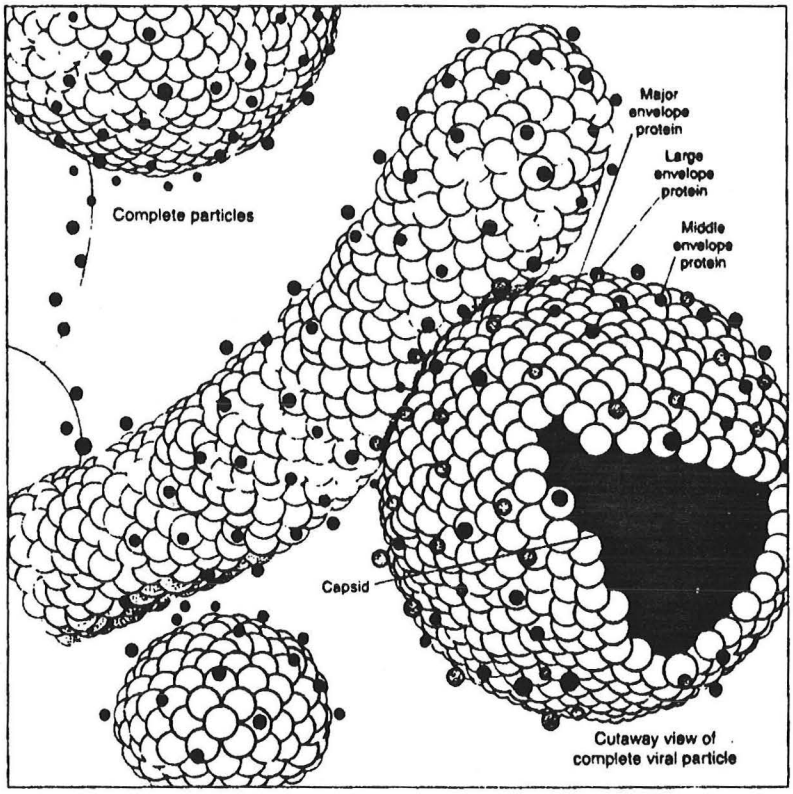
Hepatitis B: New Insights, New Therapies



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Introduction

The hepatitis B virus (HBV) was first discovered by Dr. Baruch Blumberg in 1965, only a generation ago. More than 2 billion people alive today have been exposed to this ubiquitous virus, and more than 350 million persons are currently infected (1). Worldwide, hepatitis B is a leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma, accounting for a million deaths annually. Knowledge of the intricacies of viral infection and new understanding of the molecular biology of this fascinating virus have lead to the development of a successful vaccine, and to treatments which now hold the promise of eradicating chronic infection. The present review will provide an overview of all aspects of HBV infection emphasizing the key role played by the immune system in determining outcome of clinical infection, recent developments in HBV molecular virology and treatment options.

Historical background

Like many great discoveries, serendipity played an important role in the first identification of the Australia antigen which we now know as hepatitis B surface antigen (HBsAg). In the mid-1960's, Dr. Blumberg was searching for genetically heterogeneous serum proteins when he placed in adjoining Ouchterlony wells the serum of an Australian aboriginal person and that from a New York hemophiliac. The precipitin line which formed was between the HBsAg in the Australian and the anti-HBs antibody in the multiply-transfused (and previously infected) hemophilia patient. This emphasizes one unique feature of this virus, that is, the presence of very high titers of virus during acute and chronic infections, and an even greater quantity of envelope protein (HBsAg). A lab accident linked the Australia antigen to what was then known as serum hepatitis when a laboratory worker in Dr. Blumberg's laboratory developed acute hepatitis (2). For these achievements, Dr. Blumberg was awarded the Nobel prize. Australia antigen rapidly became identified with the vector of serum hepatitis, and the development of testing for this envelope protein led to a great diminution in the incidence of post-transfusion hepatitis from approximately 30% prior to 1968 to 10% following 1970 when blood bank HBsAg screening was introduced. The remaining cases of post-transfusion hepatitis became known as non-A, non-B hepatitis and were largely cases of hepatitis C.

Milestones in hepatitis B research

Blumberg: Australia antigen 1965
Dane: Virus particles in serum 1970
Almeida: Core antigen 1971
Magnius: 'e' antigen 1975
Rizzetto: delta agent 1977
Summers: Woodchuck/duck viruses 1979
Szmuness: Vaccine 1980

In rapid succession, further discoveries from around the world widened our understanding of the complexity of this unusual virus: in 1971, the core antigen (HBcAg) was identified by Almeida at the North London Blood Center; in 1975, the hepatitis B e antigen (HBeAg) by Magnius and Espmark in Sweden; and in 1977 the delta antigen and delta virus by Rizzetto in Italy. By 1980, a vaccine prepared from partially purified serum of carrier patients was tested successfully and soon became commercially available; by the mid-1980's cell culture and animal model systems were in widespread use and the first patients had been treated with alpha interferons. In the last two years, trials of new nucleoside analogue agents have shown initial promise even as world-wide vaccine programs swing into high gear. Although a significant decrease in the worldwide incidence and prevalence of infection has yet to be made, it seems likely that the next generation will see a tremendous decrease in both the worldwide carrier rate and in the incidence of new hepatitis B infections.

Epidemiology

The global distribution of hepatitis B infection varies greatly. In areas of high prevalence, more than half the population is infected at some time or other and >8% become carriers; these areas include Southeast Asia, China and Africa. Infection in these regions is largely via neonatal (vertical) transmission or from one child to another (horizontal transmission). Areas of low endemicity include North America, Western Europe and Australia, and in these regions only a minority of inhabitants come into contact with the virus, most infections occurring in young adults due to sexual transmission or lifestyle or occupational exposure (3). It is estimated by the World Health Organization that the number of hepatitis B carriers will reach 400 million by the year 2000. As will become clear below, the vastly different outcomes of HBV infection are determined by the immune state of the host at the time of infection. The high rates of carriage in China (8-18%), for example, reflect principally transmission to the immunologically immature neonate. This situation is and will be self-perpetuating until neonatal vaccination and immunization becomes universally accepted.

Table 1. Worldwide distribution of hepatitis B infection.

	Endemicity		
	Low	Medium	High
Prevalence			
Chronic infection	<2%	2-7%	8-15%
Total infection	<20%	20-60%	>60%
Distribution	North America	Eastern Europe	Southeast Asia
	Western Europe	Southern Europe	China
	Australia	Soviet Union	Phillipines
	New Zealand	Central Asia	Indonesia
	South America (northern)	Japan	Middle East
		Israel	Africa
		South America (southern)	Amazon Basin
			Pacific Islands

In the United States, chronic HBV infection afflicts 1.25 million people. Carrier rates vary among ethnic groups, with Asian-Americans and African-Americans having slightly higher rates than the white population. Most infections in the United States result from sexual activity, injection drug use or occupational exposure in young adults. Non-sexual household contact, dialysis and blood product recipients make up much smaller groups. No identified route of exposure can be discerned in 20-30% of individuals, but this may reflect reluctance to admit to high risk behavior, or be the result of permucosal or other methods of spread. Because the virus is present in large quantities (10^8-10^{10} viral particles per ml) it is not surprising that HBV can also be detected in semen, saliva, cervical secretions and leukocytes. Respiratory, water-borne or insect-related infection has not been documented.

Of the 22,000 infants born each year to HBsAg positive mothers in the U.S., all would be at risk were it not for immunoprophylaxis of newborns. However, more than 98% of newborns receive this immunoprophylaxis in the United States, due to prenatal screening, and are protected from infection (4).

Virology

Hepatitis B virus (HBV) belongs to a family of closely related DNA viruses called the hepadnaviruses. Included in this family are the woodchuck hepatitis virus (WHV), the ground squirrel virus (GSHV) and the duck hepatitis B virus (DHBV). Several other animals also appear to have similar hepadnaviruses (5), including geese, tree squirrels, chipmunks and possibly others. All hepadnaviruses are small 42 nm particles which have similar hepatotropism and life cycles in the host. Chronic hepatitis and hepatocellular carcinoma, for example, are commonly observed in the woodchuck and less frequently in the ground squirrel and duck. It is curious in evolutionary terms that these viruses are found both in mammalian and avian species, with both groups sharing striking DNA homology. HBV does not appear to be infectious for these animals; it is not clear (but is assumed) that the reverse is also true. HBV is infectious for chimps and less uniformly for other higher primates as well.

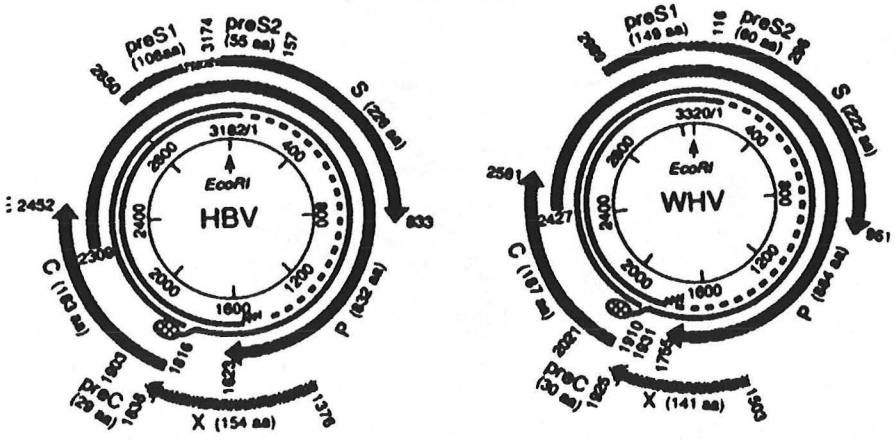


Figure 1. Diagram of HBV and WHV genomes compared.

The viral genome of HBV is a partially double-stranded circular DNA of 3200 base pairs which encodes four overlapping open reading frames: S, C, X and P, standing for the surface or envelope gene, the core gene, the X gene and the polymerase gene. Two of these have upstream regions termed pre-S and pre-C (6).

The whole virion or Dane particle is spherical, and contains a core or nucleocapsid structure enclosing the DNA. One peculiar feature of HBV is the great excess of envelope material found in the circulation, both small spheres and tubular particles with an average width of 22 nm.

Envelope, HBsAg

The Australia antigen, now known as HBsAg, is the envelope protein which is encoded by the S gene. There are several determinants on the envelope, including an 'a' determinant which is common to all HBsAg's but also d,y,w and r determinants which are subtype-specific and of epidemiologic importance. Three proteins, termed the large, middle and major proteins are produced by beginning transcription with, respectively, pre-S1, pre-S2 or the S gene alone. Pre-S1 and 2 represent some of the more immunogenic portions of HBsAg. Development of immunity to HBsAg, both cellular and humoral is protective and provides the basis for the HBV vaccines currently available.

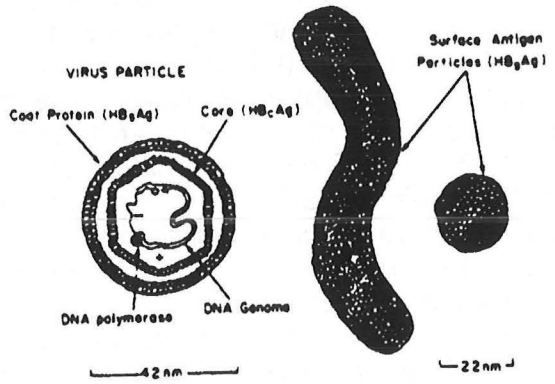


Figure 2. Schematic diagram of the hepatitis B virus.

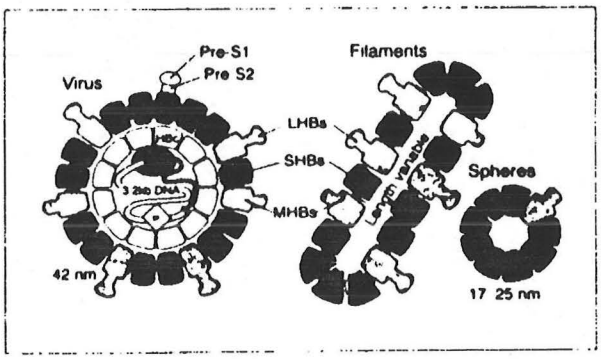


Figure 3. Close-up view displaying the various HBsAg envelope proteins.

Core, nucleocapsid, HBcAg

Using non-ionic detergents to remove the lipid-rich envelope, the core antigen (HBcAg) was first identified and is the structural basis of the nucleocapsid which encloses the viral DNA. 180 repeating subunits make up the nucleocapsid structure. The importance of the core is that it is expressed on the surface of hepatocytes, and appears to induce a cellular immune response which is crucial to cell killing. HBeAg is a soluble circulating antigen derived from HBcAg by proteolytic cleavage during active replication, and is important in immune tolerance and in modulating the immune response to the virus.

Polymerase

The longest gene encodes the DNA polymerase which also serves a reverse transcriptase function, since replication requires RNA intermediate forms.

X proteins

The X gene encodes two proteins which serve as transcriptional transactivators, aiding viral replication. These proteins may also play a role in the development of hepatocellular carcinoma. Several additional enhancer and promoter elements have also been recognized within the HBV genome.

Delta hepatitis (hepatitis D)

Rizzetto's discovery in 1977 of a passenger virus associated with hepatitis B infection added a remarkable further complexity to the story of HBV. Delta hepatitis is caused by hepatitis D virus (HDV), a defective RNA-containing passenger virus requiring helper functions provided by HBV which assembles and coats the virus with an HBsAg envelope. To some extent, HDV interferes with HBV replication and vice-versa. HDV cannot survive without HBV being present. Like HBcAg, HDV antigen (HDAg) is the nucleocapsid protein and forms multimers which encompass the viral RNA, but are then encapsidated with HBsAg of the host HBV virus subtype. HDV resembles certain plant viruses; there are no other known passenger viruses in the animal kingdom.

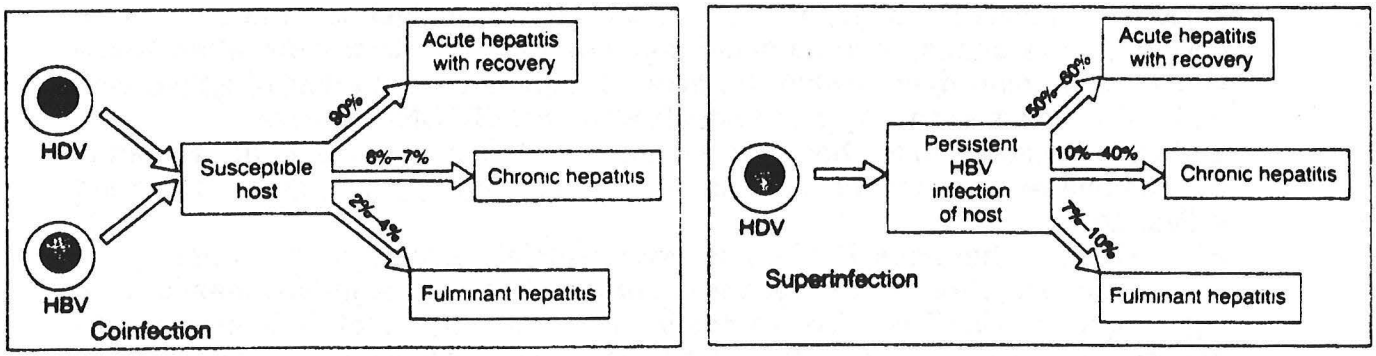


Figure 4. Sequelae of hepatitis D infection. Superinfection is generally more severe in terms of outcome than co-infection (7).

Delta infection occurs as simultaneous co-infection with hepatitis B (acute HBV and HDV), in which case the infection is usually self-limited by the eventual

eradication of hepatitis B virus. More long lasting and often severe is HDV super-infection which occurs in hepatitis B chronic carriers. The usual source of infection is intravenous drug use. There are only a few delta cases identified annually in Dallas. Delta infection is an important consideration when a patient with known chronic hepatitis B shows a worsening trend or when HBeAg is negative, but active liver disease persists. This must be distinguished from infection with HBV mutants, which can also cause more aggressive or fulminant disease.

Terminology

Here is an overview of important terms not already defined which will be used throughout this discussion:

anti-HBs antibody to the hepatitis B surface antigen, produced in response to exposure to HBsAg and confers protective immunity on the host. It is found in patients who have recovered from acute hepatitis B, and in those immunized with the hepatitis B vaccine. It tends to disappear after many years and may occasionally be undetectable in patients who have recovered fully.
anti-HBc antibody to the hepatitis B core antigen. This antibody is detected in virtually all patients who have ever been exposed to the hepatitis B virus, but unlike anti-HBs, is not protective. When it is listed as above or as 'total' anti-HBc or as IgG anti-HBc it cannot be used to distinguish acute from chronic infection. Those who carry the hepatitis B virus are anti-HBc positive, as are those who have recovered from hepatitis B.
anti-HBc IgM the IgM antibody, associated with acute infection, is helpful in distinguishing those with acute hepatitis B from chronic virus carriers. IgM antibody usually disappears within four to eight months following acute infection. Anti-HBc IgM is not absolutely reliable as a distinguishing test for acute infection, since many chronic patients in the midst of a flare of their disease will become anti-HBc IgM positive albeit with titers generally lower than those seen in acute hepatitis B.
HBeAg hepatitis B e antigen. This soluble peptide antigen is formed from pre-core and core genes, then modified and exported from liver cells when whole viruses are being made within the liver. It serves as a marker of active viral replication and is seen only in individuals who are HBV DNA positive.
anti-HBe antibody to the hepatitis B e antigen. This antibody appears as part of the immune response to the virus once replication of virus is no longer occurring in liver cells.
HBV DNA hepatitis B virus deoxyribonucleic acid, synonymous in this discussion with virus being present in serum. The gold standard measure for active viral replication. These tests vary from slot blot hybridization to polymerase chain reaction, with the latter being considerably more sensitive.
anti-HDV, anti-delta antibody serum antibody to delta is available to diagnose the presence of HDV infection. HDAg can be identified in hepatocyte nuclei on biopsy but is not measured in serum.

Life cycle of hepatitis B virus in the human host

The secret to understanding viral pathogenesis in HBV is to understand that the virus is rarely cytopathic and that virtually all of the hepatocyte injury observed is the direct result of immune-mediated damage to infected hepatocytes. An intact immune system is vital to viral clearance, although recent evidence suggests that there is also some variability in the virulence of HBV variant viruses. Nevertheless, for practical purposes, the severity of the hepatocyte injury reflects the quality of the immune response, so that those with the best immune system have the greatest likelihood of viral clearance, and the most severe liver injury. By these rules, >95% of neonates born to mothers with active viral replication (if they do not receive immunoprophylaxis) will become chronically infected, will have no immune response, normal serum aminotransferase levels and will remain tolerant to the virus for many years, finally losing tolerance in early adulthood. Likewise, about 30% of children infected beyond the neonatal period but less than six years of age will become carriers, whereas only about 3-5% of adults will remain infected six months after initial exposure, and therefore become chronically infected.

It is useful to consider the life cycle of the virus in four stages. The first stage is that of immune tolerance, and this period lasts only until the immune system responds (if indeed an immune response is forthcoming). In the healthy adult, this period lasts a maximum of three to four weeks. In the immune tolerant neonate, this period lasts for years, accounting for the large number of so-called healthy carriers worldwide. During this period, HBsAg will be positive, as well as HBeAg and HBV DNA will be present in high titer. Anti-HBc will be positive, initially both IgM and then IgG and will remain positive indefinitely. Lacking an immune response, there will be little or no aminotransferase elevations, and the patient will typically have no symptoms.

Figure 5. Four stages of hepatitis B infection.

Replicative phase		Integrative phase	
Stage 1 immune tolerant	Stage 2 active hepatitis	Stage 3 HBsAg carrier	Stage 4 immune
HBsAg+	HBsAg+	HBsAg+	HBsAg -
Anti-HBs -	Anti-HBs -	Anti-HBs -	Anti-HBs+
HBVDNA +++	HBVDNA+	HBVDNA - (pcr+)	HBVDNA -
Anti-HBc+	Anti-HBc+	Anti-HBc+	Anti-HBc+
HBeAg+	HBeAg+	HBeAg -	HBeAg -
Anti-HBe -	Anti-HBe -	Anti-HBe+	Anti-HBe+
ALT normal	ALT elevated	ALT normal	ALT normal

With time, either during the normal evolution of acute infection or over years in the chronically infected individual, immune tolerance is lost and the immune response begins constituting the second stage. With this, there is an attack by cytotoxic T cell responses against HBc epitopes on the hepatocyte surface resulting in direct cell lysis and cytokine responses which augment the inflammatory process. Anti-HBc may also augment this attack. Secretion of

HBeAg still occurs but HBV DNA titers drop as the number of infected cells declines. There is recent evidence suggesting that HBeAg in addition to HBcAg may be the target of immune attack since it also can be displayed on the hepatocyte surface. Regardless, HBeAg in the circulation serves to modulate the attack on HBcAg on the hepatocyte surface since they share common epitopes. HBeAg may be important in tolerizing individuals and in the development of the chronic carrier state. This occurs because small antigenic peptides rather than whole proteins are responsible for the HLA mediated T-cell recognition process. CD4 as well as CD8-positive cells are demonstrable in the liver biopsies of patients with chronic hepatitis B and thus are responsible for the broad cytokine responses observed. If the response is carried to completion, then all infected cells are destroyed, and replication is aborted. If the response is inadequate, then chronic infection continues. Stage 2 in the patient with acute hepatitis lasts perhaps three to four weeks, while in the patient who will go on to chronic disease it may last for ten or more years and lead to cirrhosis and its complications.

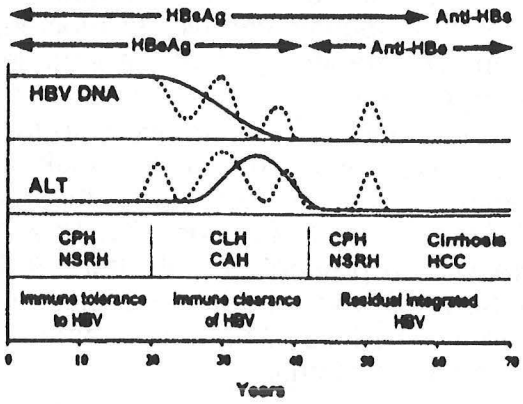


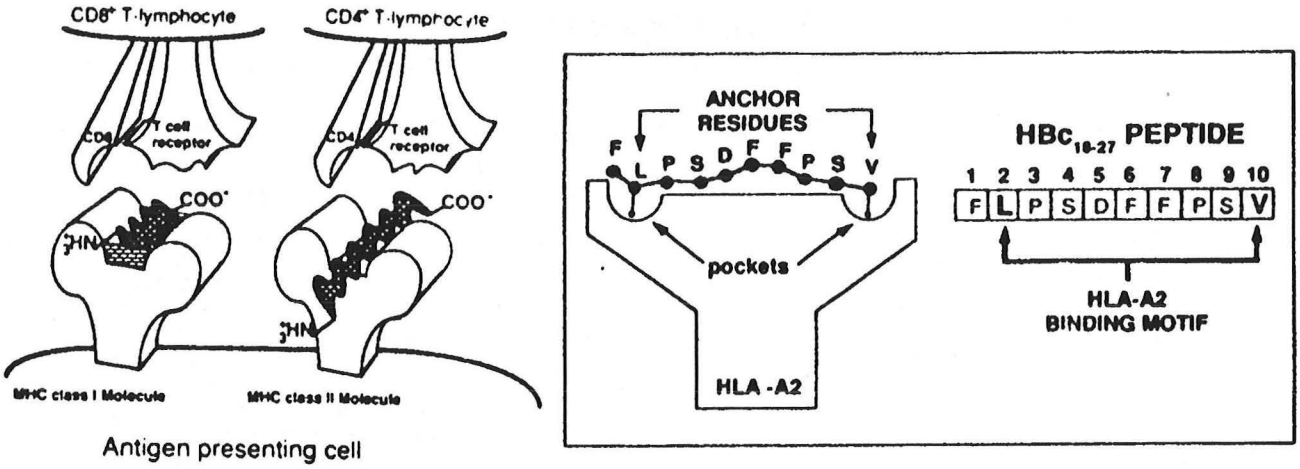
Figure 6. The same process described in Figure 5 is shown here graphically (8).

With the cessation of active viral replication, a third stage is initiated in which the patient has cleared HBeAg and seroconverted to being anti-HBe positive concomitant with the disappearance of HBV DNA from the circulation. Despite obvious diminutions in the titer of viral DNA, many of these patients remain HBV DNA positive but only when very sensitive PCR assays are used. Nevertheless, for practical purposes they no longer replicate virus, have 'cleared' their infection, and aminotransferase levels become normal. Surprisingly, these patients generally remain HBsAg positive, having integrated portions of the S gene into the host hepatocyte genome, where it often remains for years. Most patients eventually clear even the HBsAg producing cells and become HBsAg negative and anti-HBs positive, the fourth stage in the life cycle. At this point, the patient is immune, and is unlikely to become reinfected except under special circumstances. It is not clear whether significant integration of the S gene occurs in acutely infected patients, but it seems unlikely since there is little evidence of an HBsAg positive, HBV DNA negative third stage in acute infections.

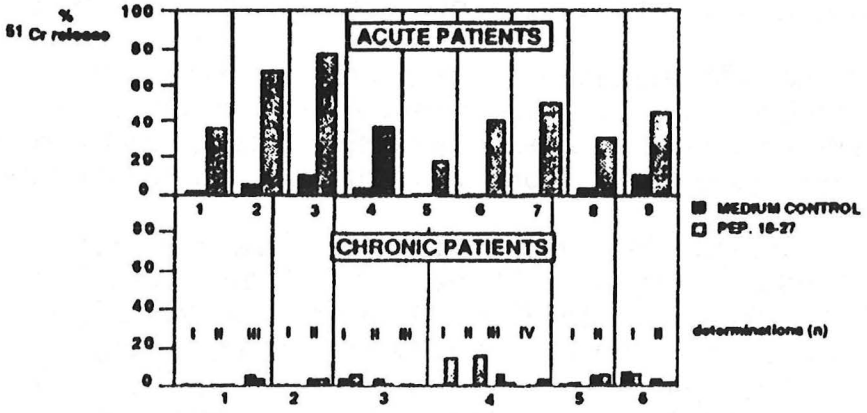
Immunopathogenesis

The immune attack directed against HBV is the cause of the liver injury, and this is mediated by a cellular response to viral antigens, specifically epitopes

of HBcAg and HBeAg on the hepatocyte surface. HLA class II restricted CD4+ cells recognize peptide fragments which are presented in the antigen groove of the MHC class II molecules on the hepatocyte. These core-related peptides are derived from extracellular antigens and are processed in the endosomes. Viral peptides are identified by the T cell receptor on the CD4+ cell, leading to stimulation of B- cell proliferation and cytokine formation. HLA class I restricted CD8+ cells recognize peptide fragments which result from intracellular HBcAg processing and presentation on the hepatocyte surface by the class I molecule. This leads to cell killing by the CD8+ cytotoxic lymphocyte (9-12).



Antigen presenting cell



CTL recognition of HBcAg peptide 18-27 in HLA-A2 positive patients with acute self-limited and chronic hepatitis B (7, 24).

Figure 7. Relationship between binding of HBcAg peptides by MHC molecules and human T cell responses in acute and chronic hepatitis B.

Recent evidence suggests that polymorphism of the MHC complex results in differences in binding affinity for the immunodominant HBcAg peptides and that this determines outcome following acute HBV infection. (13-15). Only a limited array of peptide residues in the HBcAg can gain access to the binding groove. The variable immune responses between patients who clear virus successfully or are unable to mount a sufficient immune attack (lacking any evidence of immune deficiency), depend on the match of the peptides with the specific receptor grooves. As a result, patients with chronic hepatitis B have evidence of inadequate class I and class II mediated responses when compared with those with successful clearance of the virus.

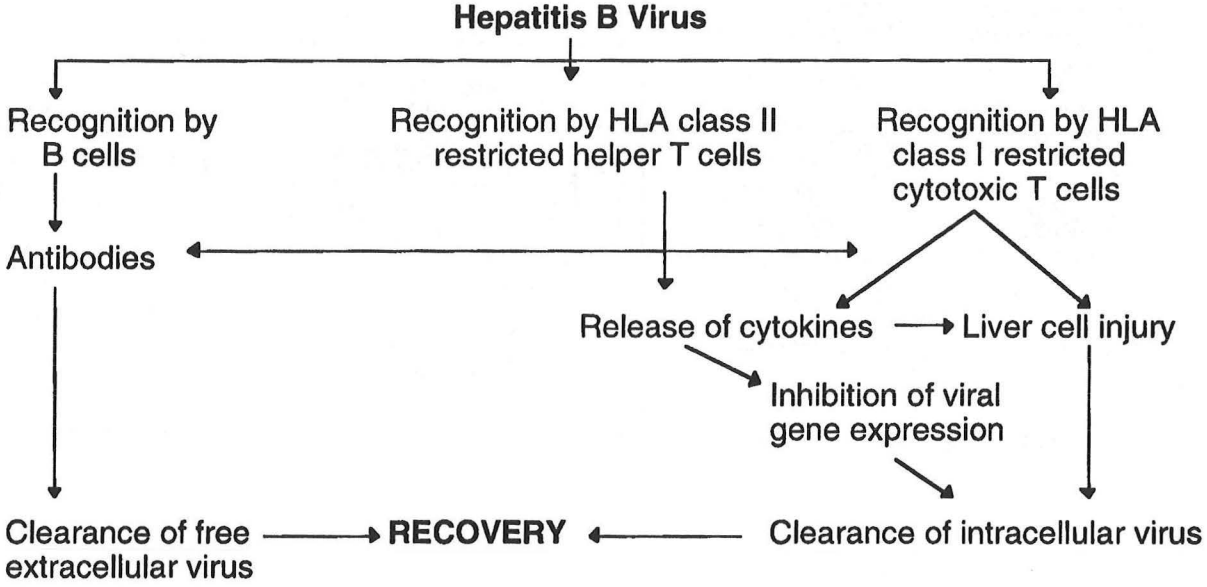


Figure 8. Proposed overall schema of the immune attack on HBV (16).

Factors affecting the evolution through the four stages described include gender, the impact of various immunosuppressive agents, other viruses such as HIV co-infection, and the appearance of HBV mutants. All these factors impact on outcome to yield a number of special situations as outlined below:

Fulminant hepatitis B: Less than 1% of those with acute hepatitis B will develop acute liver failure with coagulopathy, encephalopathy and cerebral edema. For the most part, these individuals appear to have a heightened immune response to the virus if HDV co-infection or super-infection is not present. In these patients, early clearance of HBsAg may lead to the mistaken notion that this is not acute hepatitis B. This misapprehension can be corrected by obtaining the anti-HBc IgM test which will be positive, but a high index of suspicion is necessary not to miss the obvious. Rapid clearance overall is salutary since, if these patients require transplantation as they sometimes do, they seldom infect the allograft (17).

Hepatitis B after liver transplant: Virtually all chronic hepatitis B patients requiring liver transplantation will be susceptible to reinfection of the hepatic

graft. Some hepatitis B patients under intense immunosuppression develop progressive hepatic failure due to a peculiar form of fibrosing cholestatic hepatitis almost unique to liver grafts, but also seen occasionally in the livers of bone marrow transplant or renal transplant patients (18). These patients have very high viral titers in serum and in the liver and the hepatitis is thought to be due directly to a cytopathic effect of the huge quantities of HBV particles present in the cells. These bad outcomes have precluded transplantation of HBV-infected individuals in some centers. Treatment to prevent this outcome has consisted of the use of high titer hepatitis B immune globulin (HBIG), but this must be continued almost indefinitely if an effect is to be achieved, and HBIG treatment may cost $20,000 per year. Nucleoside analogues could provide a solution to this vexing problem (see below).

Hepatitis C co-infection: Many intravenous drug users will have detectable anti-HBc and anti-HCV as well, as evidence of exposure to both parenteral hepatotropic viruses. A smaller fraction will persist in having active infection with both viruses and will therefore be HBsAg positive, HBV DNA positive, HBeAg positive as well as having detectable anti-HCV. These individuals tend to have very active liver disease, and interferon may be effective in this setting. It is unclear at this point whether each virus inhibits the replication of the other as occurs in delta virus infection.

Occult HBV infection: Rarely, patients have been identified who have mutations in the S gene sufficient enough to prevent production of detectable amounts of HBsAg despite detectable viral DNA levels. These are extremely rare as are 'vaccine escape' mutants, those who can acquire HBV infection despite being vaccinated (19,20).

HBV/HIV co-infection: Many patients have been identified with combined HIV and HBV infection. In general, HBV does not alter the outcome of HIV infection, nor does HIV enhance the evolution of HBV infection despite the obvious effect on the immune system (21).

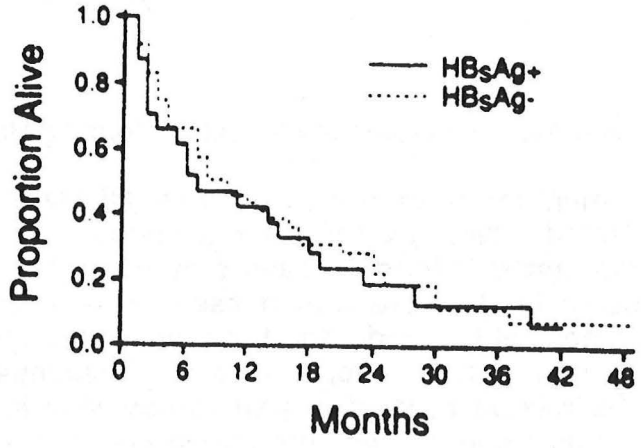


Figure 9. Survival curves for HIV patients with and without HBV co-infection (21).

Treatment of HBV in HIV positive patients will be governed by consideration of a limited life expectancy in many HIV positive individuals and the possible adverse effect of interferon therapy on CD4 counts. However, nucleoside analogues which are inhibit both viruses would solve this two-virus dilemma (see below).

Mutant HBV viruses: A small but important patient group is comprised by patients who are exceptions to the rule that clearance of HBeAg is associated with clearance of HBV DNA. The appearance of a viral mutant which is unable to produce HBeAg for export leads to seroconversion to anti-HBe without clearance of HBV DNA. HBeAg negative mutants have been associated with fulminant hepatitis B, more severe chronic hepatitis B and more rapid graft loss following transplantation (22-24). The core gene which encodes HBeAg contains two start codons, identifying the pre-core and the core regions. The most common mutation is a single nucleotide conversion in position 1896 (G to A), resulting in a stop codon (TGG to TAG) at the end of the pre-core region, thus preventing the synthesis of HBeAg, while HBcAg synthesis remains intact. Since HBeAg is an immune tolerogen, lack of circulating HBeAg may contribute to the more aggressive disease observed in these patients (25-31). Other core-related mutations yielding the same result have been observed.

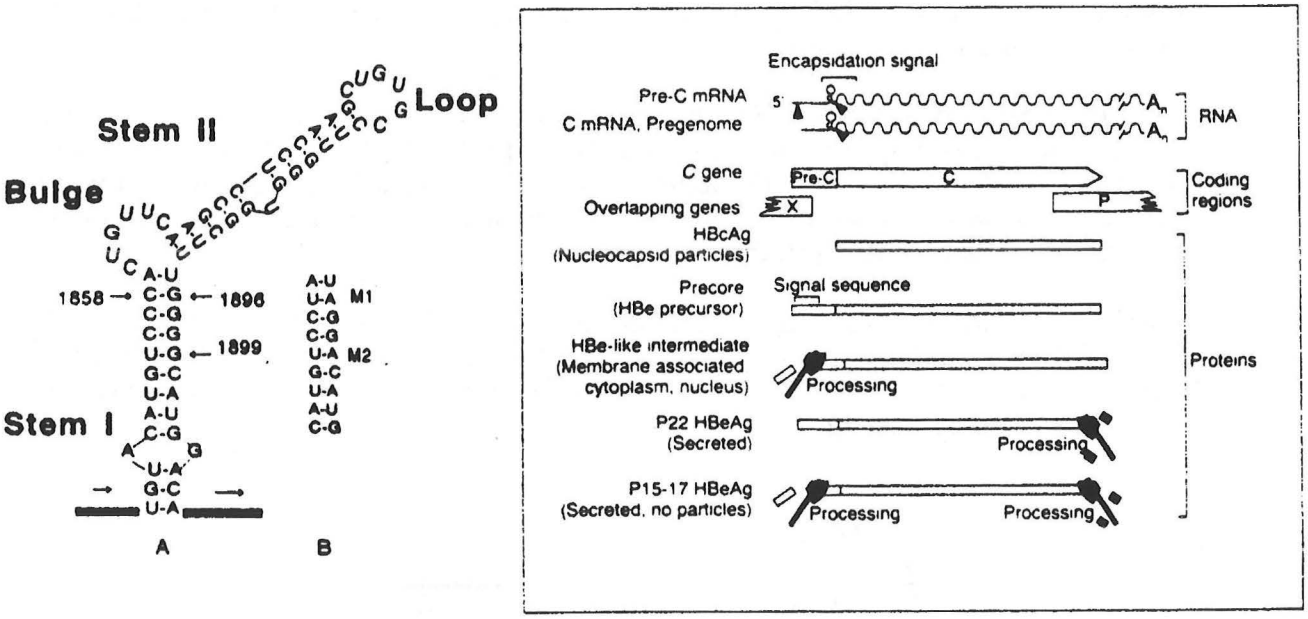


Figure 10. Common pre-core region mutation sites affecting HBeAg translation.

The development of an immune response to HBcAg or HBeAg will result in clearance of infected cells displaying these antigens, while mutant-containing cells, no longer displaying HBeAg at least, may escape immune recognition. Mutations also occur in the core region itself, with most occurring in the nucleocapsid portions which code for the peptides important in immune recognition, rather than in inter-epitope regions. Response to interferon has been thought to be related at least in part to having a low number of core mutants, whereas mutants in the pre-core region are associated with failure to clear regardless of interferon treatment.

Much remains to be understood about mutants (32). Are they the result of immune pressure, resulting from the seroconversion process as an escape phenomenon, or do they appear *de novo* and become more dominant with time because they are more virulent than the wild type virus? These questions remain to be resolved, but bring up an interesting issue. Is hepatitis B, like hepatitis C, a *quasispecies* virus, in which viral diversity resulting from errors in transcription plays a role in the strategy to escape detection?

Treatment of hepatitis B infection

Since 95% of adult infections resolve on their own, there has been little done to enhance these figures. It was learned early on however, that steroid treatment of severe acute hepatitis B patients frequently resulted in a chronic carrier state. In the early 1980's, the purine nucleosides ara-A and ara-AMP were studied as potent DNA polymerase inhibitors, but their neuromuscular toxicity (peripheral neuropathy, myopathy) and limited efficacy led to their being dropped as ineffective and risky agents. Trials using recombinant alpha interferons began in the mid-1980's and led to the FDA approval of interferon alfa 2b in 1992 as therapy for chronic hepatitis B. More recently, nucleoside analogues have been tested both as primary therapy, and as adjunctive therapy for use in combination with interferons.

What is the goal of hepatitis treatment? The hepatitis B carrier state has been defined as the presence in serum of HBsAg for more than six months. However, as the outline of the life cycle above implies, there is no need to treat patients in whom HBeAg and HBV DNA has cleared, since, even though HBsAg remains, they have inactive disease biochemically and histologically. Chronic hepatitis B then is defined as the presence of HBV DNA positivity in combination with active inflammation on biopsy and, usually, elevated aminotransferase levels. This condition leads to cirrhosis in >50% at five years. The purpose of therapy is to hasten conversion from stage 2 to stage 3: to clear essentially all hepatocytes involved in active viral replication (33). Spontaneous seroconversion occurs at a rate of approximately 5% per year, which suggests that all studies demonstrating efficacy must consider the background rate of seroconversion, and the important role of patient selection in determining outcome. Early trials included untreated control groups for this reason.

Interferon therapy

Lymphoblastoid or leukocyte-derived interferons initially used as early as 1976 (34), have now been replaced by recombinant proteins (35,36), representing naturally-occurring cytokines produced in response to viral infections. Interferons have both immunomodulatory and direct anti-viral effects, and the most thoroughly tested are alpha-interferons. In patients with chronic hepatitis B, interferon responses to stimulation of monocytes can be shown to be below normal when compared to patients with acute hepatitis who are clearing infection or to normal individuals. Interferons inhibit viral protein synthesis by induction of 2',5'-oligoadenylate synthetase, protein kinase and 2'-phosphodiesterase. Their immunomodulatory effects include both immunostimulatory and immunosuppressive ones. An important effect appears to be the induction of HLA class I antigens on the hepatocyte membrane which

promotes lysis of infected hepatocytes by CD8+ cytotoxic lymphocytes (CTL's). Additionally, interferon may increase the number of NK cells and HLA class II expression to aid recognition of infected cells by CD4+ lymphocytes in the liver. Interferon-alfa 2b treatment has become the standard of care against which other treatments will be judged. Its virtues include an approximately 40-50% remission rate in selected patients with a four month course of treatment. Remissions are generally sustained (>90%) without further treatment. The drawbacks include interferon side-effects, as well as the lack of efficacy for patients in the immune tolerant phase (as well as 50+% of stage 2 patients) who have little hepatocyte-directed attack prior to treatment.

Liver biopsy is performed prior to treatment for all patients although this practice has recently been questioned. The reasons for performing liver biopsy include: to exclude other conditions, to stage the disease and to avoid treatment in those with apparent end-stage cirrhosis. Those who would discourage use of liver biopsy argue correctly that we have defined the disease at present serologically, and that those with end-stage liver disease will also be evident on clinical grounds, and thus biopsy represents risk and expense not completely justified by the information gained.

Criteria used in consideration of patients for treatment generally include: active aminotransferase levels (> 100 IU/mL), HBV DNA less than 200 pg/mL, and an active liver biopsy. Most clinicians require that the patient be in otherwise good health, not older than 65 and not have evidence of complications of cirrhosis, such as variceal hemorrhage, ascites or encephalopathy. An observation period of several months is generally used to verify that the disease is relatively stable and not remitting spontaneously. Documentation of at least six months of total carrier state is also used to exclude patients with acute hepatitis B. Treatment is begun with 5 million units interferon alfa-2b subcutaneously daily with continuation at this dose for 16 weeks. Ten million units three times per week has also been used with virtually identical results, but may be less well tolerated in some patients. These doses of interferon are higher than those conventionally used in hepatitis C, and have, as expected, more associated side-effects.

Side effects of interferon therapy include a prominent flu-like syndrome beginning within four to six hours after the first injection. Most patients rapidly develop tachyphylaxis to this side effect, but many will continue to have fatigue, diffuse joint aches or heightened pain acuity in previous painful areas (headaches, low back pain, musculoskeletal pain) throughout their course of treatment. These symptoms rarely require dose modification or cessation of treatment. Diminution in white blood cell count and platelet counts of from 10-30% represent bone marrow suppression and can be observed without dose modification if adequate levels of formed elements are maintained (> 1000 neutrophils, > 50,000 platelets/mm^3). This may be a problem in patients with underlying cirrhosis and hypersplenism, but rarely results in dose alteration. Other less common side effects include mild hair loss, impaired concentration and frank depression. Suicidal ideation has been noted in some patients, and it is important prior to treatment to assess patients for evidence of previous depressive history, use of anti-depressants or suicidal attempts. Other minor side effects include thyroid dysfunction with either increased or decreased TSH levels as evidence of presumed subclinical thyroiditis invoked by the interferon.

Other autoimmune diseases reported to occur include autoimmune chronic hepatitis, thrombocytopenic purpura (plt counts under 10,000 mm^3). Worsening of psoriasis and rheumatoid arthritis has been observed.

Another anticipated effect of treatment is the elevation of aminotransferase levels beyond baseline, and an actual flare in the patient's hepatitis. Transaminase values in the low 1000's have been observed, but few if any fatal reactions have been reported. Thus, unlike the patient with hepatitis C who receives interferon treatment, the hepatitis B patient will not likely see a diminution in alanine aminotransferase (ALT) level with treatment and would preferably observe increased levels. This response is variable and some patients will go on to clear HBV without a discernible disease flare.

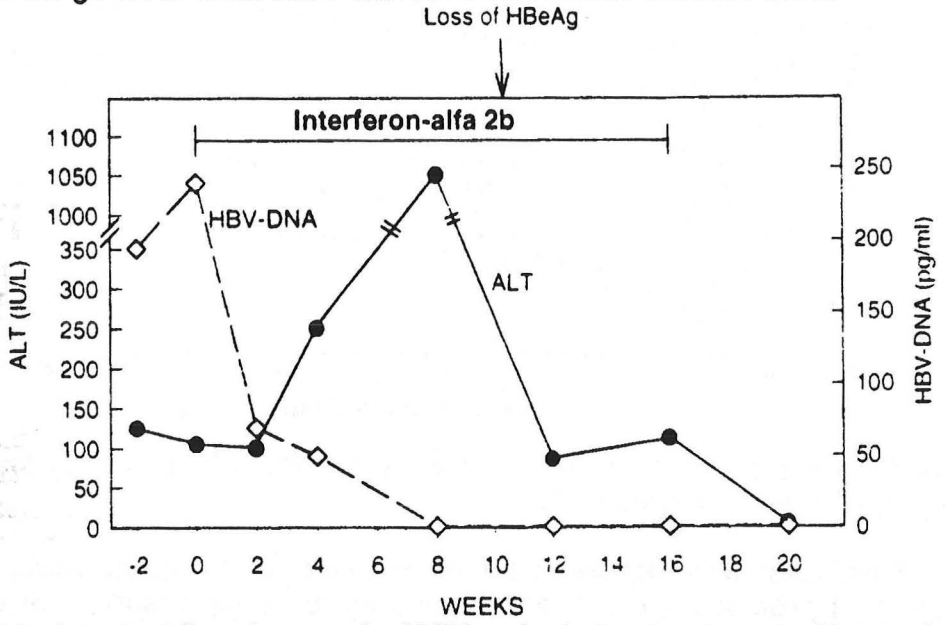


Figure 11. A typical flare is seen in ALT levels during treatment with interferon.

Similarly, patients will occasionally have their disease flare or clearance episode after completion of therapy in the six months post treatment. While these might be considered examples of spontaneous clearance, it is hard not to implicate the recent therapy in enhancing seroconversion. Once seroconversion has occurred, patients can be expected to normalize ALT levels and resolve HBV DNA to undetectable levels in rapid order, usually after a matter of days or a few weeks. About 10% of patients have been observed in some studies to have sustained disappearance of HBV DNA without HBeAg seroconversion. These patients have sustained remission and will eventually clear HBeAg but this may be a prolonged process. It is usually prudent to check both HBeAg and HBV DNA in post treatment followup for this reason.

A meta-analysis of 15 studies using a variety of interferon regimens demonstrated an overall response rate of 33% for treated patients vs. 12% for controls. Loss of the carrier state (clearance of HBsAg) was observed in 7.8% of treated individuals vs. 1.8% of control patients (36). These results are statistically significant if somewhat modest. More importantly, these responses are sustained. Only 5-10% of individuals who have seroconverted will show

reactivation over the next ten years (37). Factors predicting success include total dose >15 MU/wk, HIV-negative status, high aminotransferase levels, more active hepatitis on liver biopsy, and low HBV DNA levels (below 200 pg/ml). All these factors agree with the formulation that the likelihood that a patient will clear infection is dependent on an active immune system (33). Further seroconversion of HBsAg to anti-HBs continues as shown below.

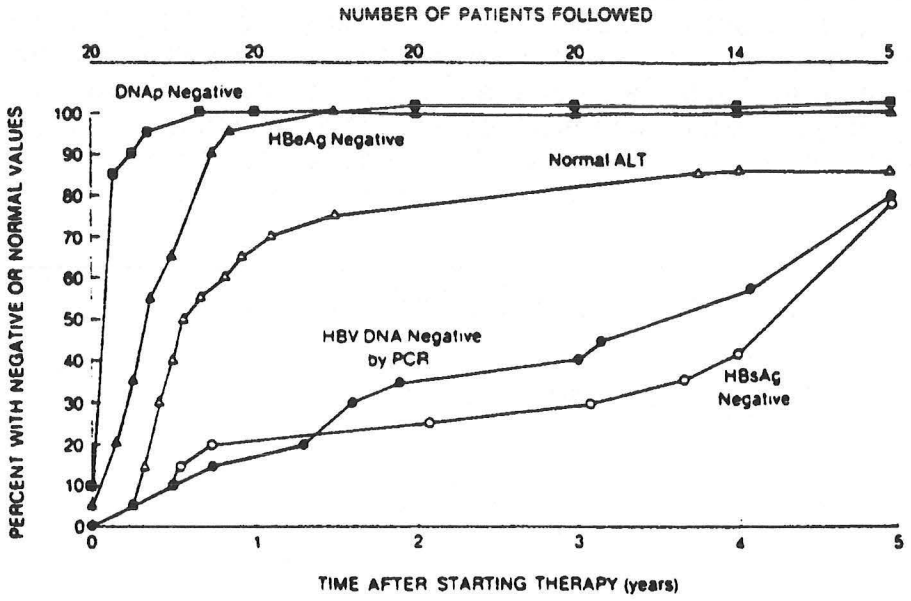


Figure 12. Clearance of serologic markers of HBV infection over time after a course of interferon treatment (37).

Treatment with interferon for a 16 week course costs roughly $5,000 exclusive of professional fees and laboratory follow-up testing. Given a 45% success rate, this may seem exorbitant. However, using the decision analysis method it can be shown that interferon treatment for chronic hepatitis B for a 35 yr old man increases life expectancy by 3.1 years or 3.4 quality-adjusted life years (38,39). This is a very favorable result.

Treatment for special groups

Decompensated cirrhosis: If 3,500 patients die annually of hepatitis B-related cirrhosis in the U.S., and if these patients are not considered good transplant candidates, then treatment which would ameliorate disease progression would be beneficial even in the advanced stage patient. Conventional treatment of these patients has been deemed inadvisable because of the likelihood of a disease exacerbation tipping the patient over into frank liver failure. A recent study has demonstrated the efficacy of low-dose titratable interferon alfa therapy for decompensated patients (40). Patients were treated with a starting dose of 500,000 unit/day for up to 24 weeks and a sustained clearance of HBV DNA was observed in 38%. The best responses were in patients classified as Child-Pugh class A (5/5 seroconverted, vs. 0/6 Child class C). Complete responders demonstrated improvement in hepatic function as measured by serum albumin

levels, bilirubin levels, and improved survival, suggesting that a subgroup of patients with decompensated but not absolute end-stage liver disease can achieve a good response to *therapy carefully conducted*, despite the use of lower doses thought previously to be ineffective.

Delta hepatitis super-infection: While HDV appears to inhibit to some extent HBV viral replication, there has been less than dramatic results in the treatment of patients with combined HBV-HDV infection. Treatment is considered to be urgently needed in this patient population, since disease progression tends to be more rapid than that observed for HBV alone (41). Treatment trials have demonstrated a decrease in ALT levels which is sustained in some patients, but essentially no eradication of the virus and virtually universal relapse once treatment was discontinued (42). Even high-dose and prolonged treatment failed to produce virologic remission.

Anti-HBe Negative Patients: Patients infected with mutant variants demonstrating high levels of HBV DNA despite anti-HBe positivity as described above have been the subject of separate treatment trials, since they cannot be followed by the usual serum markers, but have active hepatocellular disease. Three out of four treatment trials demonstrated remissions in these patient groups of from 59-90%, but with virtually universal relapse post-treatment (43). One study differs from the others in demonstrating a sustained remission of 53% at 18 months post treatment (44).

New Therapies: Nucleoside analogues

Although the successes observed with interferon therapy are dramatic, the drawbacks are obvious. As with use of interferon for hepatitis C, predicting responses to therapy is imprecise to put it mildly, making it necessary to treat many to cure some. Immune tolerant patients with normal aminotransferase levels might be considered not in need of treatment, but they remain both infectious and at risk for development of hepatitis, cirrhosis and hepatocellular carcinoma as time goes on. One form of treatment which showed initial promise was thymosin. Early studies of peptides prepared from thymus extracts, and later purified into single compounds were promising, but a controlled trial has shown no real benefit.

The search for better nucleoside-based antivirals continued. One drug with early promise was fialuridine (FIAU), which inhibits replication by direct inhibition of DNA polymerase. In 1993, fialuridine was being used in the early phase 2 trials, having shown remarkable efficacy in abolishing HBV DNA from the circulation in a four week preliminary study. However, within one week's time, seven patients became acutely ill after receiving ten to 13 weeks of treatment (45). The syndrome was uniform and peculiar: all had profound lactic acidosis and hepatic failure, accompanied by renal failure and severe coagulopathy. Two survived after liver transplantation and five succumbed. Several other patients had milder degrees of liver injury as well as pancreatitis, neuropathy and myopathy. All trials were halted and to date two investigations have yielded conflicting results as to whether the trial design was at fault or whether the outcomes were so unexpected that they could not have been

predicted. Pre-clinical studies had not demonstrated toxicity in animals and the initial clinical studies had likewise shown little evidence of toxicity. In retrospect, of 67 patients mostly with HIV infection who had been treated in pilot studies, three died of liver disease and one of pancreatitis within six months of completing the study. However, this evidence of delayed toxicity occurring as long as six months after the intial four week trial was not attributed to the drug. The histologic findings in all patients were that of microvesicular fat, a sign of mitochondrial toxicity. FIAU apparently binds to host nuclear and mitochondrial DNA, disabling mitochondrial oxidative metabolism (46,47). Similar toxicity has been attributed rarely to other nucleoside analogues such as ddI and AZT. Given the vast numbers of patients treated with these latter drugs, it seems a distinctly rare occurrence.

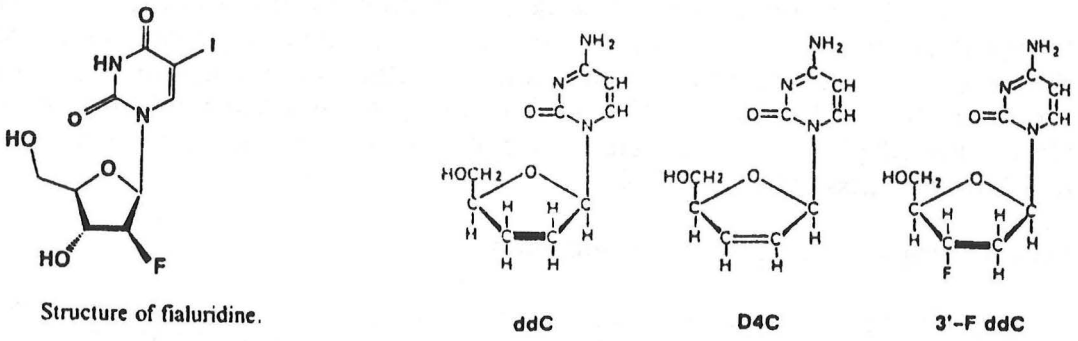


Figure 13. Chemical structures of some new nucleoside analogues.

Several other nucleoside analogues have come to light recently which have different modes of action from FIAU. These drugs appear to be active against retroviruses. Two such agents, lamivudine and famciclovir, are in clinical trials nationwide, including Southwestern Medical School's Center for Clinical Liver Diseases.

As noted previously, since hepatitis B replicates via an RNA template, the DNA polymerase for hepatitis B appears to have reverse transcriptase activity, and indeed can be shown to have homologies with retroviral reverse transcriptases. 2',3'-dideoxycytidine (ddC) has been shown to be a potent inhibitor of human immunodeficiency virus replication in cell culture and in vivo, and to have anti-viral activity against HBV in cell culture systems, probably via inhibition of DNA polymerase (48). Lamivudine, the 9(-) enantiomer of 3' thiacytidine (3TC) is a close relative of ddC, and appears to have similar effects with less toxicity (49-51). Lamivudine has been tested in > 10,000 patients for the treatment of HIV infection, and has recently been approved by the Food and Drug Administration as an alternative to or in combination with AZT for patients with AIDS (52).

A preliminary dose-finding study in patients with HBeAg-positive chronic hepatitis B, (including previous interferon failures) demonstrated complete clearance of HBV DNA from serum in all patients receiving 100 mg/day or greater within 12 weeks, the time limit of the study, and sustained remission in 19%, including several previous interferon failures (53).

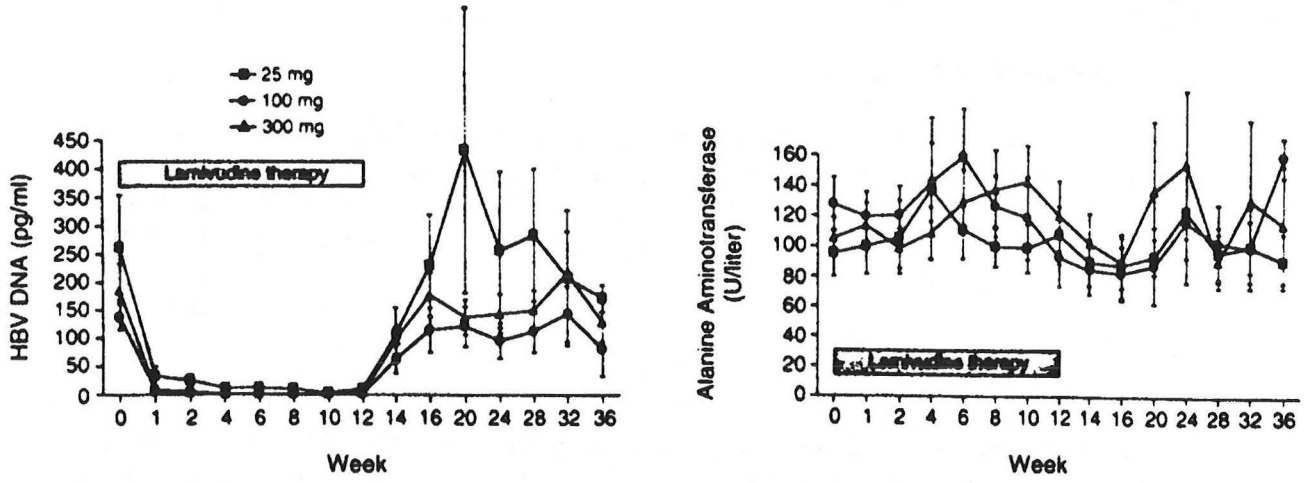


Figure 14. Changes in mean serum HBV DNA levels and ALT during and after 12 weeks of therapy with lamivudine at doses of 25, 100 and 300 mg.

Characteristically, patients in the 12 week study who showed viral clearance frequently had ALT elevations similar to those observed with interferon. This cannot readily be explained, since although the drug is anti-viral, it does not appear to be immunomodulatory. The study currently in progress employs longer courses of treatment with the aim of increasing the number of complete responses. A one year course, for example, might lead to further clearance of virus from (presumed) sequestered sites. Other protocols are underway to examine the efficacy of lamivudine in the transplant setting, since even if therapy were needed indefinitely, it would be less expensive and more effective than HBIG therapy currently in use (54).

A similar study is being undertaken using famciclovir, which has also been shown in preliminary studies to decrease HBV DNA levels in serum, presumably by inhibition of DNA polymerase (55). One new aspect is that patients with normal ALT's will be considered for treatment, a departure from previous criteria.

Hepatitis B Vaccine

Despite the availability of vaccine for hepatitis B since 1982, almost no inroads have been made in eradicating this ubiquitous infection. This was largely

the result of failure of the initial vaccine strategy (which targeted high risk groups only) to impact the overall carrier rate. High risk individuals (physicians, IVDU's, prostitutes) basically were refractory to becoming vaccinated in a timely fashion or did not follow-up with second and third injections to complete the series. As a result, the American Council on Immunization Practices in 1991 proposed a comprehensive strategy for eliminating transmission of hepatitis B in the United States The components were universal vaccination of newborns and adolescents. This strategy has been approved by the American Academy of Pediatrics. Concern has still been raised that physicians are not enthusiastic about eradication of this virus, despite the obvious dividends that will be reaped in diminishing end-stage liver disease and liver cancer, nationally and worldwide (56-58).

Other HBV-related topics

It is impossible to cover all aspects of hepatitis B-related disease in one lecture. Topics not included here but nonetheless important include the role of HBV infection in hepatocellular carcinoma (59,60), or the numerous HBV-related extrahepatic diseases (61,62). Either of these topics would be excellent for future Grand Rounds discussions.

Summary

New insights suggest that hepatitis B, although a DNA virus, shares certain features with RNA viruses, such as reverse transcriptase activity and the tendency to mutate under immune pressure. Nevertheless, viral persistence is primarily a function of the host immune response. These newly identified features underline the virus' remarkably complex strategies for viral persistence, and offer hope of a breakthrough by interrupting reverse transcription. Trials currently underway of apparently safe antivirals, (already proven in AIDS patients), may lead to dramatically improved treatment strategies for both immune competent and immunosuppressed patients.

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