on nucleoside analogues which target only a single step in the viral life cycle — DNA synthesis. While many of the new nucleoside analogues have different resistance profiles and higher potency which may limit the emergence of resistance, they also are likely to have interactions in terms of efficacy and resistance when they are used sequentially or in combination. Other steps of HBV replication deserve evaluation as targets for antiviral therapy (Fig. 1).[3,181] Several classes of compounds have been developed that interfere with the encapsidation signal (attachment of polymerase to pregenomic RNA) during viral replication, including several hetero-aryl-dihydropyrimidines and phenylpropenamides.[182,183] These compounds had favorable efficacy and toxicity profiles in pre-clinical testing but have yet to be tested in humans. Other potential targets for small molecule development are HBV receptors on the surface of hepatocytes, the HBV assembly process, the interaction of nucleocapsid and HBsAg, and the unique viral functions mediated by the HBV X protein.[184,185] Gene therapies using anti-sense, ribozyme and siRNA molecules have been designed that target and breakdown HBV RNA inside hepatocytes. Several of these approaches have shown promise *in vitro* and in animal models.[186] The major obstacle with these approaches is delivery into the hepatocyte and the appropriate site where HBV replicates. Recent advances in delivery technology may render solutions to this critical issue.

Another potential future approach to therapy of chronic hepatitis B is by modulation of the immune response that is detective in persons with chronic infection. Immunomodulatory agents that nonspecifically enhance host–immune responses include multiple cytokines with antiviral actions, such as interferon gamma, interleukin (IL)-2, IL-12, IL-18. Studies of these molecules in humans have shown little effect on viral replication or disease activity.[181] Toll-like receptor ligands to activate innate immunity or inhibitors of IL-10 or programmed death 1 (PD1) expression on T cells are alternative and attractive strategies for modulation of the immune response.[187,188] These have yet to be applied to patients with chronic hepatitis B. Finally, therapeutic vaccination including use of dendritic cell and T cell-based vaccines is being developed to target and induce HBV-specific T cell response, which has been shown to play a pivotal role in viral clearance. Thus, an array of approaches to therapy are being pursued which ultimately may be beneficial either alone or in combination with peginterferon or nucleoside analogue therapy of hepatitis B.

Recommendations for Future Research

Despite more than 40 years of research, fundamental questions remain regarding HBV infection and its optimal management. The underlying reasons for the evolution from acute to chronic HBV infection remain unclear. How does HBV evade immune clearance or induce tolerance to its protein antigens in the liver? What specific defects or changes in the innate and/or adaptive immune responses to HBV account for viral persistence? Can these processes be manipulated to promote recovery or response to antiviral therapy?

Once chronic infection is established, clinical disease and outcome vary greatly, yet the reasons for this variability are not clear. What factors determine the degree of hepatocellular injury during chronic hepatitis B? What factors control the level of viral replication? What underlying mechanisms account for spontaneous clearance of HBV that can occur during chronic infection? And what factors lead to resolution of disease activity in some patients and evolution to progressive fibrosis, cirrhosis, and end-stage liver disease in others?

Six agents are now available for treating hepatitis B, all of which are effective in the short-term. Nevertheless, practical management is plagued by uncertainties. Which patients should be treated? At what stage of infection or disease? With which agent or combination of agents? For how long? Which criteria should be used to continue therapy, or switch to other therapies, or stop altogether?

The two-and-a-half day workshop allowed for answering of some questions regarding management of hepatitis B, but succeeded more in focusing questions of what should to be addressed in future research. What are the most important clinical questions to be addressed and how can research help resolve them within the next 5 to 10 years?

Studies of natural history of chronic hepatitis B need to be combined with prospective analyses of virological, genetic, and immunological factors using state-of-the-art methodology. Studies of viral genetic variation and diversity and changes in serum levels combined with analysis of

the state and levels of replicative forms of HBV in liver should be combined with prospective studies of innate and adaptive immune responses to HBV antigens using both peripheral blood and hepatic infiltrating mononuclear cells. Assessment of genetic factors, particularly of genes that regulate the adaptive and innate immune response, should be combined with these studies, but most important is to correlate these findings with careful clinical phenotypes of disease.

Four phases of chronic hepatitis B have been defined: two marked by immune control (immune tolerant phase and inactive carrier state) and two by immunological injury (HBeAg-positive and -negative chronic hepatitis B). Therapy of hepatitis B has focused largely on the phases without immune control with resulting liver injury and high viral replication. Nevertheless, the role of treatment during the immune tolerant phase and even the inactive carrier state deserves prospective analysis. Animal models of chronic HBV infection do not adequately reflect these four phases of infection and cannot help in defining pathogenesis, natural history or therapy. Few prospective studies in humans have adequately addressed the clinical, viral, immunological and host genetic factors that accompany and account for these four phases of infection.

Recommendations for therapy of hepatitis B are difficult and are limited by the lack of reliable information on the long-term safety and efficacy of therapies.[125] The uncertainty surrounding these recommendations indicates that further studies of therapy of hepatitis B are needed — not aimed at proving efficacy, but at placing therapies in context of how they should be used, in which patients, at what phase of infection, to what endpoint and using what factors to judge success or failure. Important current issues include.

- Which patients with chronic hepatitis B should be treated? Should criteria for therapy be based upon ALT elevations (any abnormality, greater than twice elevated, greater than five-times elevated)? HBV DNA (and what levels)? Liver histology? Or a combination of the three?
- What is the role of combination therapy versus monotherapy of chronic hepatitis B? This issue is particularly important in regards to the new, more potent nucleoside analogues (entecavir, tenofovir, telbivudine), as it is already clear that combination therapy is preferable to lamivudine or adefovir monotherapy in patients with HBeAg and/or high HBV DNA levels.
- What is the role of peginterferon? While several trials have shown a high rate of loss of HBeAg and sustained suppression of HBV DNA after peginterferon therapy, the overall response rate (30%-40%) is low. Should all patients receive a course of peginterferon before being started on nucleoside analogues? Or only patients with favorable predictive factors for a response, such as HBeAg-positivity, genotype A or B, or marked ALT elevations?
- What should be the criteria for judging success of therapy? Should the goal be to maintain HBV DNA below a certain level? Normalization of ALT levels? Or improvement in liver histology?
- Similarly, what should be the criteria for switching therapy? From peginterferon to nucleoside analogues? Or from one nucleoside class (L-nucleosides, PMEA derivative, guanosine derivative) to another?
- Should therapy be stopped after HBV DNA has been undetectable for a certain period? Or only once HBeAg becomes undetectable? Or only after seroconversion to anti-HBe? Or only if HBsAg becomes undetectable?

At present these issues are only partially resolved and recommendations for therapy must be based on imperfect information and extrapolation of results.

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