# Hepatitis B in 2005:
# What Every Physician Should Know

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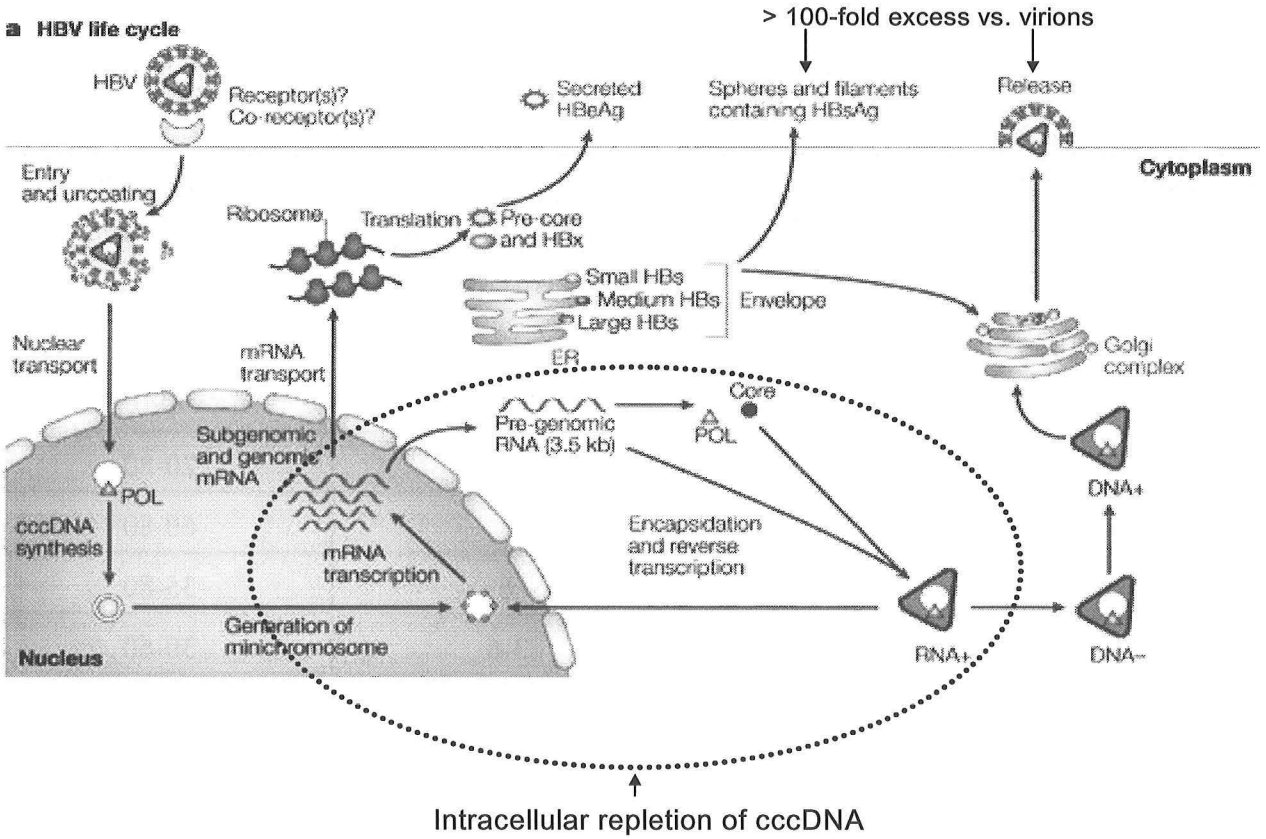
cycle that facilitates its capacity for a long-term host-parasite relationship in which the chronically infected but otherwise healthy host serves as a highly infectious vector throughout the peak years of sexual and reproductive activity. The significant liver diseases that develop in 15-40% of chronic carriers usually develop later in life (median age for cirrhosis - 46, median age for HCC - 63) following prolonged attempts by the host immune system to clear the virus from the liver via cytopathic mechanisms [6, 9, 11].

Hepadnaviruses are lipoprotein enveloped viruses that attach to and penetrate hepatocytes via yet to be delineated receptor mechanisms. The viral envelope is then removed and the nucleocapsid released into the cytosol. Cytoplasmic cores are then transported to the cell nucleus where the HBV genomic DNA is converted from a partially double stranded, relaxed circular DNA into a "repaired" double stranded covalently closed circular DNA (cccDNA). HBV cccDNA resides

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in the nucleus of the infected hepatocyte where it is organized into nucleosomes that form long lived viral minichromosomes [9,10]. Host RNA polymerase II generates a series of genomic and subgenomic transcripts from cccDNA that are transported to the cytoplasm where translation yields viral envelope, core and polymerase proteins as well as the accessory proteins preC and the hepatitis B X polypeptides [9,10]. Nucleocapsids assemble in the hepatocyte cytosol with incorporation of a single molecule of genomic RNA. This genomic RNA is reverse-transcribed into viral DNA via the multifunctional activity of the HBV polymerase protein. There are four HBV polymerase dependent steps in this process: 1.) RNA-dependent DNA polymerase activity that produces the minus-strand

HBV DNA, 2.) Priming of minus-strand DNA synthesis via covalent binding of Tyr-63 in the polymerase protein to the first nucleotide (G) of the minus-strand, 3.) DNA-dependent DNA polymerase activity that produces positive-strand HBV DNA, and 4.) Ribonuclease H activity that cleaves the RNA in the RNA-DNA hybrids during reverse transcription [9,10]. As detailed later in this review, the nucleoside and nucleotide analogs that have been developed as antiviral drugs for therapy of chronic hepatitis B target one or more of these HBV polymerase dependent functions [12, 13]. The resulting viral cores that now contain the partially double stranded, relaxed circular form of HBV viral DNA either enter the endoplasmic reticulum to be enveloped and exported from the cell via vesicular transport as infectious viral particles or, are recycled into the nucleus for conversion into cccDNA. This ability of the virus to replenish relatively stable cccDNA and viral minichromosomes via an entirely intracellular process independent of cycles of reinfection and largely sheltered from the host immune system is viewed as one of the unique properties of hepadnaviruses that permit maintenance of chronic infection [9, 10, 13].

The HBV genome has only four open reading frames. Those for the polymerase and X proteins each produce only a single gene product. In contrast, the preS-S (presurface-surface) region of the HBV genome encodes three viral surface proteins (S, pre-S2 or M, and pre-S1 or L) by differential initiation of translation at each of three in-frame initiation codons. Similarly, the pre-C-C (pre-core-core) open reading frame, via differential initiation of translation at two in-frame initiation codons, encodes a pre-C polypeptide, subsequently processed to form the hepatitis B early antigen (HBeAg), and the core protein which is the major polypeptide of the nucleocapsid.

The pre-S1 or long surface protein is thought to play key roles in the binding of the virus to host cell receptors and in the assembly and release of the virion from the cell [9, 10]. The functions of the pre-S2 or medium surface and the S or short surface protein, which is produced in greatest abundance, are less well defined although the S protein is know to be essential for virion formation [9, 14]. Each of the surface proteins is incorporated into the mature viral particles in ratios of approximately 7:1:2, S:M:L. One of the most striking characteristics of the HBV viral replication cycle is that in addition to production of 40-42 nm diameter virions, there is simultaneous production of > 100 fold greater numbers of 20 nm spheres along with somewhat less abundant 20-22 nm filamentous structures. These non-infectious spheres and filaments consist of host-derived lipids and virus-derived surface proteins in ratios of 89% S: 10% M: 1% L for the spheres and 70% S: 10%M: 20% L for the filamentous structures [15]. At early stages of the host B cell response to HBsAg, no anti-HBs can be detected in the serum of infected hosts despite the presence of anti-HBs producing B cells in the peripheral blood [16]. This is attributed to the presence of an abundant excess of HBsAg expressing "decoy" particles that overwhelm and/or divert the neutralizing antibody response from interaction with infectious virions [16].

The second pre-C-C HBV open reading frame with multiple in-frame initiation codons also produces protein products that appear to play an important role in interactions between the virus and the host immune response [17]. In contrast to the L, M and S hepatitis B surface proteins that are all structural components of intact virions, the HBeAg produced by post-translational processing of the polypeptide product of the pre-C initiation codon is an accessory protein that is not incorporated into the virion at any stage of viral replication. In addition, HBeAg is not essential for viral replication *in vitro* and is it not required for infection of new hosts [9, 10, 15]. However, the synthesis of HBeAg is required for establishment of chronic infection by HBV in the woodchuck and multiple clinical

observations and experimental results implicate HBeAg as a "tolerogenic" protein that interferes with initiation and/or maintenance of host immune responses to the essential core protein of HBV [10, 17]. The presence of a leader sequence with secretion signals in the pre-C polypeptide and an arginine rich COOH-terminal region in core proteins that is removed from the pre-C polypeptide by post-translational processing results in striking differences in final tertiary structure of the two pre-C-C ORF products. HBeAg is secreted from the host hepatocyte to circulate freely in host blood and body fluids while HBc forms a polymeric RNA-DNA binding protein found in abundance only in the infected hepatocyte nucleus or within viral core particles. Antibody responses to these two viral proteins are quite distinct [17]. HBcAg is capable of eliciting both T cell independent and T cell dependent antibody responses whereas HBeAg elicits only T cell dependent antibody responses [17]. However, the majority of the C-terminal portion of HBeAg and the N-terminal portion of HBc (≥ 160 amino acids depending upon genotype and degree of HBeAg processing) are identical in amino acid sequence. HBeAg contains only 10 N-terminal amino acids not found in HBcAg and up to 34 of the COOH terminal amino acids of HBcAg are not found in the heterogeneous population of HBeAg proteins secreted from HBV infected hepatocytes [15]. These unique sequences appear to be only weakly immunogenic as the dominant CD4+ and CD8+ T cell epitopes of HBeAg and HBcAg appear to arise entirely from the region of common amino acid sequence [17]. For this reason there is significant overlap and interaction between the T cell responses to these two viral polypeptides.

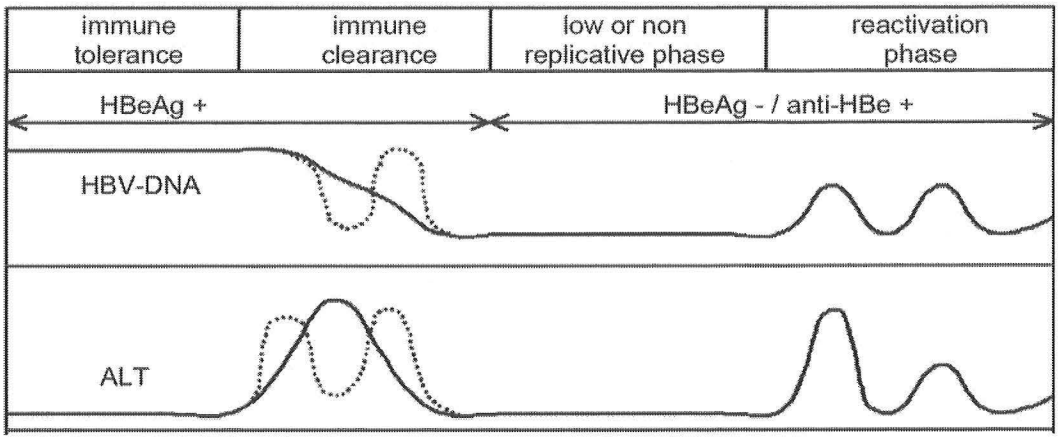
HBeAg has been found to cross the human placenta [18, 19]. Exposure of mice to HBeAg or its peptide fragments either in utero or in the perinatal period has been found to be highly tolerogenic leading to long term T cell tolerance not only to HBeAg but also to HBcAg [17]. The tolerogenic nature of HBeAg in humans is supported by the linkage of some common source outbreaks of fulminant acute hepatitis B to HBeAg negative HBV viral strains [20-22]. In contrast, there have been no reports of humans primarily infected with HBeAg negative variants who progressed to chronic infection [17].

HBeAg is not solely a "tolerogenic" protein. In contrast to infections in neonates that usually lead to chronic infections, when initial HBV infections occur in immunocompetent adults, both T and B cell responses to HBeAg usually evolve during the first several weeks to months after infection and are associated with brisk Th1 responses to shared HBeAg and HBcAg epitopes [17]. In addition, later in life, individuals infected with HBV during childhood begin to mount T and B cell responses to HBeAg which usually correlate with flares of hepatitis and significant declines in levels of viremia [6, 11, 23]. Indeed,, in some individuals chronically infected with genotypes B-E, HBeAg negative mutants appear to be selected late in their clinical course by immune pressure directed against HBeAg [17]. HBeAg negative mutants of wild type HBV can arise by several mechanisms. A single-base substitution (G→A) at nt 1896 introduces a translational stop codon (TGG →TAG) in the second last codon (codon 28) of the pre-core region and thus totally disrupts HBeAg production without significant interference with translation of core [10]. This codon is part of a highly conserved stem loop structure with nt G1896 forming a base pair with nt 1858. In HBV genotypes B, D, E, G and some strains of C, the 1858 nt is a thymidine and the stop codon mutation actually stabilizes the stem loop structure by permitting T-A pairing. In contrast in HBV genotype A, genotype F and some genotype C strains, nt 1858 is a cytidine and stop codon mutations are rarely observed. Mutations of the basic core promoter (BCP) at position 1762 (A→T) and 1764 (G→A) when present in combination decrease HBeAg production by up to 70% without altering transcription of pregenomic RNA or the translation of core protein [10].

In addition to the likely immunoregulatory roles of the non-infectious HBsAg containing particles and soluble HBeAg produced by HBV infected cells, the single most important host factor responsible for the incidence of chronic infection by the HBV virus relates to the age at which humans are initially infected. Individuals infected prior to 1 year of age when the adaptive immune responses have yet to fully mature exhibit a >90% incidence of chronic infection. For children aged 1-5 the risk of chronic infection is 30% while for older children and adults the risk for chronic infection falls to about 2% [24]. The natural history of HBV infection also varies greatly depending upon age of initial infection and, to a lesser extent, on the genotype of the infecting HBV virus. In immunocompetent adult humans or primates infected with HBV, a period of high levels of viremia, readily detectable circulating HBsAg and antibody responses to HBcAg usually precedes by several weeks the development of T cell cytokine responses that lead to significant reduction in viremia via the antiviral effects of IFN-$\gamma$, and TNF$\alpha$ [11, 25]. The onset of ALT elevations correlates closely with the development of CD8+ cytotoxic T cell responses which appear to play an essential role in the final clearance of HBV infected hepatocytes [25]. With onset of neutralizing anti-HBs responses, humans and primates generally develop lifelong resistance to repeat HBV infection. However, for prolonged time intervals after apparent resolution of acute infections, sensitive PCR based assays may still detect HBV DNA sequences in peripheral blood for years after resolution of acute hepatitis [26, 27]. Blood from such individuals has not been found to be routinely infectious in chimpanzees [27], but the occasional transmission of hepatitis B by anti-HBc(+), HBsAg (-) blood transfusions is well documented. Furthermore, when HBsAg(-), anti-HBc (+), anti-HBs (+/-) individuals are used as organ donors, HBV naive recipients of the their livers but not kidneys or hearts commonly develop HBV infection [28, 29]. Very rarely, when such HBsAg(-), anti-HBc(+), anti-HBs(+) individuals are profoundly immunosuppressed, recurrent HBV viremia and clinical hepatitis may recur. Thus, even after resolution of acute Hepatitis B and loss of HBsAg, many if not most humans continue to harbor small amounts of residual HBV, likely in the form of cccDNA in long lived hepatocytes, and this may account for the presence of lifelong ant-HBc responses in such individuals [11, 26].

Despite a very small risk of recurrent liver disease in individuals who have cleared conventional markers of HBV infection, the vast majority of liver disease develops in individuals who remain HBsAg(+) and serum HBV DNA positive. Current understanding of the natural history of chronic HBV infection suggests that there are four stages of such infection [6, 8]. An initial immune tolerant phase is marked by high levels of viremia (typically $>>10^7$ copies/ml), HBsAg and HBeAg but normal ALT levels and a host immune response largely limited to antibody responses to HBcAg [11, 17]. This immune tolerant phase may extent well into the 2nd, 3rd or even 4th decades of life among those individuals infected as neonates and is usually associated with normal or near normal liver histology. The next phase of chronic hepatitis B is marked by onset of elevated ALT levels and declines but not disappearance of HBV DNA (still $> 10^6$ copies/ml) and HBeAg from the circulation [6, 8]. This phase of chronic hepatitis B is marked histologically by the development of hepatitis characterized by periportal or lobular lymphocytic infiltrates, hepatocyte apoptosis, and over time, the development of fibrosis and cirrhosis if the immune system is not able to adequately suppress HBV replication during this immune clearance phase of the disease [6, 8, 11]. In this phase of the disease, as during acute hepatitis, CD8+ cytotoxic T cells appear to be the primary mediators of hepatocyte injury as they attempt to clear HBV infected hepatocytes from the liver of the infected host [11, 23].

## Phases of Chronic Hepatitis B Infection




| | | | | |
|---|---|---|---|---|
| **Histology:** | Normal or mild CH | Mod/Severe CH<br>?<br>Cirrhosis | Normal or<br>Inactive fibrosis | Mod/Severe CH<br>?<br>Cirrhosis |
| **Diagnosis:** | HBeAg Positive Chronic Hepatitis B | | Inactive Carrier<br>(HBsAg Positive)<br>or<br>Resolved Hepatitis B<br>(HBsAg Negative) | HBeAg Negative<br>Chronic Hepatitis B |

Modified from Fattovich, G. Seminars in Liver Diseases, 23: 47-58, 2003.

Following an immune clearance phase, many chronically infected individuals enter a low-replicative, inactive carrier phase marked by very low levels of viremia (< $10^4$ copies/ml), normal ALT levels, loss of HBeAg and development of anti-HBe responses and inactive liver histology [6, 8, 11]. Over time some of these individuals will also lose HBsAg and develop anti-HBs. However, the maintenance of this low-replicative, inactive disease state in HBsAg (+) inactive carriers appears to be dependent upon active immune suppression of viral replication. Following a variety of immunosuppressive therapies, a significant fraction of such individuals will show evidence of recurrent high levels of viremia associated with often high ALT levels and histologically severe hepatitis [8,11,30,31]. Immunosuppressive regimens containing corticosteroids are especially predisposed towards inducing such reactivations [31] likely because the HBV genome contains a glucocorticoid responsive element (GRE) encoding two tandem repeats of the GRE core hexanucleotide TGTTCCT [10]. Even in the absence of exogenous immunosuppression, a significant fraction of HBsAg (+), inactive carriers from HBV endemic areas of the world, where genotypes B-D predominate, will develop a reactivation of HBeAg(-) hepatitis marked by somewhat lower levels of viremia than seen in the immune clearance phase (usually still > $10^5$ copies/ml) but levels of biochemical and histologic activity comparable to that observed during the immune clearance phase of HBeAg positive chronic hepatitis B [6]. This form of reactivation hepatitis appears to largely reflect immune selection of an HBeAg negative mutant of a susceptible HBV genotype [17].

Therapy of chronic hepatitis B has been attempted since the 1980's with either immunomodulatory agents (Type I or Type II interferons, thymosin α) or nucleoside/nucleotide

analogs. Several of the initial nucleoside analogs with anti-HBV efficacy (adenine arabinoside, fialuridine, and lobucavir) were abandoned after discovery of unacceptable side effects [32]. Other agents were found to have limited efficacy (interferon -γ, thymosin-α, famciclovir) and have never been adopted for therapy in the U.S. or Europe. Thymosin-α, however, has been licensed for HBV therapy in more than 30, predominately Asian, countries [32]. Since the early 1990's, efficacy of standard interferon-α therapy for ≥16 weeks has been demonstrated in European and North American, HBeAg positive patients with biochemically active (>2 x ULN ALT) chronic hepatitis [33]. During interferon-α therapy, most chronic HBV patients do not exhibit improvement in biochemical measures or in hepatitis activity and 20-40% actually exhibit ALT elevations or, more rarely, icteric flares of hepatitis [8]. However, by the end of therapy and 6 months of additional follow-up, about 1/3 of interferon-α treated HBeAg positive, chronic hepatitis B patients exhibit seroconversion to a HBeAg negative, anti-HBe positive, inactive stage of chronic HBV infection. Such HBeAg seroconversions are associated with sustained normalization of ALT, histologic remission of liver disease, decrease of HBV DNA levels undetectable by assays sensitive to about $3 \times 10^5$ copies/ml and, during prolonged followup, a decreased risk of hepatitis B cirrhosis-related complications and mortality [33-35]. Initially 8% of interferon-α treated White or African-American patients lose HBsAg during or shortly after interferon-α therapy. During long term followup, 65-71% of White or African-American patients with interferon-α induced seroconversion to HBeAg negative, anti-HBe positive status lose HBsAg as well and in 80-94% of cases such chronic hepatitis remissions have been found to be "durable" over 4-8 years of follow-up [34, 35].

Efficacy of standard interferon-α therapy for HBeAg positive chronic hepatitis in Asian patients has been more difficult to demonstrate [36, 37]. In addition, while long courses of standard interferon-α therapy for the HBeAg negative variant of chronic hepatitis B have been successful in achieving ALT normalization and HBV DNA viral load decreases to $< 10^5$ copies/ml while patients remain on therapy, rates of sustained remissions after therapy discontinuation have been very disappointing in this HBeAg negative variant of chronic hepatitis B [32]. However, more recently, pegylated interferon-α products with a prolonged *in vivo* half-life and greater ease of administration have been found to have significant efficacy in both European and Asian patients with HBeAg positive or HBeAg negative chronic hepatitis B [38-40]. The highest response rates to pegylated interferon-α therapy are observed in HBeAg positive patients infected with genotype A HBV strains [39,40] with 47-52% being HBeAg negative, anti-HBe positive 6 months after completion of 48 weeks of pegylated interferon-α therapy. Genotype A is most prevalent in Europe, Africa and in North American among residents of European or African descent [10] and rarely associated with development of HBeAg negative mutants [10, 41]. Of note, the overall sustained response rate to 48 weeks of pegylated interferon-α therapy for HBeAg negative chronic hepatitis B as defined by HBV DNA $< 2 \times 10^4$ copies/ml and ALT < ULN 6 months after completion of therapy was 36% [38]; a rate not different from the 28-30% sustained seroconversion / response rate seen observed in the genotype B, C and D patients entered in trails of pegylated interferon therapy of HBeAg positive hepatitis [39, 40]. Thus, in part, the disparities in chronic hepatitis B response rates to interferon therapy among different ethnic groups likely reflects geographic variations in genotype distribution and the different sensitivity of the various HBV genotypes to interferon based immunomodulatory therapy. Of note however, in a recent large, international trial of pegylated interferon-α therapy of HBeAg positive chronic hepatitis B, significant differences in rates of loss of detectable serum HBsAg were observed between European (17%) and Asian (2%) patients; a difference seemingly out of proportion to differences in response rates of different HBV genotypes.

Despite demonstrated "efficacy" in therapy of chronic hepatitis B, interferon-α therapy only induces sustained remissions (HBeAg / anti-HBe seroconversion, low HBV DNA levels, nl ALT) in a minority of patients. Use of this therapy is also associated with multiple side effects including flu-like symptoms, leukopenia, thrombocytopenia, anorexia and depression [32,38,40,42]. Moreover, the hepatitis flares induced by this immunomodulatory therapy may be fatal in patients with advanced cirrhosis [8, 32, 42]. Thus, use of this therapy is contraindicated in the patient subset most in need of therapy.

Fortunately, over the past 7 years an increasing number of nucleoside or nucleotide analogs with efficacy against HBV have been identified [12, 43]. These agents have far fewer side effects than interferon [43], have proved to be both safe and efficacious in patients with decompensated cirrhosis [44] and have been found to have utility in preventing HBV "re-activation" following chemotherapy or other forms of immunosuppressive therapy [31, 45-50]. Moreover, lamivudine, the first nucleoside analog approved by the FDA for chronic hepatitis B therapy, has also proven effective in preventing infection of donor livers following orthotopic liver transplantation for chronic hepatitis B induced cirrhosis [51] and, most recently, has been noted to decrease risk of mother to child transmission of HBV infection when administered to pregnant women with high HBV viral loads [52]. Finally, in contrast to the differences in therapeutic efficacy of interferon-α therapy among different ethnic groups or among patients infected with different genotypes, nucleos(t)ide analog therapy appears equally efficacious against all HBV genotypes [53, 54] and has similar efficacy in all ethnic groups [53]. Thus, with availability of this new class of antiviral agents, there are now far more opportunities for therapeutic intervention in the course of this chronic infection.

Three nucleos(t)ide analogs have been approved for HBV therapy by the FDA - lamivudine (Epivir-HBV), a nucleoside analog of cytidine; adefovir (Hepsera), a nucleotide analog of adenosine monophosphate; and entecavir (Baraclude), a nucleoside analog of guanosine. In addition, emtricitabine (Emtriva), a nucleoside analog of cytosine and tenofvir (Viread), a nucleotide analog of adenosine, have FDA approval for HIV therapy. While not yet FDA approved for HBV therapy, these two drugs have demonstrated efficacy in treatment of chronic hepatitis B in controlled trials [55, 56]. Furthermore, multiple additional nucleos(t)ide analogs with promising levels of anti-HBV efficacy are in Phase II or phase III clinical trials [12]. These include agents such as telbivudine, a β-L configuration nucleoside analog of thymidine [57] that achieves significantly greater $\log_{10}$ reductions in serum HBV DNA levels than lamivudine (6 $\log_{10}$ vs. 4.5 $\log_{10}$) [58], and clevudine, an L-nucleoside pyrimidine analog that has been distinguished by a very slow rebound in HBV DNA levels after treatment is stopped [59, 60].

Lamivudine, the first nucleoside analog approved for therapy of chronic HBV, inhibits HBV (and HIV) replication *in vivo* and results in rapid declines in serum HBV DNA levels during the first month of therapy [61]. By the end of one year of therapy with lamivudine, 100mg per day, 41-72% of patients with HBeAg positive chronic hepatitis B achieve normalization of ALT levels and 49-56% exhibit significant improvement in liver histology. However, only 16-18% achieve an HBeAg / anti-HBe seroconversion [61-65] after 1 year of therapy, and, when therapy is stopped, patients who have not achieved an HBeAg / anti-HBe seroconversion relapse, occasionally with a significant flare of their hepatitis [65]. HBeAg / anti-HBe seroconversions are achieved most frequently 50-60%) in patients with > 5 fold increases in per-therapy ALT levels, less often (28-30%) in those with 2-5 fold elevated ALT levels, rarely (10-20%) in those with 1-2 fold ALT elevations and almost never in

those with normal ALT levels prior to therapy.

When lamivudine therapy is extended to three years duration, up to 40% of HBeAg positive chronic hepatitis B patients achieve an HBeAg / anti-HBe seroconversion which, if therapy is continued for another 3-6 months after development of anti-HBe, appears to result in a sustained remission (HBV DNA $< 10^5$ copies/ml, ALT normal, inactive histology) in 50-80% of cases. However, in patients who do not achieve HBeAg / anti-HBe seroconversion on lamivudine therapy, HBV virions with mutations resulting in methionine to valine or isoleucine substitutions at position 204 in the HBV DNA polymerase (rtM204V/I) appear and manifest with increases in serum HBV DNA followed by rises in ALT levels. Incidence of resistance mutations increases with duration of therapy and occurs at a rate of 16-32% after 1 year, and 38%, 53%, 66% and 69% after 2, 3, 4, or 5 years of therapy, respectively and at an even higher rate in patients co-infected with HIV with 90% of such co-infected patients developing lamivudine resistance mutations after 4 years of therapy [66-68]. Lamivudine resistant HBV is initially a "less aggressive" of "fit" virus with lower rates of replication but additional "compensatory" mutations (rtL180M, rtV173L) develop [13,66] and over time disease relapse occurs.

Despite the limitations imposed by drug resistance, lamivudine has proven to be a very well tolerated drug, even in patients with decompensated cirrhosis. In patients with advanced fibrosis or cirrhosis, decreased rates of liver disease decompensation and hepatocellular carcinoma have been observed with long term lamivudine therapy [44]. Lamivudine therapy also often has dramatic impacts on the disease course of patients awaiting liver transplant for decompensated chronic hepatitis B induced cirrhosis. Use of lamivudine in such patients has been associated with significant ($\geq$3 points) improvement in Child-Pugh-Turcotte scores in about 60% of patients, and when lamivudine therapy is continued post-liver transplant in conjunction with Hepatitis B Immune Globulin (HBIG) therapy, rates of recurrent disease are significantly decreased [51, 69].

Adefovir, the second nucleos(t)ide analog approved by the FDA for HBV therapy is a "failed" HIV drug, largely because it is a high-affinity substrate for the human renal organic anion transporter 1 (hOAT1). Significant nephrotoxicity occurred at doses of 60-120 mg per day in clinical trials for HIV therapy. Fortunately, however, adefovir only rarely causes nephrotoxicity at doses of 10 mg per day which still achieve significant efficacy against HBV infection [70-73]. Adefovir therapy has exhibited efficacy similar to that of lamivudine in treatment of HBeAg positive chronic hepatitis B [70] and is equally efficacious in treatment of wild type and lamivudine resistant Hepatitis B infection [13, 71]. Mutant HBV viruses with resistance to adefovir are also selected during long term therapy but at a slower rate with only 3% of patients treated for 2 years and 6% of patients treated for 3 years exhibiting appearance of adefovir resistant HBV viruses. These adefovir resistant mutations occur at positions rtN236T or rtA181V [13, 73]. Both lamivudine and adefovir therapy are efficacious for HBeAg chronic hepatitis B [72, 74]. However, nearly all patients with HBeAg negative chronic hepatitis B relapse after suppressive nucleos(t)ide therapy is discontinued [8, 43, 73] and those on chronic lamivudine therapy exhibit high rates of resistance mutations over time [66-68]. In contrast, with prolonged adefovir therapy for HBeAg negative chronic hepatitis B, ever increasing numbers of patients achieve biochemical remissions with normal ALT in about ~70% of patients after 2 or 3 years of therapy; virologic remissions with HBV DNA $< 10^3$ copies/ml in 79% of patients after 3 years of therapy; and associated histologic improvement [73]. Thus, in addition to being used widely as a second line drug in lamivudine resistant HBeAg positive or HBeAg negative chronic hepatitis

B, adefovir has been increasingly viewed as the preferred therapy for HBeAg negative chronic hepatitis B [42].

Table 2
Comparison of FDA Approved Treatments for Chronic Hepatitis B

| | PEG-IFN-α | Lamivudine | Adefovir | Entecavir |
|---|---|---|---|---|
| Duration of Rx<br>HBeAg+ Chr Hep<br>HBeAg- Chr Hep | <br>6-12 months<br>1 year | <br>≥ 1 year<br>> 1 year | <br>≥ 1 year<br>≥ 3 years | <br>≥ 1 year<br>> 1 year |
| Route | SubQ | Oral | Oral | Oral |
| Side Effects | Multiple | Negligible | Rare Renal | Negligible |
| Drug Resistance | Not Known | 1 yr ~ 20%<br>5 yr ~ 70% | 2 yr ~ 3%<br>3 yr ~ 6% | 1 yr ~0% naive,<br>7% Lam Resist. |
| Normalization of ALT* | 39% | 41-72% | 48% | 68% |
| HBeAg Seroconversion | 32% | 16-19% | 12% | 21% |
| Loss of HBsAg | 3-8% | <1% | 0% | < 1% |
| Cost per Month** | $1300 | $205 | $546 | $710 |

*At the end of 48 weeks of therapy for HBsAg positive, chronic hepatitis B.
**Thompson Healthcare, Average Wholesale Price.

Within the past 6 months, the FDA has approved use of entecavir, 0.5 mg or 1 mg per day for treatment of chronic hepatitis B (www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4049B1_02_FDA-Background-Memo.pdf), and published results of two clinical trials have demonstrated efficacy of the FDA approved drugs HIV drugs emtricitabine [55], 200 mg per day and tenofovir [56], 300 mg per day for treatment of chronic hepatitis B. Entecavir has exhibited higher efficacy than lamivudine in treatment of HBeAg positive chronic hepatitis B [43] and tenofovir has been found to have higher efficacy than adefovir in treatment of lamivudine resistant chronic hepatitis B [56]. Both entecavir and tenofovir have *in vitro* efficacy in treatment of HBV viral constructs containing both lamivudine and adefovir resistance mutations [75] and entecavir at a higher dose of 1 mg per day is effective *in vivo* in treating lamivudine resistant HBV (www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4049B1_02_FDA-Background-Memo.pdf). Thus, as summarized in table 2, there are now multiple nucleos(t)ide analogs available for therapy of HBV infected patients with elevated aminotransferase levels or histologic evidence of moderate or severe inflammation or advanced fibrosis.

None of the drugs currently available for therapy of chronic hepatitis B have demonstrated efficacy in altering the course of infection in "immunotolerant" patients with high HBV DNA levels but normal aminotransferases and histologically mild disease. Similarly, such therapies are not known to accelerate the course of HBsAg clearance in inactive carriers who are already HBeAg negative, anti-HBe positive and usually have HBV DNA levels < $10^4$ copies per ml. All practice

guidelines recommend against attempting long term antiviral therapy in such patients [7,8,32,42] as there is concern that little will be accomplished other than selection of drug resistant HBV mutants. However, there are now two circumstances in which use of lamivudine prophylaxis may be of benefit to such patients or their children.

In HBV endemic regions of Asia, 10-22% of patients with lymphomas or other malignancies are HBsAg positive [30, 50]. When such patients are treated with chemotherapy, 20-25% exhibit clinically apparent hepatitis B reactivation [30, 50] which is associated with development of jaundice in about 50% of cases and evolves into fatal acute liver failure in about 25% of cases [30]. Multiple published studies have now reported a > 80% reduction in HBV reactivation and associated liver disease morbidity in patients placed on lamivudine, 100 mg per day immediately prior to chemotherapy and continued on this antiviral agent until 8-26 weeks after completion of chemotherapy [31,47-50]. Preemptive therapy has been found to be superior to therapy started after HBV reactivation [46, 76]. Practice guidelines now recommend that all patients, or at least all patients "at risk" for HBV infection (see Table 1), be screened for HBsAg prior to chemotherapy or immunosuppressive therapy and, if found to be positive, suggest that such patients should be placed on lamivudine, 100 mg per day before the start of chemotherapy or immunosuppressive therapy and continued on lamivudine for at least 6 weeks and up to 6 months after completion of such therapy [8, 32].

For more than 15 years, it has been the standard of care to administer HBIG and HBV vaccination to neonates born to HBsAg positive mothers. This therapy has been associated with a significant reduction in vertical transmission rates. However, this therapy fails to prevent transmission in 10-30% of cases where the mother is HBeAg positive [77, 78] with the highest risk in those cases where maternal HBV DNA levels are > 3 x $10^8$ copies per ml [77]. In women with chronic hepatitis B who are on lamivudine therapy prior to pregnancy, rates of mother to child transmission have been found to be much lower in cases where the mother continued lamivudine therapy throughout pregnancy than in cases where the mother discontinued lamivudine prior to the end of pregnancy [52]. Several small published studies [52, 78, 79] have now suggested that in pregnancies where the maternal HBV DNA level is extremely high (> $10^8$ - $10^9$ copies per ml), administration of prophylactic lamivudine during the last 4-6 weeks of pregnancy is associated with a 50% or greater reduction in neonatal infection [78-80]. The results of a large ongoing, multicenter trial of lamivudine prophylaxis have been presented in abstract form and suggest that, in pregnant women with high HBV DNA levels (> 1000 Meg/ml by the Chiron assay), administration of lamivudine, 100 mg per day from week 32 of pregnancy until 4 weeks post-partum reduces the rate of vertical transmission from 39% to 18% [81]. Lamivudine has been used previously in women with HIV infection and is classified by the FDA as pregnancy category C. The safety and toxicity data listed by the U.S. Department of Health and Human Services indicates rates of birth defects in children born to U.S. woman receiving lamivudine during the pregnancy (2.8%) appear no higher than in the general U.S. population (3.1%) in studies with sufficient patient numbers to detect at least a two-fold increase in birth defects (www.aidsinfo.nih.gov/guidelines/default_db2.asp?id=51). The Asian-Pacific consensus statement on the management of hepatitis B recommends that women who become pregnant while on therapy for hepatitis B may continue the therapy during the pregnancy but make no firm recommendations regarding use of lamivudine or other nucleos(t)ide analogs to prevent HBV transmission [32].

Table 3
Chronic Hepatitis B: Treatment Indications

| Diagnosis | ALT | HBV DNA* | Histology | Therapy |
|---|---|---|---|---|
| Inactive HBsAg Carrier, HBeAg-, anti-HBe+ | nl | $< 10^4$ | No hepatitis | None except prophylaxis during immunosuppression |
| Chronic HBV Infection HBeAg+, anti-HBe- | nl | $> 10^5$ | No hepatitis | None except prophylaxis, Observe, reconsider when ↑ ALT |
| Chronic Hepatitis B HBeAg+, anti-HBe- | ↑2-fold | $> 10^5$ | Mod / Severe Chr Hepatitis | IFN or nucleos(t)ide |
| Chronic Hepatitis B HBeAg-, anti-HBe+ | ↑2-fold | $> 10^4$ | Mod / Severe Chr Hepatitis | IFN or nucleos(t)ide |
| Cirrhosis HBeAg+/-, anti-HBe+/- | nl or ↑ | $> 10^4$ | Cirrhosis | Nucleos(t)ide or transplant |
| Cirrhosis HBeAg+/-, anti-HBe+/- | nl | $< 10^4$ | Cirrhosis | Transplant when decompensated |

*Copies per ml, arbitrary values, consensus of references 7,8,32 and 42.

In summary, the recent development of a "wealth" of therapies for chronic hepatitis B has provided physicians with many opportunities to intervene in a disease ranked as the 10th leading cause of mortality worldwide. Listed in Table 3 above are a summary of consensus guidelines regarding uses of currently available therapies for chronic hepatitis B induced liver diseases [7,8,32,42]. Proper use of these therapies will require appropriate screening of high risk individuals as detailed in Table 1. The duration of therapy and the role of combined therapy have yet to be fully defined.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