



WHO/CDS/CSR/LYO/2002.2:Hepatitis B

Hepatitis B

World Health Organization
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Response

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Hepatitis B - an introduction

Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. Since the development of jaundice is a characteristic feature of liver disease, a correct diagnosis can only be made by testing patients' sera for the presence of specific anti-viral antigens or antibodies.^{15, 23, 31}

Of the many viral causes of human hepatitis few are of greater global importance than hepatitis B virus (HBV).^{10, 15, 23, 31}

Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world.^{6, 10, 15, 23, 31}

The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC). In addition, HBV carriers can transmit the disease for many years.^{10, 23, 30, 31}

Infection occurs very often in early childhood when it is asymptomatic and often leads to the chronic carrier state.

More than 2 000 million people alive today have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus.^{6, 15, 23, 38, 51} Three quarters of the world's population live in areas where there are high levels of infection.

Every year there are over 4 million acute clinical cases of HBV, and about 25% of carriers, 1 million people a year, die from chronic active hepatitis, cirrhosis or primary liver cancer.⁵¹

Hepatitis B has also been called type B hepatitis, serum hepatitis, homologous serum jaundice.^{23, 31}

What causes the disease?

Hepatitis B is caused by the hepatitis B virus (HBV), an enveloped virus containing a partially double stranded, circular DNA genome, and classified within the family hepadnavirus.^{10, 15, 23, 30, 31}

The virus interferes with the functions of the liver while replicating in hepatocytes. The immune system is then activated to produce a specific reaction to combat and possibly eradicate the infectious agent. As a consequence of pathological damage, the liver becomes inflamed.

HBV may be the cause of up to 80% of all cases of hepatocellular carcinoma worldwide, second only to tobacco among known human carcinogens.^{15, 38, 51}

How is HBV spread?

One should not judge by appearance: most infected people look perfectly healthy and have no symptoms of disease, yet may be highly infectious.



HBV is transmitted through percutaneous or parenteral contact with infected blood, body fluids, and by sexual intercourse.^{10, 11, 15, 23}

HBV is able to remain on any surface it comes into contact with for about a week, e.g. table-tops, razor blades, blood stains, without losing infectivity.^{15, 31}

HBV does not cross the skin or the mucous membrane barrier. Some break in this barrier, which can be minimal and insignificant, is required for transmission.³¹

HBV is a large virus and does not cross the placenta, hence it cannot infect the fetus unless there have been breaks in the maternal-fetal barrier, e.g. via amniocentesis. Still, pregnant women who are infected with HBV can transmit their disease to their babies at birth. If not vaccinated at birth, many of these babies develop lifelong HBV infections, and many develop liver failure or liver cancer later in life.²³

Sexual intercourse with multiple partners or with persons who have multiple partners can be dangerous. Hepatitis B is the only sexually transmitted infection for which there is a protective vaccine.²³

All persons who are hepatitis B surface antigen (HBsAg, [LINK TO PAGE 21](#)) positive are potentially infectious. The many millions of people around the world who become HBV carriers are a constant source of new infections for those who have never contracted the virus.³¹

Blood is infective many weeks before the onset of the first symptoms and throughout the acute phase of the disease. The infectivity of chronically infected individuals varies from highly infectious (HBeAg positive) to often sparingly infectious (anti-HBe positive).

Who is susceptible to infection?

Susceptibility is general. Only people who have been vaccinated successfully or those who have developed anti-HBs antibodies after HBV infection are immune to HBV infection.

Persons with congenital or acquired immunodeficiency including HIV infection, and those with immunosuppression including those with lymphoproliferative disease, and patients treated with immunosuppressive drugs including steroids and by maintenance haemodialysis are more likely to develop persistent infection with HBV.

Following acute HBV infection, the risk of developing chronic infection varies inversely with age. Chronic HBV infection occurs among about 90% of infants infected at birth, 25-50% of children infected at 1-5 years of age and about 1-5% of persons infected as older children and adults. Chronic HBV infection is also common in persons with immunodeficiency.^{10, 15, 23, 31}

Where is HBV a problem, globally?

The world can be divided into three areas where the prevalence of chronic HBV infection is high (>8%), intermediate (2-8%), and low (<2%).^{23, 42}



High endemicity areas include south-east Asia and the Pacific Basin (excluding Japan, Australia, and New Zealand), sub-Saharan Africa, the Amazon Basin, parts of the Middle East, the central Asian Republics, and some countries in eastern Europe. In these areas, about 70 to 90% of the population becomes HBV-infected before the age of 40, and 8 to 20% of people are HBV carriers.¹⁵

In countries such as China, Senegal, Thailand, infection rates are very high in infants, and continue through early childhood. At that stage, the prevalence of HBsAg in serum may exceed 25%. In other countries such as Panama, Papua New Guinea, Solomon Islands, Greenland, and in populations such as Alaskan Indians, infection rates in infants are relatively low and increase rapidly during early childhood.¹⁵

Low endemicity areas include North America, Western and Northern Europe, Australia, and parts of South America. The carrier rate here is less than 2%, and less than 20% of the population is infected with HBV.^{15, 23}

The rest of the world falls into the intermediate range of HBV prevalence, with 2 to 8% of a given population being HBV carriers.

When is hepatitis B contagious?

The most important mode of HBV transmission globally is perinatal, from the mother to her newborn baby. If a pregnant woman is an HBV carrier and is also HBeAg-positive, her newborn baby has a 90% likelihood to be infected and become a carrier. Of these children, 25% will die later from chronic liver disease or liver cancer.¹⁵

Another important mode of HBV transmission is from child to child during early life resulting from blood contact.¹¹

All patients with acute hepatitis B are HBeAg positive, and therefore highly infectious and careless contact with their blood or body fluids can lead to HBV infection.

HBeAg-positive specimens contain high concentrations of infectious virions and HBV DNA, in contrast to anti-HBe positive samples, in which the number of hepatitis B virions is substantially reduced.

Why is there no treatment for the acute disease?

There is no specific treatment for acute viral hepatitis B.²³

Hepatitis B is a viral disease, and as such, antibiotics are of no value in the treatment of the infection.

The use of adrenocorticosteroids in the management of acute, uncomplicated hepatitis B is not indicated because they have no effect on the resolution of the underlying disease process, and may increase the rate of relapse. Early treatment of acute hepatitis B with steroids may result in the development of persistent infection. Corticosteroid therapy is only to be used in patients with chronic active hepatitis who are symptomatic, HBsAg negative ([LINK TO PAGE 21](#)), and who have severe histologic lesions in liver biopsies.³¹



The therapeutic effectiveness of interferon on the course and prognosis of acute hepatitis B is not known.²³

Haemodialysis, exchange transfusions, cross-perfusion, and immune globulin (IG) containing high titres of anti-HBs (HBIG) do not affect favourably the course of fulminant hepatitis.

Therapy for acute hepatitis B should be supportive and aimed at maintaining comfort and adequate nutritional balance.²³

Specific antiviral drugs such as lamivudine, a second generation nucleoside analogue, are available, and others are under development, but these drugs have not been evaluated for the treatment of acute hepatitis B.



The hepatitis B virus HBV

The hepatitis B virus, a hepadnavirus, is a 42 nm partially double stranded DNA virus, composed of a 27 nm nucleocapsid core (HBcAg), surrounded by an outer lipoprotein coat (also called envelope) containing the surface antigen (HBsAg).^{10, 11, 15, 23, 30, 31}

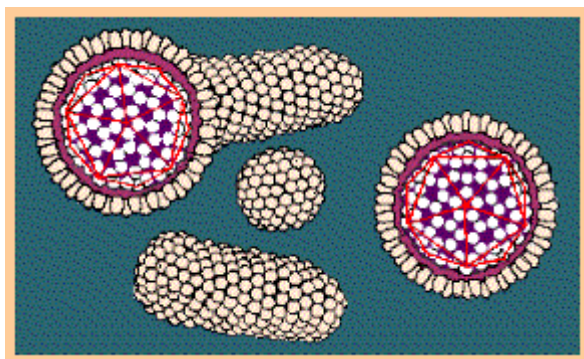
The family of hepadnaviruses comprises members recovered from a variety of animal species, including the woodchuck hepatitis virus (WHV), the ground squirrel hepatitis virus (GSHV), and the duck HBV. Common features of all of these viruses are enveloped virions containing 3 to 3.3 kb of relaxed circular, partially duplex DNA and virion-associated DNA-dependent polymerases that can repair the gap in the virion DNA template and have reverse transcriptase activities. Hepadnaviruses show narrow host ranges, growing only in species close to the natural host, like gibbons, African green monkeys, rhesus monkeys, and woolly monkeys.^{15, 30, 31}

Hepatocytes infected in vivo by hepadnaviruses produce an excess of noninfectious viral lipoprotein particles composed of envelope proteins. Persistent infections display pronounced hepatotropism.¹⁵

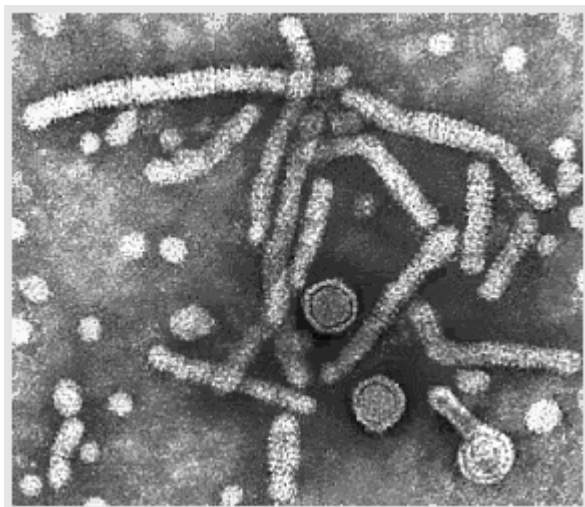
Mammalian hepadnaviruses fail to propagate in cell culture.^{23, 30, 31}

Intracellular HBV is non-cytopathic and causes little or no damage to the cell.^{6, 10, 15, 23}

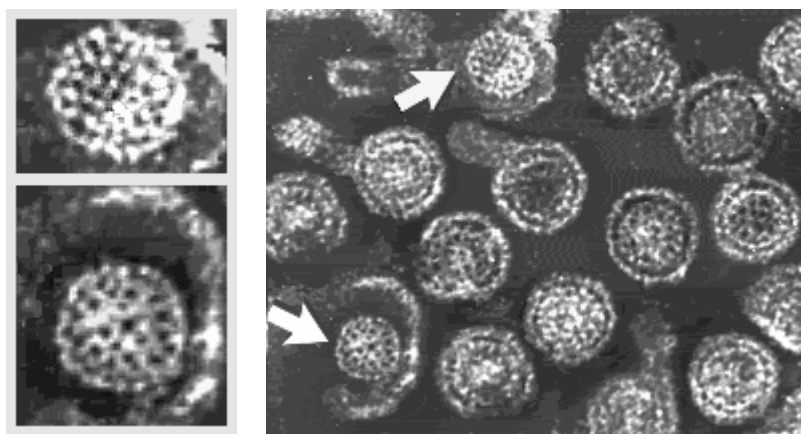
Electron Microscopy (EM) picture and schematic representation of the hepatitis B virion



A diagrammatic representation of the hepatitis B virion and the surface antigen components



Virions are 42nm in diameter and possess an isometric nucleocapsid or "core" of 27nm in diameter, surrounded by an outer coat approximately 4nm thick. The protein of the virion coat is termed "surface antigen" or HBsAg. It is sometimes extended as a tubular tail on one side of the virus particle. The surface antigen is generally produced in vast excess, and is found in the blood of infected individuals in the form of filamentous and spherical particles. Filamentous particles are identical to the virion "tails" - they vary in length and have a mean diameter of about 22nm. They sometimes display regular, non-helical transverse striations.



A group of hepatitis B virions (right) and enlargements of the two exposed cores (indicated by arrows).

From: University of Cape Town, South Africa. <http://www.uct.ac.za/depts/mmi/stannard/hepb.html>

The hepatitis B virus life cycle

The HBV virion binds to a receptor at the surface of the hepatocyte.¹⁰

A number of candidate receptors have been identified, including the transferrin receptor, the asialoglycoprotein receptor molecule, and human liver endonexin. The mechanism of HBsAg binding to a specific receptor to enter cells has not been established yet.

Viral nucleocapsids enter the cell and reach the nucleus, where the viral genome is delivered.^{6, 10, 13, 23}

In the nucleus, second-strand DNA synthesis is completed and the gaps in both strands are repaired to yield a covalently closed circular (ccc) supercoiled DNA molecule that serves as a template for transcription of four viral RNAs that are 3.5, 2.4, 2.1, and 0.7 kb long.^{6, 10, 23, 31}

These transcripts are polyadenylated and transported to the cytoplasm, where they are translated into the viral nucleocapsid and precore antigen (C, pre-C), polymerase (P), envelope L (large), M (medium), S (small)), and transcriptional transactivating proteins (X).^{6, 10, 23, 31}

The envelope proteins insert themselves as integral membrane proteins into the lipid membrane of the endoplasmic reticulum (ER).

The 3.5 kb species, spanning the entire genome and termed pregenomic RNA (pgRNA), is packaged together with HBV polymerase and a protein kinase into core particles where it serves as a template for reverse transcription of negative-strand DNA. The RNA to DNA conversion takes place inside the particles.^{10, 23}

The new, mature, viral nucleocapsids can then follow two different intracellular pathways, one of which leads to the formation and secretion of new virions, whereas the other leads to amplification of the viral genome inside the cell nucleus.^{10, 23}

In the virion assembly pathway, the nucleocapsids reach the ER, where they associate with the envelope proteins and bud into the lumen of the ER, from which they are secreted via the Golgi apparatus out of the cell.^{10, 23}

In the genome amplification pathway, the nucleocapsids deliver their genome to amplify the intranuclear pool of covalently closed circular DNA (cccDNA).^{10, 23}

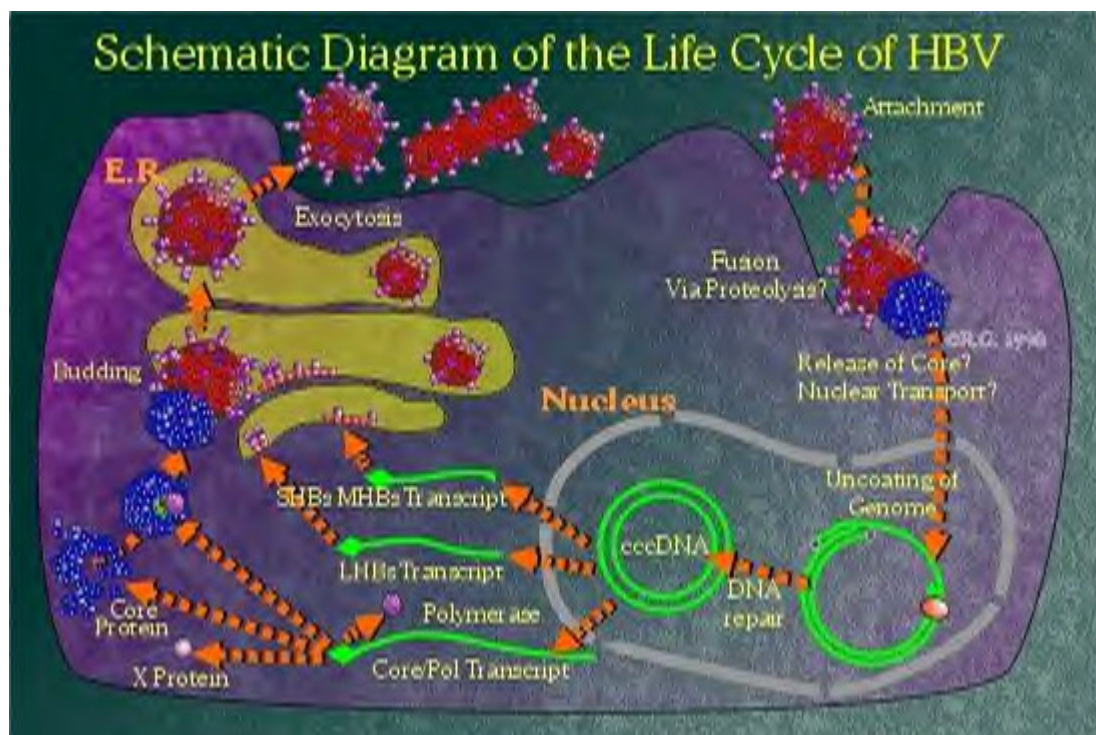
The precore polypeptide is transported into the ER lumen, where its amino- and carboxy-termini are trimmed and the resultant protein is secreted as precore antigen (eAg).

The X protein contributes to the efficiency of HBV replication by interacting with different transcription factors, and is capable of stimulating both cell proliferation and cell death.^{10, 23}

The HBV polymerase is a multifunctional enzyme. The products of the P gene are involved in multiple functions of the viral life cycle, including a priming activity to initiate minus-strand DNA synthesis, a polymerase activity, which synthesizes DNA by using either RNA or DNA templates, a nuclease activity which degrades the RNA strand of RNA-DNA hybrids, and the packaging of the RNA pregenome into nucleocapsids.^{6, 10, 23} Nuclear localisation signals on the polymerase mediate the transport of covalently linked viral genome through the nuclear pore.^{6, 10}



Scheme of genome replication



From: <http://www.globalseve.net/~harlequin/HBV/hbvcycle.htm>

Morphology and physicochemical properties

Ultrastructural examination of sera from hepatitis B patients shows three distinct morphological forms.¹⁵

The most abundant are small, spherical, noninfectious particles, containing HBsAg, that measure 17 to 25 nm in diameter. Concentrations of 10^{13} particles per ml or higher have been detected in some sera. These particles have a buoyant density of 1.18 g/cm^3 in CsCl, reflecting the presence of lipids, and a sedimentation coefficient that ranges from 39 to 54 S.^{15, 31}

Tubular, filamentous forms of various lengths, but with a diameter comparable to that of the small particles, are also observed. They also contain HBsAg polypeptides.^{15, 31}

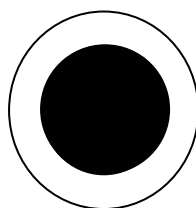
The third morphological form, the 42 nm hepatitis B virion, is a complex, spherical, double shelled particle that consists of an outer envelope containing host-derived lipids and all S gene polypeptides, the large (L), middle (M), and small (S) surface proteins, also known as pre-S1, pre-S2 and HBsAg. Within the sphere is an electron-dense inner core or nucleocapsid with a diameter of 27 nm. The nucleocapsid contains core proteins HBcAg, a 3.2 kb, circular, partially double stranded viral DNA genome, an endogenous DNA polymerase (reverse transcriptase) enzyme, and protein kinase activity.^{15, 23, 31}



The sera of infected patients may contain as many as 10^{10} infectious virions per ml. The complete virion has a buoyant density of about 1.22 g/cm^3 in CsCl and a sedimentation coefficient of 280 S in sucrose gradients.¹⁵

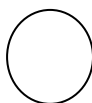
Schematic representation of viral particles found in serum of HBV-infected people

Infectious HBV particle:



- 42 to 47 nm double-shelled particle. Outer envelope containing lipid and three forms of HBsAg
- 27 nm nucleocapsid made of 180 copies of core protein, containing the polymerase and HBV DNA

Empty noninfectious particles:



- 22 nm spheres and filaments of variable length containing lipid and mainly one form of HBsAg usually present in 10 000 to 1 000 000-fold excess over Dane particles.

Titres of the virus in the blood can range between $<10^4/\text{ml}$ and $>10^9/\text{ml}$.

The envelope can be removed with nonionic detergents, liberating the inner core, the nucleocapsid of 27 nm. The major structural protein of the core is the C protein, a 21 kD basic phosphoprotein called hepatitis B core antigen (HBcAg).

Within the core are the viral DNA, a DNA polymerase, and a protein kinase.

The 22 nm spheres and filaments lack nucleic acid altogether and hence are noninfectious. These particles are highly immunogenic and induce a neutralizing anti-HBs antibody response.

The number of subviral particles can exceed that of virions by a factor of 10^3 to 10^5 .

Genome and proteins

HBV virion DNA is a relaxed circular, partially duplex molecule of 3.2 kb, whose circularity is maintained by 5' cohesive ends.^{15, 31}

The positions of the 5' ends of both strands map to the regions of short (11 nucleotides) direct repeats (DRs) in viral DNA. The 5' end of the minus strand DNA maps within the repeat termed DR1, while plus strand DNA begins with DR2. These repeats are involved in priming the synthesis of their respective DNA strands.⁶

The viral minus strand is unit length and has protein covalently linked to its 5' end.

The viral plus strand is less than unit length and has a capped oligoribonucleotide at its 5' end. The single-stranded region or gap is of fixed polarity but variable length.³¹

A virion-associated polymerase can repair this gap and generate a fully duplex genome.

Negative strand DNA is the template for the synthesis of the viral mRNA transcripts. HBV DNA has a very compact coding organization with four partially overlapping open reading frames (ORFs) that are translated into seven known proteins. Noncoding regions are not present.

Four separate viral promoters have been identified, driving expression of a) genomic, P, and pre-C and C RNAs, b) L protein mRNA, c) M and S protein mRNAs, and d) X protein mRNA. They are referred to as the genomic, pre-S1, S, and X promoters, respectively.

Two major classes of transcripts exist: genomic and subgenomic. The subgenomic RNAs function exclusively as messenger RNAs (mRNAs) for translation of envelope and X proteins. The genomic RNAs are bifunctional, serving as both the templates for viral DNA synthesis and as messages for ORF pre-C, C, and P translation.^{6, 23}

ORF P encodes the viral polymerase and the terminal protein found on minus strand DNA. ORF C encodes the structural protein of the nucleocapsid and the HBeAg, and ORF S/pre-S encodes the viral surface glycoproteins. The product of ORF X is a poorly understood regulatory protein that enhances the expression of heterologous and homologous cellular genes in trans.^{6, 31}

Classic HBsAg, which contains the S domain only, is also called the S-protein (24 kD). Two other proteins share the C-terminal S domain, but differ by length and structure of their N-terminal (pre-S) extensions. The large L protein (39 kD) contains the pre-S1, the pre-S2 region and the S region, and the medium M protein (31 kD) contains the pre-S2 and the S region only. HBsAg is the most abundant of the S-related antigens. The L and M proteins are expressed at levels of about 5-15% and 1-2% compared with S protein.³¹

The glycosylation of the S domain gives rise to two isoforms of each protein. In addition, the M protein contains an N-linked oligosaccharide on its pre-S2-specific domain, and the L protein carries a myristic acid group in amide linkage to its amino-terminal glycine residue. While the function of M protein is still obscure, L proteins play a role in viral assembly and infectivity.³¹

The three envelope glycoproteins are not distributed uniformly among the various HBV particle types. Subviral 22 nm particles are composed predominantly of S proteins, with variable amounts of M proteins and few or no L proteins. Virus particles are enriched for L proteins. L proteins carry the receptor recognition domain, which allows efficient binding to cell surface receptors.



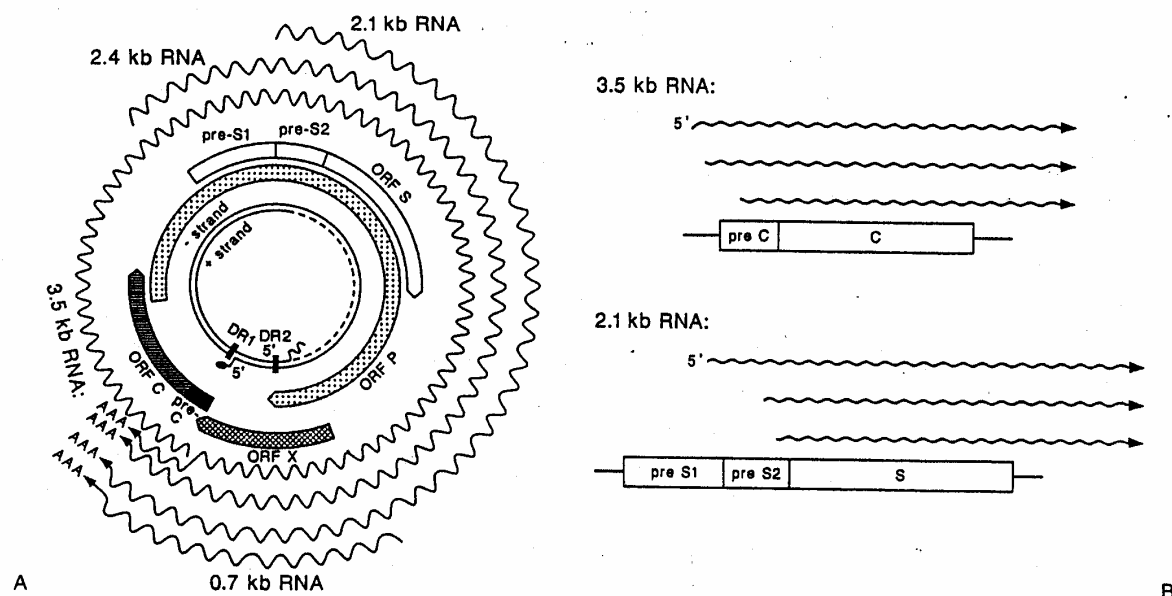
Two in-frame AUG codons are present in ORF C. Classic HBcAg (21 kD) is the product of initiation from the more internal start codon, while initiation at the upstream AUG produces a C-related protein that is not incorporated into virions but instead is independently secreted from cells, accumulating in serum as an immunologically distinct antigen known as HBeAg (16-18 kD). The function of HBeAg is still unknown.³¹

HBcAg is the most conserved polypeptide among the mammalian hepadnaviruses with 68% homology between HBV and GSHV and 92% between GSHV and WHV. Core proteins spontaneously assemble into forms resembling core particles.

The polymerase protein is a DNA-dependent DNA polymerase, a reverse transcriptase, an RNase H, and it binds to the 5' end of HBV DNA, acting thus as a primer for reverse transcription of the pregenome, an RNA intermediate, to form negative strand DNA.³¹ Furthermore, it plays important roles in the encapsidation of the viral pregenomic RNA. The polymerase protein is quite immunogenic during both acute and chronic infection.⁶

ORF X encodes the protein X (17 kD), a transactivator for the viral core and S promoters. The X protein is the least-conserved protein among hepadnaviruses with only 33% amino acid homology between GSHV and HBV, and 71% between the two rodent viruses.⁶

HBV coding organization



From: Ganem D and Schneider RJ. *Hepadnaviridae: The Viruses and Their Replication*. In: Knipe DM et al., eds. *Fields Virology*, 4th ed. Philadelphia Williams & Wilkins, 2001:2923-2969,¹⁰ with permission (<http://lww.com>).

A: Diagrammatic representation of the hepatitis B virus coding organization. *Inner circle* represents virion DNA, with dashes signifying the single-stranded genomic region; the locations of DR1 and DR2 sequence elements are as indicated. *Boxes* denote viral coding regions, with *arrows* indicating direction of translation. *Outermost wavy lines* depict the viral RNAs identified in infected cells, with arrows indicating direction of transcription. **B:** Fine structure of the 5' ends of the pre-C/C transcripts (*top*) and pre-S2/S transcripts (*bottom*) relative to their respective open reading frames.¹⁰

Hepatitis B virus DNA and hepatocellular carcinoma

More than 85% of hepatocellular tumours examined harbor integrated HBV DNA, often multiple copies per cell. The viral DNA integrants are usually highly rearranged, with deletions, inversions, and sequence reiterations all commonly observed. Most of these rearrangements ablate viral gene expression, but the integrations alter the host DNA.^{10, 31, 52}

Interestingly, tumours are clonal with respect to these integrants: every cell in the tumour contains an identical complement of HBV insertions. This implies that the integration event(s) preceded the clonal expansion of the cells. How integration is achieved is still not well understood. Since integration is not an obligatory step in the hepadnaviral replication cycle, and hepadnaviruses have no virus-encoded integration machinery, HBV DNA is probably assimilated into the nucleus by host mechanisms.^{10, 23}

There is no similarity in the pattern of integration between different tumours, and variation is seen both in the integration site(s) and in the number of copies or partial copies of the viral genome.⁵²

The molecular mechanisms by which hepadnaviruses predispose to malignancy are still unknown.⁵²



Direct models

In the direct models, HBV DNA makes direct genetic contributions to the lesion by either providing cis-acting sequences deregulating host growth genes, or by providing trans-acting factors that interfere with cellular growth control.¹⁰

Indirect models

In the indirect models, HBV genes and their products make no direct genetic contribution to the transforming event. Rather, HBV-induced liver injury triggering a series of host responses that lead to liver cell regeneration increases the probability of mutation and malignant transformation.¹⁰

A better understanding of the immunologic mechanisms of liver cell injury could allow the development of therapeutic agents that would control these responses.

HBV mutants

Naturally occurring envelope, precore, core, and polymerase variants have been described.^{11, 15, 23}

Envelope antigenic variants may have a selective advantage over wild type under immune selection pressure, as observed in some cases after hepatitis B IG (HBIG) treatment or HBV vaccination. An epidemiological shift has not been observed yet.

A number of precore mutations preventing HBeAg synthesis have been identified in HBeAg negative carriers. The most frequent variant has a G to A point mutation at nucleotide 83 (mutant HBV83, nucleotide 1896 of the genome, amino acid 144) in the precore region, introducing a stop codon at codon 28.^{15, 23} The HBV83 mutant is predominantly found in Mediterranean and Asian countries but is uncommon in North America and Northern Europe. Precore mutants are found in patients with fulminant hepatitis or chronic active hepatitis, but also in asymptomatic carriers.¹¹

HBV core gene mutations have been reported in patients from Japan, Hong Kong, United States, and Italy. Most of the mutations are concentrated in the middle-third of the core gene, but although many of these mutations are located in regions that harbor B and T cell epitopes, they have not been proven to result in loss of immune recognition.

In rare patients where the function of the polymerase gene is impaired, additional compensatory mutations were found that minimized the impact of the impaired function of the polymerase.

HBV is far more heterogeneous than is generally thought. The HBV genome seems not to be characterized by a single representative genomic molecule, but by a pool of genomes which differ both in structure and function.

The public health importance of mutant hepatitis B viruses is currently under debate. Further studies and a strict surveillance to detect the emergence of these viruses are crucial for a correct evaluation of the effectiveness of current immunization strategies.^{23, 52, 53}



Nomenclature of hepatitis B

HBV	hepatitis B virus (complete infectious virion)	The 42 nm, double-shelled particle, originally called the Dane particle, that consists of a 7 nm thick outer shell and a 27 nm inner core. The core contains a small, circular, partially double-stranded DNA molecule and an endogenous DNA polymerase. This is the prototype agent for the family Hepadnaviridae.
HBsAg	hepatitis B surface antigen (also called envelope antigen)	The complex of antigenic determinants found on the surface of HBV and of 22 nm particles and tubular forms. It was formerly designated Australia (Au) antigen or hepatitis-associated antigen (HAA).
HBcAg	hepatitis B core antigen	The antigenic specificity associated with the 27 nm core of HBV.
HBeAg	hepatitis B e antigen	The antigenic determinant that is closely associated with the nucleocapsid of HBV. It also circulates as a soluble protein in serum.
Anti-HBs, anti-HBc, and anti-HBe	Antibody to HBsAg, HBcAg, and HBeAg	Specific antibodies that are produced in response to their respective antigenic determinants.

From: Hollinger FB and Liang TJ. Hepatitis B Virus. In: Knipe DM et al., eds. *Fields Virology*, 4th ed., Philadelphia, Lippincott Williams & Wilkins, 2001:2971-3036,¹⁵ with permission (<http://lww.com>).

Antigenicity

All three coat proteins of HBV contain HBsAg, which is highly immunogenic and induces anti-HBs (humoral immunity). Structural viral proteins induce specific T-lymphocytes, capable of eliminating HBV-infected cells (cytotoxic T-cells; cellular immunity).^{6, 15}

HBsAg is heterogeneous antigenically, with a common antigen designated a, and two pairs of mutually exclusive antigens, d and y, and w (including several subdeterminants) and r, resulting in 4 major subtypes: adw, ayw, adr and ayr.^{23, 30, 31}



The distribution of subtypes varies geographically.³⁰ Because of the common determinants, protection against one subtype appears to confer protection to the other subtypes, and no difference in clinical features have been related to subtypes.

In the US, northern Europe, Asia, and Oceania, the d determinant is common, but the y determinant is found at lower frequency. The d determinant to the near exclusion of y is found in Japan. The y determinant, and rarely d, are found in Africa and in Australia aborigines. y is also frequently found in India and around the Mediterranean. In Europe, the US, Africa, India, Australia, and Oceania, the w determinant predominates. In Japan, China, and Southeast Asia, the r determinant predominates. Subtypes adw, ady, and adr are each found in extensive geographic regions of the world. Subtype ayr is rare in the world, but it is commonly found in small populations in Oceania.^{23, 52}

The c antigen (HBcAg) is present on the surface of core particles. HBcAg and core particles are not present in the blood in a free form, but are found only as internal components of virus particles.^{23, 30}

The core antigen shares its sequences with the e antigen (HBeAg), identified as a soluble antigen, but no crossreactivity between the two proteins is observed.^{30, 31}

Viral oligopeptides of 8-15 amino acids are loaded on host cell MHC-class I molecules and are transported to the surface of the cell. HBV-specific T-lymphocytes can then detect infected cells and destroy them. This cell deletion triggered by inflammation cells may result in acute hepatitis. When the infection is self-limited, immunity results. If HBV is not eliminated, a delicate balance between viral replication and immunodefence prevails which may lead to chronic hepatitis and liver cirrhosis. In chronically infected cells the HBV DNA may integrate into the host cell DNA. As a long term consequence, integration may lead to hepatocellular carcinoma.^{15, 23, 52}

Stability

The stability of HBV does not always coincide with that of HBsAg.¹⁵

Exposure to ether, acid (pH 2.4 for at least 6 h), and heat (98°C for 1 min; 60°C for 10 h) does not destroy immunogenicity or antigenicity. However, inactivation may be incomplete under these conditions if the concentration of virus is excessively high.¹⁵

Antigenicity and probably infectivity are destroyed after exposure of HBsAg to 0.25% sodium hypochlorite for 3 min.¹⁵

Infectivity is lost after autoclaving at 121°C for 20 min or dry heat treatment at 160°C for 1 h.^{15, 31}

HBV is inactivated by exposure to sodium hypochlorite (500 mg free chlorine per litre) for 10 min, 2% aqueous glutaraldehyde at room temperature for 5 min, heat treatment at 98°C for 2 min, Sporicidin (Ash Dentsply, York, PA) (pH 7.9), formaldehyde at 18.5 g/l (5% formalin in water), 70% isopropylalcohol, 80% ethyl alcohol at 11°C for 2 min, Wescodyne (a iodophor disinfectant, American Sterilizer Co., Erie, PA) diluted 1:213, or combined β -propiolactone and UV irradiation.^{15, 45}

HBV retains infectivity when stored at 30°C to 32°C for at least 6 months and when frozen at –15°C for 15 years. HBV present in blood can withstand drying on a surface for at least a week.^{15, 31}



The disease

The course of hepatitis B may be extremely variable.³¹ Hepatitis B virus infection has different clinical manifestations depending on the patient's age at infection and immune status, and the stage at which the disease is recognized.

During the incubation phase of the disease (6 to 24 weeks), patients may feel unwell with possible nausea, vomiting, diarrhea, anorexia and headaches. Patients may then become jaundiced although low grade fever and loss of appetite may improve. Sometimes HBV infection produces neither jaundice nor obvious symptoms.^{15, 31}

The asymptomatic cases can be identified by detecting biochemical or virus-specific serologic alterations in their blood. They may become silent carriers of the virus and constitute a reservoir for further transmission to others.

Most adult patients recover completely from their HBV infection, but about 5 to 10%, will not clear the virus and will progress to become asymptomatic carriers or develop chronic hepatitis possibly resulting in cirrhosis and/or liver cancer.³¹ Rarely, others may develop fulminant hepatitis and die.

People who develop chronic hepatitis may develop significant and potentially fatal disease.³¹

In general, the frequency of clinical disease increases with age, whereas the percentage of carriers decreases.

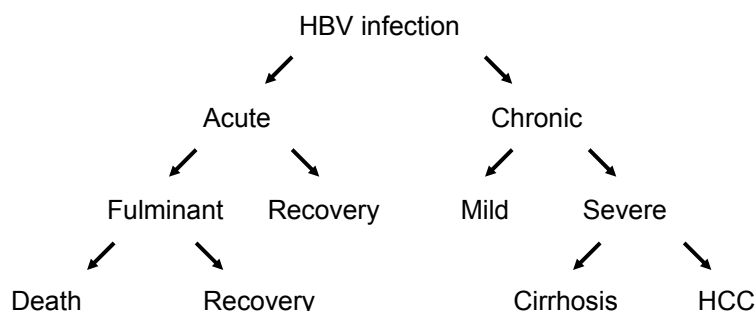
Worldwide, about 1 million deaths occur each year due to chronic forms of the disease.³⁹

Persistent or chronic HBV infection is among the most common persistent viral infections in humans. More than 350 million people in the world today are estimated to be persistently infected with HBV. A large fraction of these are in eastern Asia and sub-Saharan Africa, where the associated complications of chronic liver disease and liver cancer are the most important health problems.³¹

A small number of long-established chronic carriers apparently terminate their active infection and become HBsAg-negative (about 2%/year).

Survivors of fulminant hepatitis rarely become infected persistently, and HBsAg carriers frequently have no history of recognized acute hepatitis.

Spectrum of liver disease after HBV infection



From: Chisari FV and Ferrari C. *Viral Hepatitis*. In: Nathanson N et al., eds. *Viral Pathogenesis*, Philadelphia, Lippincott - Raven, 1997:745-778,⁶ with permission (<http://www.com>).

The infecting dose of virus and the age of the person infected are important factors that correlate with the severity of acute or chronic hepatitis B.^{23, 31}

Only a small proportion of acute HBV infections are recognized clinically. Less than 10% of children and 30-50% of adults with acute HBV infection will have icteric disease.⁵¹

Primary HBV infection may be associated with little or no liver disease or with acute hepatitis of severity ranging from mild to fulminant.³¹

HBV infection is transient in about 90% of adults and 10% of newborn, and persistent in the remainder.²³

Most cases of acute hepatitis are subclinical, and less than 1% of symptomatic cases are fulminant.³¹

Worldwide, about 350 million people are estimated to be infected chronically with HBV.³⁹

Persistent HBV infection is sometimes associated with histologically normal liver and normal liver function, but about one third of chronic HBV infections are associated with cirrhosis and HCC.³¹

Clinical phases of acute hepatitis B infection

The acute form of the disease often resolves spontaneously after a 4-8 week illness. Most patients recover without significant consequences and without recurrence. However, a favourable prognosis is not certain, especially in the elderly who can develop fulminating, fatal cases of acute hepatic necrosis. Young children rarely develop acute clinical disease, but many of those infected before the age of seven will become chronic carriers.^{6, 15, 23, 30, 31}

The incubation period varies usually between 45 and 120 days, with an average of 60 to 90 days. The variation is related to the amount of virus in the inoculum, the mode of transmission and host factors.^{6, 15, 23, 31}

The hallmark of acute viral hepatitis is the striking elevation in serum transaminase (aminotransferase) activity. The increase in aminotransferases, especially ALT, during acute hepatitis B varies from a



mild/moderate increase of 3- to 10-fold to a striking increase of >100-fold. The latter does not necessarily imply a poor prognosis.

In patients with clinical illness, the onset is usually insidious with tiredness, anorexia, vague abdominal discomfort, nausea and vomiting, sometimes arthralgias and rash, often progressing to jaundice. Fever may be absent or mild.^{6, 15, 23, 31}

The icteric phase of acute viral hepatitis begins usually within 10 days of the initial symptoms with the appearance of dark urine followed by pale stools and yellowish discoloration of the mucous membranes, conjunctivae, sclerae, and skin. Jaundice becomes apparent clinically when the total bilirubin level exceeds 20 to 40 mg/l. It is accompanied by hepatomegaly and splenomegaly. About 4-12 weeks thereafter, the jaundice disappears and the illness resolves with the development of natural, protective antibodies (anti-HBs), in about 95% of adults.¹⁵

The larger the virus dose, the shorter the incubation period and the more likely that icteric hepatitis will result. The largest virus doses received by patients may occur in transfusions of infectious blood.³¹

In most cases, no special treatment or diet is required, and patients need not be confined to bed.

Acute hepatitis B is characterized by the presence of anti-HBc IgM serum antibodies converting to IgG with convalescence and recovery, and the transient (<6 months) presence of HBsAg, HBeAg, and viral DNA, with clearance of these markers followed by seroconversion to anti-HBsAg and anti-HBeAg. More than 90% of adult-onset infection cases fall into this category. The remaining 5 to 10% of adult-onset infection and over 90% of cases of neonatal infection become chronic, and may continue for the life span of the patient.²³

A small percentage of persons die from acute HBV.

Clinical features of chronic hepatitis B

Although most adult patients recover completely from an acute episode of hepatitis B, in a significant proportion, 5 to 10%, the virus persists in the body. This figure is much higher in children: 70 to 90% of infants infected in their first few years of life become chronic carriers of HBV.^{23, 31}

Hepatitis B causes about 4 million acute infections worldwide each year. An estimated 350 million persons worldwide are chronic carriers of HBV, with 100 million carriers in China and 1 million carriers in the USA.⁴⁰ Of persistent HBsAg carriers, 70% have chronic persistent hepatitis (see below), and 30% have chronic active hepatitis (see below).²³

Chronic hepatitis can cause serious destructive diseases of the liver and it contributes greatly to the worldwide burden of the disease.²³

Chronic hepatitis generally develops over many years during which individual patients will pass through a number of disease states.

Surprisingly, some of the patients infected persistently may have no clinical or biochemical evidence of liver disease, while others may show signs of easy fatigability, anxiety, anorexia, and malaise.^{15, 23}

Chronic hepatitis B is a prolonged (>6 months) infection with persistent serum levels of HBsAg and IgG anti-HBcAg and the absence of an anti-HBsAg antibody response. HBV DNA and HBeAg are often



detectable at high concentrations, but may disappear if viral replication ceases or if mutations occur that prevent the synthesis of the viral precore protein precursor of HBeAg. The associated inflammatory liver disease is variable in severity. It is always much milder than in acute hepatitis B, but it can last for decades and proceed to cirrhosis, and it is associated with a 100-fold increase in the risk of developing a hepatocellular carcinoma.^{15, 31}

Three phases of viral replication occur during the course of HBV infection, especially in patients with chronic hepatitis B.¹¹

High replicative phase. In this phase HBsAg, HBeAg, and HBV DNA are present and detectable in the sera. Aminotransferase levels may increase, and moderate inflammatory activity is histologically apparent. The risk of evolving to cirrhosis is high.

Low replicative phase. This phase is associated with the loss of HBeAg, or a decrease or loss of the HBV DNA concentrations, and with the appearance of anti-HBe. Histologically, a decrease in inflammatory activity is evident. Serologic changes like the loss of HBV DNA and HBeAg are referred to as seroconversion.

Nonreplicative phase. Markers of viral replication are either absent or below detection level, and the inflammation is diminished. However, if cirrhosis has already developed, it persists indefinitely.

The laboratory abnormalities consist of elevation of the ALT, ranging from normal to 200 IU/l in up to 90% of patients. Transaminases, serum bilirubin, albumin, and gammaglobulin values are mild to markedly elevated, and autoimmune antibodies such as antinuclear antibody, anti-smooth muscle antibody and antimitochondrial antibody may be present.¹⁵

Sustained increases in the concentrations of the aminotransferases together with the presence of HBsAg for >6 months is regarded as indicative of chronic hepatitis.

Up to 20% of the chronic persistent hepatitis cases progress to cirrhosis. This a serious liver disease associated with chronic and often widespread destruction of liver substance occurring over a period of several years.

In cirrhosis, liver cells die and are progressively replaced with fibrotic tissue leading to nodule formation. The internal structure of the liver is deranged leading to the obstruction of blood flow and decrease in liver function. This damage is caused by recurrent immune responses stimulated by the presence of the virus. Because liver inflammation can be totally symptomless, progression of inflammation to cirrhosis can occur without the knowledge of the patient.

Therefore most carriers are contagious but some are not. This is determined by the presence of HBV DNA.

Globally, HBV causes 60 - 80% of the world's primary liver cancers.³⁸

It is estimated that, in men, the lifetime risk of death from chronic disease which leads to cirrhosis and/or hepatocellular carcinoma is between 40 and 50%. In women the risk is about 15%, placing chronic hepatitis B infections among the 10 leading causes of death in men.



HBV and hepatocellular carcinoma (HCC)

A number of HBV patients with chronic hepatitis will develop hepatocellular carcinoma^{15, 31}. Persons at increased risk of developing HCC include adult male and chronic hepatitis B patients with cirrhosis who contracted hepatitis B in early childhood²³. Only about 5% of patients with cirrhosis develop HCC. On the other hand, between 60 and 90% of HCC patients have underlying cirrhosis.^{15, 30, 31}

The incidence of HCC varies with geography, race, age, and sex. HCC is responsible for 90% of the primary malignant tumours of the liver observed in adults. Worldwide, it is the seventh most frequent cancer in males and ninth most common in females. Liver cancer is the cause of more than 500 000 deaths annually throughout the world, with a male:female ratio of 4:1. The frequency of HCC follows the same general geographic distribution pattern as that of persistent HBV infection. The age distribution of patients with clinically recognized tumours suggests that these tumours appear after a mean duration of about 35 years of HBV infection.^{15, 31}

Patients who develop HCC as a result of malignant transformation of hepatocytes have a mean 5-year survival rate of 25 to 60%.¹⁵ This variation depends on the size of the tumour, its resectability, and the presence or absence of α -fetoprotein (AFP). Non-resectable tumours have a mean survival rate of 5 months for AFP-positive tumours and of 10.5 months for AFP-negative tumours.¹⁵

When serum α -fetoprotein (AFP) followed serially in HBsAg carriers rises significantly above the patient's own baseline ($>100 \mu\text{g/ml}$), HCC can often be detected by liver scanning or ultrasound procedures at a stage when the tumour can be cured by surgical resection.³¹ This suggests that HBsAg carriers should have regular serial serum AFP determinations and ultrasound examinations (at 6 months intervals for those above 40 years). Both these tests are recommended to be repeated regularly for all HBsAg carriers with cirrhosis.³¹

HBV causes 60-80% of the world's primary liver cancer, and primary liver cancer is one of the three most common causes of cancer deaths in males in East and South-east Asia, the Pacific Basin, and sub-Saharan Africa.³¹

Primary liver cancer is the eighth most common cancer in the world.³¹ Up to 80% of liver cancers are due to HBV. When HCC presents clinically, the disease is fatal. The median survival frequency of HCC patients is less than 3 months. However, if the cancer is detected early, there is a 85% chance of a cure. Treatment involves surgery, hepatic irradiation, and anticancer drugs.

Progression to fulminant hepatitis B

Fulminant hepatitis B is a rare condition that develops in about 1% of cases. It is caused by massive necrosis of liver substance and is usually fatal.^{15, 23}

Survival in adults is uncommon, prognosis for children is rather better. Remarkably, the few survivors usually recover completely without permanent liver damage and no chronic infection.^{15, 31}

Patients infected with precore mutants often manifest severe chronic hepatitis, early progression with cirrhosis, and a variable response to interferon therapy. It may have an association with fulminant hepatic failure.⁵²

Genetic heterogeneity of HBV, coinfection or superinfection with other viral hepatitis agents, or host immunological factors, may be associated with the development of fulminant hepatitis B.^{15, 31}



A rapid fall in ALT and AST in patients with fulminant hepatic failure may be erroneously interpreted as a resolving hepatic infection, when in fact hepatocytes are being lost and the outcome is fatal.¹¹

Extrahepatic manifestations of hepatitis B

Extrahepatic manifestations of hepatitis B are seen in 10-20% of patients as

- ♦ **transient** serum sickness-like syndrome^{15, 23, 31}

with fever (<39°C), skin rash (erythematous, macular, macopapular, urticarial, nodular, or petechial lesions), polyarthritis (acute articular symmetrical inflammation, painful, fusiform swelling of joints of hand and knee, morning stiffness. Symptoms usually precede the onset of jaundice by a few days to 4 weeks and subside after onset of jaundice and may persist throughout the course of the disease. No recurrent or chronic arthritis occurs after recovery.

Immune complexes (e.g. surface antigen-antibody) are important in the pathogenesis of other disease syndromes characterized by severe damage of blood vessels:³¹

- ♦ **acute necrotizing vasculitis (polyarteritis nodosa)**^{15, 31}

with high fever, anemia, leucocytosis, arthralgia, arthritis, renal disease, hypertension, heart disease, gastrointestinal disease, skin manifestations, neurologic disorders. Highly variable disease with mortality rate of 40% within 3 years unless treated. The diagnosis is established by angiography.

- ♦ **membranous glomerulonephritis**^{15, 31}

is present in both adults and children. Remission of nephropathy occurs in 85 to 90% of cases over a period of 9 years and is associated with clearance of HBeAg from serum.

- ♦ **papular acrodermatitis of childhood** (Gianotti-Crosti syndrome)¹⁵

a distinctive disease of childhood. Skin lesions, lentil-sized, flat, erythematous, and papular eruptions localized to the face and extremities, last 15 to 20 days. The disease is accompanied by generalized lymphadenopathy, hepatomegaly, and acute anicteric hepatitis B of ayw subtype.

Immune complexes have been found in the sera of all patients with fulminant hepatitis, but are seen only infrequently in nonfulminant infections. Perhaps complexes are critical factors only if they are of a particular size or of a certain antigen-to-antibody ratio.⁵²

Why only a small proportion of patients with circulating complexes develop vasculitis or polyarteritis is still not clear.

Coinfection or superinfection with HDV

Hepatitis Delta virus (HDV) is a defective virus that is only infectious in the presence of active HBV infection. HDV infection occurs as either coinfection with HBV or superinfection of an HBV carrier. Coinfection usually resolves. Superinfection, however, causes frequently chronic HDV infection and chronic active hepatitis. Both types of infections may cause fulminant hepatitis.^{3, 31}

Routes of transmission are similar to those of HBV.³

Preventing acute and chronic HBV infection of susceptible persons by vaccination will also prevent HDV infection.^{3, 23}

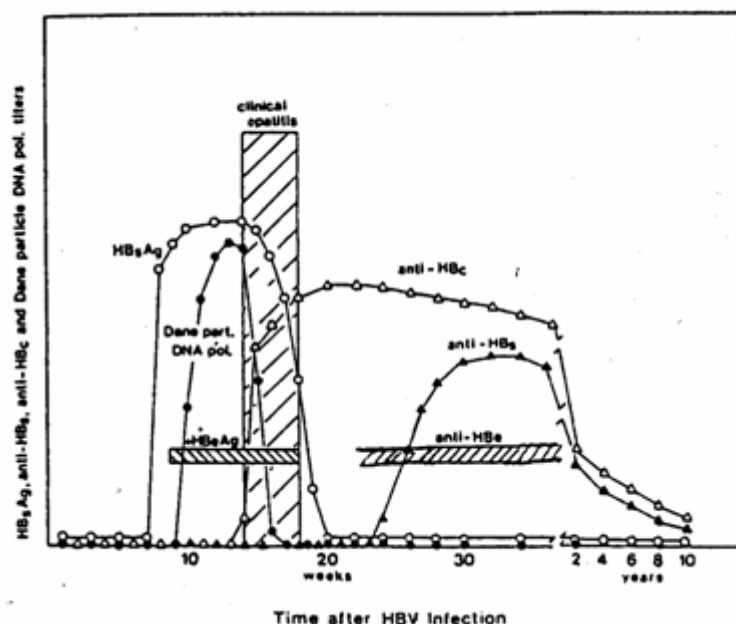
Lamivudine, an inhibitor of HBV-DNA replication, is not beneficial for the treatment of chronic hepatitis D.²²



Patterns of viral infection

Several patterns of infection define the spectrum of responses to HBV.³¹

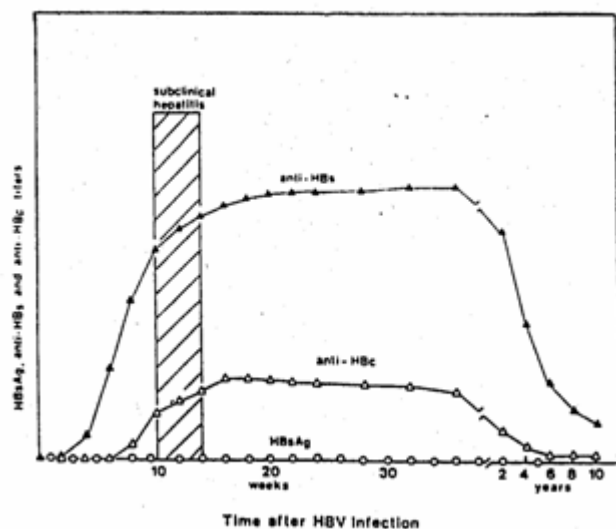
Self-limited HBsAg-positive primary HBV infection



From: Robinson WS. Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennett JE, and Dolin R, eds. *Principles and Practice of Infectious Diseases*, 4th ed. New York, Churchill Livingstone, 1995:1406-1439,³¹ with permission.

Figure 1 primary Schematic representation of viral markers in the blood through a typical course of self-limited HBsAg-positive HBV infection.³¹ This is the most common pattern of primary infection in adults.

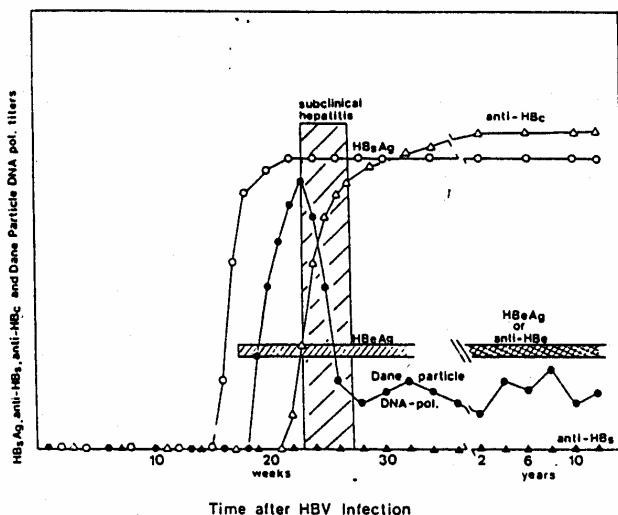
Self-limited primary infection without detectable serum HBsAg



From: Robinson WS. Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennett JE, and Dolin R, eds. *Principles and Practice of Infectious Diseases*, 4th ed. New York, Churchill Livingstone, 1995:1406-1439,³¹ with permission.

Figure 2 Schematic representation of the serologic response through a typical course of HBsAg-negative primary HBV infection.³¹ A significant number of patients with acute self-limited primary HBV infection never have detectable HBsAg in the blood.

HBsAg-positive persistent HBV infection



From: Robinson WS. Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennett JE, and Dolin R, eds. *Principles and Practice of Infectious Diseases*, 4th ed. New York, Churchill Livingstone, 1995:1406-1439,³¹ with permission ().

Figure 3 Schematic representation of viral markers in the blood through a typical course of HBV infection that becomes persistent.³¹ Patients who remain HBsAg-positive for 20 weeks or longer after primary infection are very likely to remain positive indefinitely and be designated chronic HBsAg carriers.³¹



Diagnosis

Large-scale screening for HBV infection

Diagnosis of hepatitis is made by biochemical assessment of liver function. Initial laboratory evaluation should include: total and direct bilirubin, ALT, AST, alkaline phosphatase, prothrombin time, total protein, albumin, globulin, complete blood count, and coagulation studies.^{15, 31}

Diagnosis is confirmed by demonstration in sera of specific antigens and/or antibodies. Three clinical useful antigen-antibody systems have been identified for hepatitis B:

- hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs)
- antibody (anti-HBc IgM and anti-HBc IgG) to hepatitis B core antigen (HBcAg)
- hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe)

Tests specific for complete virus particles or DNA and DNA polymerase-containing virions, and for HDAG and HDV RNA in liver and serum are available only in research laboratories.³¹

HBsAg can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious.^{15, 23, 31}

Very early in the incubation period, pre-S1 and pre-S2 antigens are present. They are never detected in the absence of HBsAg. Hepatitis B virions, HBV DNA, DNA polymerase, and HBeAg are then also detected. The presence of HBeAg is associated with relatively high infectivity and severity of disease.^{15, 31}

Anti-HBc is the first antibody to appear. Demonstration of anti-HBc in serum indicates HBV infection, current or past. IgM anti-HBc is present in high titre during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis. This test may therefore reliably diagnose acute HBV infection. IgG anti-HBc generally remains detectable for a lifetime.^{15, 23, 31}

Anti-HBe appears after anti-HBc and its presence correlates to a decreased infectivity. Anti-HBe replaces HBeAg in the resolution of the disease.^{15, 23, 31}

Anti-HBs replaces HBsAg as the acute HBV infection is resolving. Anti-HBs generally persists for a lifetime in over 80% of patients and indicates immunity.^{15, 23, 31}

Acute hepatitis patients who maintain a constant serum HBsAg concentration, or whose serum HBeAg persists 8 to 10 weeks after symptoms have resolved, are likely to become carriers and at risk of developing chronic liver disease.¹⁵ (LINK TO PAGE 27)

A complication in the diagnosis of hepatitis B is the rare identification of cases in which viral mutations change the antigens so they are not detectable.

Small-scale screening for HBV infection

Immunofluorescence studies, in situ hybridization, immunohistochemistry, and thin-section electron microscopy are used to examine pathological specimens for the presence of HBV-associated antigens or particles, providing information about the relationship between HBV DNA replication and HBV gene expression.¹⁵



Within the hepatocyte, HBsAg localizes in the cytoplasm, and HBcAg is seen in the nucleus and/or the cytoplasm. Detection of complete virions in the liver is uncommon.¹⁵

DNA hybridization techniques and RT-PCR assays have shown that almost all HBsAg/HBeAg-positive patients have detectable HBV DNA in their serum, whereas only about 65% of the HBsAg/anti-HBe-reactive patients are positive. All patients who recover from acute hepatitis B are negative for HBV DNA. On the other hand, some patients infected chronically who have lost their HBsAg remain HBV DNA positive.^{15, 31}

HBV serological markers in hepatitis patients

The three standard blood tests for hepatitis B can determine if a person is currently infected with HBV, has recovered, is a chronic carrier, or is susceptible to HBV infection.^{15, 23, 31}

Assay results			Interpretation
HBsAg	anti-HBs	anti-HBc	
+	-	-	Early acute HBV infection
+	+/-	+	Acute or chronic HBV infection. Differentiate with IgM-anti-HBc. Determine level of infectivity with HBeAg or HBV DNA.
-	+	+	Indicates previous HBV infection and immunity to hepatitis B.
-	-	+	Possibilities include: past HBV infection; low-level HBV carrier; time span between disappearance of HBsAg and appearance of anti-HBs; or false-positive or nonspecific reaction. Investigate with IgM anti-HBc, and/or challenge with HBsAg vaccine. When present, anti-HBe helps validate the anti-HBc reactivity.
-	-	-	Another infectious agent, toxic injury to the liver, disorder of immunity, hereditary disease of the liver, or disease of the biliary tract.
-	+	-	vaccine-type response.

From: Hollinger FB and Liang TJ. Hepatitis B Virus. In: Knipe DM et al., eds. *Fields Virology*, 4th ed., Philadelphia, Lippincott Williams & Wilkins, 2001:2971-3036,¹⁵ with permission (<http://lww.com>).

Interpretation of HBV serologic markers in patients with hepatitis.¹⁵



Host immune response

There is little evidence that humoral immunity plays a major role in the clearance of established infection. Cell-mediated immune responses, particularly those involving cytotoxic T-lymphocytes (CTLs), seem to be very important.^{30, 31}

CD8-positive, class I major histocompatibility complex (MHC)-restricted CTLs directed against HBV nucleocapsid proteins are present in the peripheral blood of patients with acute, resolving hepatitis B. Such cells are barely detectable in the blood of patients with chronic HBV infection, suggesting that the inability to generate such cells may predispose to persistent infection, although their absence from the blood in chronic infection may be due to their sequestration elsewhere.

CTLs against envelope glycoprotein determinants, that are often CD4-positive, class II MHC-restricted, have also been detected.

Primary infection leads to an IgM and IgG response to HBcAg shortly after the appearance of HBsAg in serum, at onset of hepatitis. Anti-HBs and anti-HBe appear in serum only several weeks later, when HBsAg and HBeAg are no longer detected, although in many HBsAg-positive patients, HBsAg-anti-HBs complexes can be found in serum.^{23, 30, 31}

Serological markers of HBV infection

During HBV infection, the serological markers vary depending on whether the infection is acute or chronic.^{11, 23, 31}

Antigens	Antibodies
HBsAg Hepatitis B surface antigen is the earliest indicator of acute infection and is also indicative of chronic infection if its presence persists for more than 6 months. It is useful for the diagnosis of HBV infection and for screening of blood. Its specific antibody is anti-HBs.	anti-HBs This is the specific antibody to hepatitis B surface antigen. Its appearance 1 to 4 months after onset of symptoms indicates clinical recovery and subsequent immunity to HBV. Anti-HBs can neutralize HBV and provide protection against HBV infection.
HBcAg Hepatitis B core antigen is derived from the protein envelope that encloses the viral DNA, and it is not detectable in the bloodstream. When HBcAg peptides are expressed on the surface of hepatocytes, they induce an immune response that is crucial for killing infected cells. The HBcAg is a marker of the infectious viral material and it is the most accurate index of viral replication. Its specific antibody is anti-HBc.	anti-HBc This is the specific antibody to hepatitis B core antigen. Antibodies to HBc are of class IgM and IgG. They do not neutralize the virus. The presence of IgM identifies an early acute infection. In the absence of HBsAg and anti-HBs, it shows recent infection. IgG with no IgM may be present in chronic and resolved infections. Anti-HBc testing identifies all previously infected persons, including HBV carriers, but does not differentiate carriers and non-carriers.
HBeAg Hepatitis B e antigen appearing during weeks 3 to 6 indicates an acute active infection at its most infectious period, and means that the patient is infectious. Persistence of this virological marker beyond 10 weeks shows progression to chronic infection and infectiousness. Continuous presence of anti-HBe indicates chronic or chronic active liver disease. HBeAg is not incorporated into virions, but is instead secreted into the serum. Mutant strains of HBV exist that replicate without producing HBeAg. HBeAg's function is uncertain. Its specific antibody is anti-HBe.	anti-HBe This is the specific antibody to hepatitis B e antigen. During the acute stage of infection the seroconversion from e antigen to e antibody is prognostic for resolution of infection. Its presence in the patient's blood along with anti-HBc and in the absence of HBsAg and anti-HBs indicates low contagiousness and convalescence.³¹
HBxAg Hepatitis B x antigen is detected in HBeAg positive blood in patients with both acute and chronic hepatitis. HBxAg is a transcriptional activator. It does not bind to DNA. Its specific antibody is anti-HBx.	anti-HBx This is the specific antibody to hepatitis B x antigen. It appears when other virological markers are becoming undetectable.
HBV DNA HBV DNA is detectable by PCR as soon as 1 week after initial infection, but the test is generally only performed for research purposes or to detect mutants that escape detection by current methods.	
HBV DNA polymerase Tests for the presence of HBV DNA polymerase, detectable within 1 week of initial infection, are only performed for research purposes.	



Serological test findings at different stages of HBV infection and in convalescence

Stage of infection	anti-HBc					
	HBsAg	anti-HBs	IgG	IgM	HBeAg	anti-HBe
late incubation period	+	-	-	-	+ or -	-
acute hepatitis B or persistent carrier state	+	-	+	+	+	-
HBsAg-negative acute hepatitis B infection	-	-	-	+	-	-
recovery with loss of detectable anti-HBs	-	-	+	-	-	-
healthy HBsAg carrier	+	-	+++	+ or -	-	+
chronic hepatitis B, persistent carrier state	+	-	+++	+ or -	+	-
HBV infection in recent past, convalescence	-	++	++	+ or -	-	+
HBV infection in distant past, recovery	-	+ or -	+ or -	-	-	-
recent HBV vaccination, repeated exposure to antigen without infection, or recovery from infection with loss of detectable anti-HBc	-	++	-	-	-	-

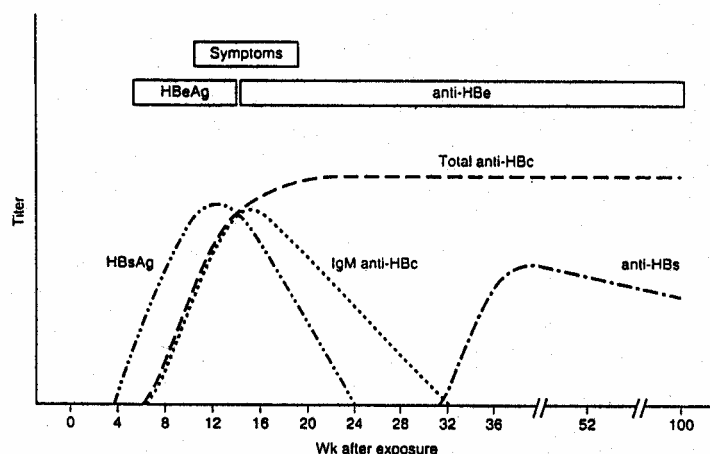
From: Robinson WS. Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennett JE, and Dolin R, eds. *Principles and Practice of Infectious Diseases*, 4th ed. New York, Churchill Livingstone, 1995:1406-1439,³¹ with permission.

Hepatitis B virus serological markers in different stages of infection and convalescence.^{23, 31, 52}



Serological and clinical patterns of acute or chronic HBV infections

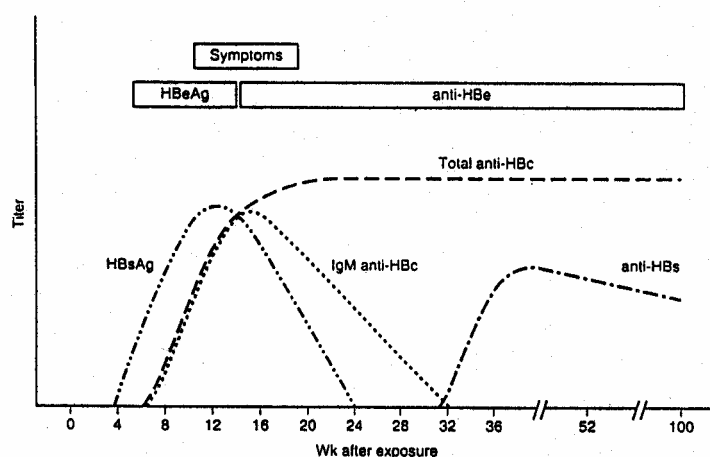
acute HBV infection



From: Mahoney FJ and Kane M. Hepatitis B vaccine. In: Plotkin SA and Orenstein WA, eds. *Vaccines*, 3rd ed. Philadelphia, W.B. Saunders Company, 1999:158-182,¹⁵ with permission.

Titre of hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), IgM anti-HBc, and antibody to hepatitis B surface antigen (anti-HBs) in patients with acute hepatitis B with recovery.²³

chronic HBV infection



From: Mahoney FJ and Kane M. Hepatitis B vaccine. In: Plotkin SA and Orenstein WA, eds. *Vaccines*, 3rd ed. Philadelphia, W.B. Saunders Company, 1999:158-182,¹⁵ with permission.

Titre of hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), and IgM anti-HBc during progression to chronic hepatitis B virus infection.²³



Interpretation of hepatitis B markers

Marker	Infection		
	acute	chronic	past
HBsAg	+	+	-
HBeAg	+ early, then -	+/-	-
anti-HBs	-	-	+
anti-HBc IgM	+	-	-
anti-HBc IgG	+	+	+
anti-HBe	- early, then +	+/-	+
HBV DNA	+ early, then -	+/-	-
ALT	increased (marked)	increased (mild-moderate)	normal

From: Gitlin N. *Hepatitis B: diagnosis, prevention, and treatment. Clinical Chemistry*, 1997, 43(8(B)):1500-1506,¹¹ with permission.



Discordant or unusual hepatitis B serological profiles requiring further evaluation

Repeat testing of the same sample or possibly of an additional sample is advisable when tests yield discordant or unusual results.¹⁵

HBsAg positive / anti-HBc negative	An HBsAg-positive response is accompanied by an anti-HBc negative reaction only during the incubation period of acute hepatitis B, before the onset of clinical symptoms and liver abnormalities.
HBsAg positive / anti-HBs positive / anti-HBc positive	Uncommon, may occur during resolution of acute hepatitis B, in chronic carriers who have serious liver disease, or in carriers exposed to heterologous subtypes of HBsAg.
anti-HBc positive only	Past infection not resolved completely
HBeAg positive / HBsAg negative	Unusual
HBeAg positive / anti-HBe positive	Unusual
anti-HBs positive only in a nonimmunized person	It may be a result of passive transfer of anti-HBs after transfusion of blood from a vaccinated donor, in patients receiving clotting factors, after IG administration, or in newborn children of mothers with recent or past HBV infection. Passively acquired antibodies disappear gradually over 3 to 6 months, whereas actively produced antibodies are stable over many years. Apparently quite common when person has forgotten his/her immunization status!

Mutant proteins from mutant HBV strains may escape diagnostic detection. The presence of different serological markers should therefore be tested for a correct diagnosis. Diagnostic kits should contain antibodies against a variety of mutant proteins, if perfection is the goal.

Prevalence

HBV occurs worldwide.^{23, 31}

The highest rates of HBsAg carrier rates are found in developing countries with primitive or limited medical facilities.²³



In areas of Africa and Asia, widespread infection may occur in infancy and childhood. The overall HBsAg carrier rates may be 10 to 15%.

The prevalence is lowest in countries with the highest standards of living, such as Great Britain, Canada, United States, Scandinavia, and some other European Nations.

In North America infection is most common in young adults. In the USA and Canada, serological evidence of previous infection varies depending on age and socioeconomic class. Overall, 5% of the adult USA population has anti-HBc, and 0.5% are HBsAg positive.

In developed countries, exposure to HBV may be common in certain high-risk groups (see section on Risk groups).

Adults infected with HBV usually acquire acute hepatitis B and recover, but 5 to 10% develop the chronic carrier state. Infected children rarely develop acute disease, but 25 to 90% become chronic carriers. About 25% of carriers will die from cirrhosis or primary liver cancer as adults.^{3, 23}

In the past, recipients of blood and blood products were at high risk (for HBV infection). Over the last 25 years, testing blood donations for HBsAg has become a universal requirement. Testing procedures have made major progress in sensitivity in the last 15-20 years. However 19% of countries reported that they were not testing all blood donations for HBsAg (WHO Global Database on Blood Safety, unpublished data). In the many countries where pretransfusion screening of blood donations for HBsAg is carried out systematically, the residual risk of HBV transmission is minimal. Moreover, plasma derived medicinal products (including antihaemophilic factors) undergo additional viral inactivation and removal procedures resulting in greatly reduced or no transmission of HBV by these products.

However, the risk is still present in many developing countries. Contaminated and inadequately sterilized syringes and needles have resulted in outbreaks of hepatitis B among patients in clinics and physicians' offices. Occasionally, outbreaks have been traced to tattoo parlors and acupuncturists. Rarely, transmission to patients from HBsAg positive health care workers has been documented.⁴¹

Reductions in the age-related prevalence of HBsAg in countries where hepatitis B is highly endemic and universal immunization of infants has been adopted suggest that it may be possible to eradicate HBV from humans.

Hepatitis B vaccines have been available since 1982 and have been used in hundreds of millions of individuals with an outstanding record of safety and impact on the disease. Carriage of HBV has already been reduced from high prevalence to low prevalence in immunized cohorts of children in many countries.



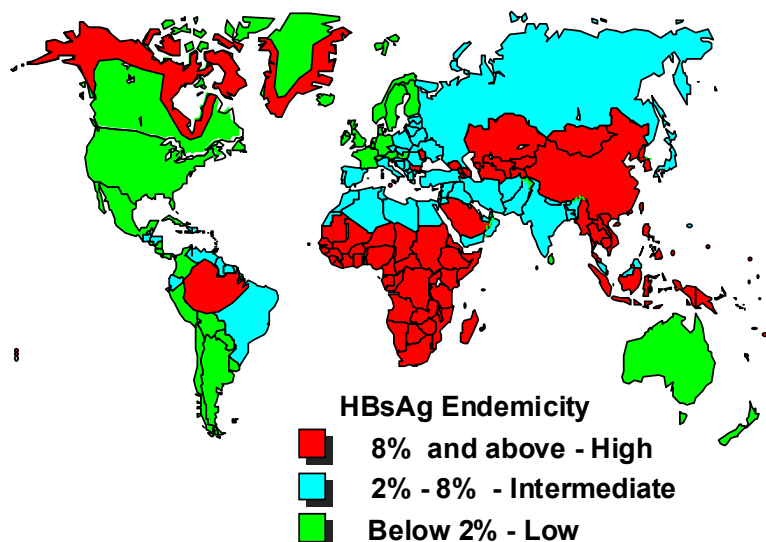
Prevalence of hepatitis B in various areas

Area	% of population positive for infection			
	HBsAg	anti-HBs	neonatal	childhood
Northern, Western, and Central Europe, North America, Australia	0.2-0.5	4-6	rare	infrequent
Eastern Europe, the Mediterranean, Russia and the Russian Federation, Southwest Asia, Central and South America	2-7	20-55	frequent	frequent
Parts of China, Southeast Asia, tropical Africa	8-20	70-95	very frequent	very frequent

From: Zuckerman AJ. Hepatitis Viruses. In: Baron S, eds. *Medical Microbiology*, 4th ed. Galveston, TX, The University of Texas Medical Branch at Galveston, 1996:849-863,⁵² with permission.



World distribution map



From: World Health Organization. *Introduction of hepatitis B vaccine into childhood immunization services, 2001*, Geneva, WHO, WHO/V&B/01.31

Geographical distribution of chronic hepatitis B virus infection. (Note: The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.)

Pathogenesis

HBV infection contracted early in life may lead to chronic hepatitis, then to cirrhosis, and finally to HCC, usually after a period of 30 to 50 years. Once infected with HBV, males are more likely to remain persistently infected than women, who are more likely to be infected transiently and to develop anti-HBs.

It is possible that in man HBV is not carcinogenic by a direct viral mechanism. Instead, the role of HBV may be to cause chronic liver cell damage with associated host responses of inflammation and liver regeneration that continues for many years. This pathological process, especially when leading to cirrhosis, may be carcinogenic without involving a direct oncogenic action of the virus. No viral oncogene, insertional mutagenesis, or viral activation of oncogenic cellular genes has been demonstrated.³⁰

The expression of HBV proteins and the release of virions precedes biochemical evidence of liver disease. Moreover, large quantities of surface antigen can persist in liver cells of many apparently healthy persons who are carriers. HBV is therefore not directly cytopathic.⁶

Three mechanisms seem to be involved in liver cell injury during HBV infections.

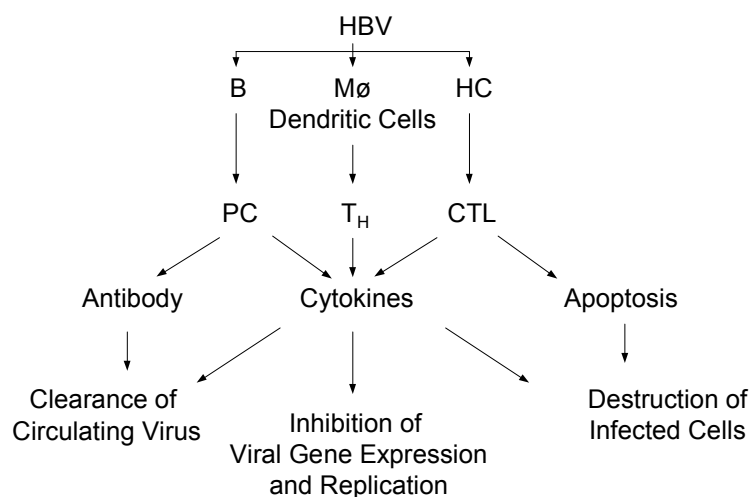
The first is an HLA class I restricted cytotoxic T-cell (CTL) response directed at HBcAg/HBeAg on HBV-infected hepatocytes.^{23, 30, 31}



A second possible mechanism is a direct cytopathic effect of HBcAg expression in infected hepatocytes.^{23, 30, 31}

A third possible mechanism is high-level expression and inefficient secretion of HBsAg.³¹

Hypothetical course of immunopathogenesis of hepatitis B virus (HBV)



From: Chisari FV and Ferrari C. *Viral Hepatitis*. In: Nathanson N et al., eds. *Viral Pathogenesis*, Philadelphia, Lippincott - Raven, 1997:745-778,⁶ with permission (<http://www.com>).

Eradication of HBV infection depends on the coordinate and efficient development of humoral and cell-mediated immune responses against HBV proteins. Antibodies secreted by plasma cells (PC) derived from antigen-specific B cells (which usually recognize viral antigens in their native conformation) are mostly responsible for the neutralization of free circulating viral particles, Cytotoxic T cells (CTL) that recognize endogenous viral antigens in the form of short peptides associated with human leukocyte antigen (HLA) class I molecules on the surface of the infected hepatocytes (HC) are the main effectors for the elimination of intracellular virus. They can do this by at least two different mechanisms: direct attachment to the cell membrane, causing the infected cell to undergo apoptosis; and the release of soluble cytokines that can downregulate viral gene expression, leading to the elimination of intracellular virus without destruction of the infected cell. Both humoral and cytotoxic functions are more or less stringently regulated by the helper effect of the CD4⁺ T cells (T_H) that recognize exogenous viral antigens, released or secreted by liver cells, in the form of short peptides that associate with HLA class II molecules in the endosomal compartment of professional antigen-presenting cells such as B cells, macrophages (MØ), and dendritic cells.

Transmission

Currently, there are four recognized modes of transmission:^{15, 39}

1. From mother to child at birth (perinatal)
2. By contact with an infected person (horizontal)
3. By sexual contact
4. By parenteral (blood-to-blood) exposure to blood or other infected fluids.



There is considerable variation between areas, countries and continents as to the age at which most transmission takes place.

There can be carriers with or without hepatitis.³¹

There is no convincing evidence that airborne infections occur and faeces are not a source of infection, since the virus is inactivated by enzymes of the intestinal mucosa or derived from the bacterial flora. HBV is not transmitted by contaminated food or water, insects or other vectors.^{15, 31}

HBsAg has been found in all body secretions and excretions. However, only blood, vaginal and menstrual fluids, and semen have been shown to be infectious.^{15, 23, 30, 31}

Transmission occurs by percutaneous and permucosal exposure to infective body fluids. Percutaneous exposures that have resulted in HBV transmission include transfusion of unscreened blood or blood products, sharing unsterilized injection needles for iv drug use, haemodialysis, acupuncture, tattooing and injuries from contaminated sharp instruments sustained by hospital personnel.^{15, 23, 31}

Sexual and perinatal HBV transmission usually result from mucous membrane exposures to infectious blood and body fluids. Perinatal transmission is common in hyperendemic areas of south-east Asia and the far East, especially when HBsAg carrier mothers are also HBeAg positive.^{15, 23, 31}

Infection may also be transmitted between household contacts and between sexual partners, either homosexual or heterosexual, and in toddler-aged children in groups with high HBsAg carrier rates.^{15, 23}

Immune globulins, heat-treated plasma protein fraction, albumin and fibrinolysin are considered safe when manufactured appropriately.

HBV is stable on environmental surfaces for at least 7 days, and indirect inoculation of HBV can occur via inanimate objects like toothbrushes, baby bottles, toys, razors, eating utensils, hospital equipment and other objects, by contact with mucous membranes or open skin breaks.³¹

Infectious HBV can be present in blood without detectable HBsAg, so that the failure to detect antigen does not exclude the presence of infectious virus.³¹

The source of infection cannot be identified in about 35% of cases.

The natural reservoir for HBV is man.³⁸ Closely related hepadnaviruses have been found in woodchucks and ducks, but they are not infectious for humans.¹⁰

The reuse of the same, unsterilized needle and syringe for vaccination of many different children accounts for many unnecessary HBV infections.^{31, 41}

People depending on repeated transfusion should be vaccinated against HBV.

HBV is about 100 times more infectious than HIV.³⁸

The role of non-human primates in the transmission of HBV

The only non-human primates that can develop productive HBV infection are the great apes (e.g. chimpanzees, orang-utans and gorillas). Chimpanzees have served as the model for the study of HBV infection for over 20 years.¹⁵



Although chimpanzees may be infected in nature, there is no evidence that they are important sources for human infections, because transmission from infected individuals requires specific patterns of intimate contact.³¹

Gibbons are susceptible to HBV and have been infected successfully experimentally and also naturally by contact in captivity.³⁵

Experimental infections of woolly monkeys, tamarins, and other primate species have generally been unsatisfactory.

Risk groups

Here is a list of groups of people who are at risk of contracting HBV:^{15, 31}

- infants born to infected mothers
- young children in day-care or residential settings with other children in endemic areas
- sexual/household contacts of infected persons
- health care workers
- patients and employees in haemodialysis centres^{4, 41}
- injection drug users sharing unsterile needles⁴¹
- people sharing unsterile medical or dental equipment
- people providing or receiving acupuncture and/or tattooing with unsterile medical devices
- persons living in regions or travelling to regions with endemic hepatitis B⁵⁰
- sexually active heterosexuals
- men who have sex with men

Frequent and routine exposure to blood or serum is the common denominator of healthcare occupational exposure. Surgeons, dentists, oral surgeons, pathologists, operating room and emergency room staff, and clinical laboratory workers who handle blood are at the highest risk.³¹

HBV infection is the major residual posttransfusion risk in developed countries because of the long window period, HBV mutants, the low viraemia (difficulties for PCR on pooled samples) and the very high infectivity.

Over one-third of patients with acute hepatitis B do not have readily identifiable risk factors.³

Efforts to vaccinate persons in the major risk groups have had limited success because of the difficulties in identifying vaccination candidates belonging to high risk groups. Moreover, regulations have to be developed to ensure the implementation of vaccination programs.^{3, 37}

High risk persons should be post-tested within 1-2 months of receipt of the third dose of HBV vaccine, to identify good responders to vaccination. This policy is cost-saving since adequate responders do not need to be retested or given HBIG whenever they later are exposed to HBV. They also do not need to be offered booster doses of vaccine periodically.



Surveillance and control

Hepatitis B disease surveillance procedures should include

- monitoring disease incidence
- determination of sources of infection and modes of transmission by epidemiological investigation
- detection of outbreaks
- spread containment
- identification of contacts of case-patients for postexposure prophylaxis

Hepatitis B disease control measures should include

- immunization, the most effective and cost-saving means of prevention.
- education of high risk groups and health care personnel to reduce the risk of contracting the virus and to reduce the chances for transmission to others, as well as to promote acceptance of vaccination schemes.
- screening of blood and blood products to reduce the chance that the blood supply system may contain pathogens like HBV.

Surveillance systems for hepatitis B vary in their methods and completeness. In many countries notification of HBV infections is mandatory. However, case definitions vary, laboratory confirmation is not always used, reporting systems differ, and distinctions are not always made between the types of viral hepatitis. In addition, underreporting of HBV infection is commonplace. Surveillance systems need therefore to be strengthened and standardized.

For better standardization of surveillance systems, countries should follow the case definition of viral hepatitis B recommended by WHO:

- **A clinical case of acute viral hepatitis** is an acute illness that includes the discrete onset of symptoms and jaundice or elevated serum aminotransferase levels (>2.5 times the upper limit of normal)
- **A confirmed case of hepatitis B** is a suspected case that is laboratory confirmed: HBsAg positive or anti-HBc-IgM positive, and anti-HAV-IgM negative.

The serological quality of the test used is crucial for firm diagnosis of infection. Countries without ready access to these tests may choose methods to detect HBsAg such as reverse passive haemagglutination (RPHA) or latex bead technology that are inexpensive. While not quite as sensitive as radioimmunoassay (RIA) antigen tests or enzyme-linked immunoabsorbent assay (ELISA), these tests are far better than not testing at all.¹⁵

Regardless of the availability of serological tests, all countries are advised to report all cases of jaundice and suspected viral hepatitis. Countries with laboratory facilities can differentiate further between hepatitis A, B, C, and other types of hepatitis. Surveillance reports should be submitted on a regular basis.



Endemicity

There are no seasonal preferences for primary HBV infections.^{15, 30}

Hepatitis B is highly endemic in all of Africa, some parts of South America, Alaska, northern Canada and parts of Greenland, eastern Europe, the eastern Mediterranean area, south-east Asia, China, and the Pacific Islands, except Australia, New Zealand and Japan. In most of these areas, 5 to 15% of the population are chronically infected carriers of HBV, and in some areas may also carry HDV, which may lead to severe liver damage.^{23, 42}

Even in low endemicity countries such as the USA, mortality from HBV was five times that from *Haemophilus influenzae* b (Hib) and ten times that from measles before routine vaccination of children was introduced.

Incidence/Epidemiology

The hepatitis B virus is a ubiquitous virus with a global distribution.^{15, 38}

Hepatitis B is one of the world's most common and serious infectious diseases. It is estimated that more than one third of the world's population has been infected with the hepatitis B virus. About 5% of the population are chronic carriers of HBV, and nearly 25% of all carriers develop serious liver diseases such as chronic hepatitis, cirrhosis, and primary hepatocellular carcinoma. HBV infection causes more than one million deaths every year.^{15, 23, 30, 39}

The HBsAg carrier rate varies from 0.1 to 20% in different populations around the world. The incidence of the HBsAg carrier state in populations is related most importantly to the incidence and age of primary infection.²³

In low-risk areas of the world, the highest incidence of the disease is seen in teenagers and young adults. Despite the low incidence of disease seen in the general population, certain groups who are sexually promiscuous or who have frequent contact with blood or blood products have a high rate of HBV infection. Nevertheless, the availability of an effective vaccine, optimized blood donor screening, and better sterilization procedures for blood derivatives have lowered substantially the infection risk.⁷⁵

In endemic areas of Africa and Asia, different epidemiological patterns are seen. In these regions, most infections occur in infants and children as a result of maternal-neonatal transmission or close childhood contact, although percutaneous exposure with contaminated needles or following unsafe injections is always a possibility in these countries.^{15, 23}

The chronic liver disease and HCC associated with HBV infections are among the most important human health problems in high-prevalence regions.



Trends

There is no seasonal trend similar to that observed in hepatitis A infections.¹⁵

Epidemics are unusual unless associated with contaminated blood or blood products, or the use of nonsterile injection equipment.

Evaluations of infant vaccination programs need to compare vaccination coverage data with population-based serological analyses, since most HBV infection in young children are asymptomatic and are therefore not detected in surveillance studies of acute disease. A decline in the prevalence of chronic disease is on the other hand a major indicator of program success and infection reduction.²³

A reduction in the prevalence of chronic HBV infection after implementation of infant immunization programmes has been demonstrated in high endemicity areas like Alaska, Taiwan, Indonesia, Polynesia, and the Gambia.²³

The implementation of routine infant immunization will eventually achieve broad-population-based immunity to HBV infection and prevent HBV transmission among all age groups. However, it is only in the longer term that infant immunization in countries that have adopted the HBV vaccination programme will affect the incidence of hepatitis B and the severe consequences of chronic infections.³⁷

Costs

Hepatitis B is a significant health problem and vaccination saves both money and lives. Consideration of epidemiological and economic data shows that universal vaccination strategies are cost-effective even in countries with a low prevalence of hepatitis B. Hepatitis B prevention programmes incorporating universal immunization of newborns and/or adolescents have been highly successful in Spain and Italy, and their success offers an exemplary model for other countries.³⁹

Even in low HBV endemicity areas of the world it is more cost-saving for the society to follow prevention programmes against HBV infection for the younger age groups than to face an increase in chronic liver disease among adults.³⁷

The cost of vaccines has fallen dramatically since the early 1980s, to the point that paediatric-dose vaccine in quantities of several hundred thousand can be found for less than US\$ 1 per dose in developing countries. However, even at US\$ 0.5 per dose, a three-dose series costs more than the other six childhood vaccines recommended by the WHO Expanded Programme on Immunization (EPI) combined (BCG, three doses of DTP, four doses of OPV, and measles vaccines). Cost therefore remains the primary obstacle to worldwide control of hepatitis B.²³

In the USA, the price of vaccination per dose is estimated at US\$ 41 if given by a general practitioner, US\$ 15 if administered through an existing childhood immunization programme, and US\$ 17 if given through the school medical system.³⁷

Immune prophylaxis

In 1974, a special lot of high-titred human hepatitis B IG designated HBIG was introduced. HBIG is similar to conventional IG preparations except that it is prepared from plasma preselected for a high titre of anti-



HBs (>100 000 IU/ml of anti-HBs by RIA). The process used to prepare HBIG inactivates and eliminates HIV from the final product. There is no evidence that HIV can be transmitted by HBIG.^{3, 15, 23, 31}

HBIG protects by passive immunization if given shortly before or soon after exposure to HBV. The protection is immediate, but it lasts only 3 to 6 months. HBIG is not recommended as pre-exposure prophylaxis because of high cost, limited availability, and short-term effectiveness. HBIG is generally not affordable in developing countries.^{15, 23, 31}

HBIG should be given to adults within 48 h of HBV exposure.²³

Maternal-neonatal transmission of HBV and the subsequent development of chronic hepatitis B in infected children has been reduced drastically, when HBIG was given to newborn babies of HBV carrier mothers in conjunction with the first dose of HB vaccine.^{15, 23}

HBV vaccination and one dose of HBIG, administered within 24 h after birth, are 85 to 95% effective in preventing both HBV infection and the chronic carrier state. HB vaccine administered alone beginning within 24 h after birth, is 70-95% effective in preventing perinatal HBV infection.²³

Routine infant immunization programmes have shown that the currently available vaccines confer as much protection upon the infants as does a combination of vaccine and HBIG. Therefore, the additional expenses for the administration of HBIG can be avoided.²³

With the availability of a vaccine against hepatitis B and mandatory screening of blood donors for HBsAg and anti-HBc, there is little justification for the use of HBIG in preexposure prophylaxis, except for individuals failing to respond to vaccine, or in patients with disorders that preclude a response (e.g. agammaglobulinaemia).^{15, 23, 31}

However, situations exist where postexposure prophylaxis is essential or desirable. The effectiveness appears to diminish rapidly if administration is delayed for more than 3 days. Passive immunization is now generally combined with active immunization induced by vaccine, providing immediate protection and more durable immunity.²³

Safety of immune globulin

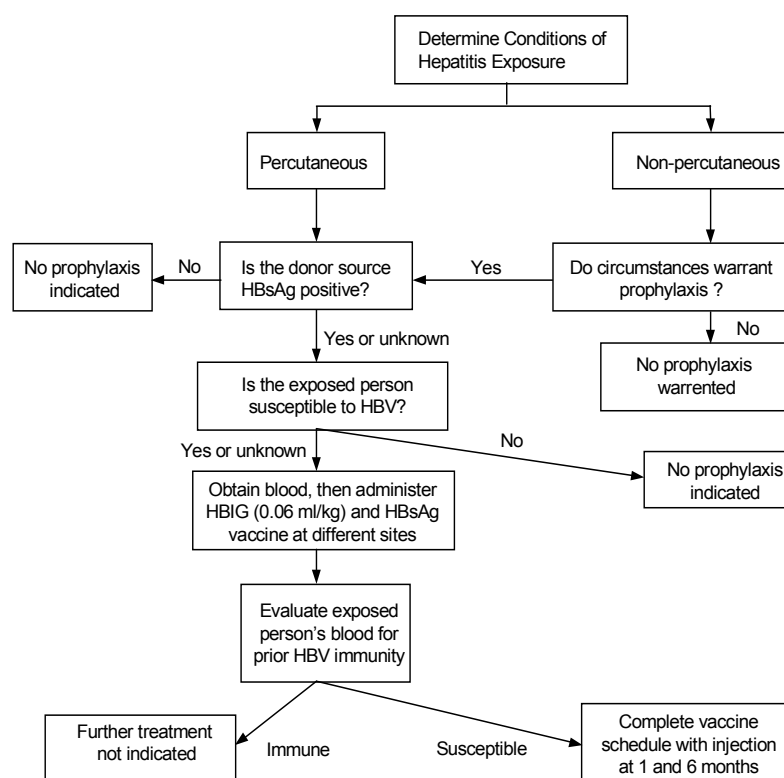
In 1972, routine screening of plasma donors for HBsAg was introduced, resulting in a sharp decline in the concentration of HBsAg inadvertently added to donor pools destined for IG production.¹⁵

Since 1977, all tested lots of commercial IG contain anti-HBs at a titer of at least 1:100 by RIA.¹⁵

Side effects associated with the administration of IG are rare.¹⁵



Postexposure prophylaxis



From: Hollinger FB and Liang TJ. *Hepatitis B Virus*. In: Knipe DM et al., eds. *Fields Virology*, 4th ed., Philadelphia, Lippincott Williams & Wilkins, 2001:2971-3036,¹⁵ with permission (<http://www.com>).

Algorithm for postexposure prophylaxis of healthcare personnel exposed to a potentially infectious source of HBV.

Abbreviation: HBIG, high-titred specific hepatitis B immune globulin.¹⁵

Vaccines

Hepatitis B is a vaccine-preventable disease, but although global control of hepatitis B is achievable, it has not been attained yet.^{5, 36, 37} In fact, a large pool of carriers and the burden of their disease remains, so that efforts must necessarily continue to treat the various stages of disease.

HB vaccine is the first and currently the only vaccine against a major human cancer. Vaccination is the most effective tool in preventing the transmission of HBV and HDV. Vaccines are composed of the surface



antigen of HBV (HBsAg), and are produced by two different methods: plasma derived or recombinant DNA. When administered properly, hepatitis B vaccine induces protection in about 95% of recipients.⁵

A safe and effective vaccine against HBV infection has been available for 20 years. HB vaccine is effective in preventing HBV infections when it is given either before exposure or shortly after exposure. At least 85%-90% of HBV-associated deaths are vaccine-preventable.

Despite the availability of a vaccine, worldwide infection persists.

Systematic hepatitis B vaccination of newborns renders the screening of pregnant women for HBsAg-status before delivery superfluous.²³

WHO recommends that hepatitis B vaccine be included in routine immunization services in all countries. The primary objective of hepatitis B immunization is to prevent chronic HBV infections which result in chronic liver disease later in life. By preventing chronic HBV infections, the major reservoir for transmission of new infections is also reduced,

Plasma-derived vaccines

These vaccines, derived from the plasma of HBsAg-positive donors, consist of highly purified, formalin-inactivated and/or heat-inactivated, alum-adsorbed, hepatitis B subviral particles (22 nm) of HBsAg that are free of detectable nucleic acid, and, therefore, noninfectious.^{15, 23}

The first plasma-derived hepatitis B vaccines manufactured in the USA and in France were licensed in 1981-1982 (Heptavax B®, Merck & Co., Hevac B®, Institut Pasteur). They contain 20 µg/ml HBsAg and the preservative thimerosal at a concentration of 1:20 000.^{3, 15, 31}

Plasma-derived HB vaccines are no longer produced in North America or western Europe, but several hundred million doses are produced in the Republic of Korea, China, Vietnam, Myanmar, India, Indonesia, Iran and Mongolia.^{15, 23, 31}

More than 200 million doses of plasma-derived vaccines have been distributed globally, and the safety record is impressive. Local reactions are generally insignificant clinically and are limited to mild pain or discomfort at the injection site in up to 25% of the vaccine recipients.¹⁵

Recombinant DNA yeast-derived or mammalian cell-derived vaccines

In the mid-1980s, an alternative, genetically engineered vaccine became available. The new technologies offer manufacturers a shorter production cycle (12 instead of 65 weeks), batch-to-batch consistency, and continuous supply of material, allowing the replacing of plasma-derived vaccines available on the market.^{15, 31}

In recombinant DNA technology, the S gene (pre-S1, pre-S2, S) is cloned and isolated, inserted into an expression plasmid and introduced into yeast (*S. cerevisiae*) or mammalian (Chinese hamster ovary, CHO) cells. The desired protein(s) is(are) expressed and assembled into 22 nm antigenic particles.^{15, 23, 31}

As on natural HBsAg particles, the a epitope that elicits the most important immune response is exposed on the surface of artificial particles. Natural and artificial particles differ in the glycosylation of HBsAg.^{15, 23}

The only mammalian cell-derived vaccine available is GenHevac B® (Pasteur Mérieux Connaught, 1993). GenHevac B® contains both preS2 and S proteins.¹⁵



The two major yeast-derived hepatitis B vaccines that are licensed in most countries are Engerix-B® (SmithKline Beecham, 1992) and Recombivax HB® (Merck & Co.). Both recombinant products contain nonglycosylated HBsAg particles (only S protein) that have been physicochemically purified, adsorbed on aluminium hydroxide, and preserved with thimerosal. Only Recombivax HB® is treated with formaldehyde.¹⁵

The yeast-derived HB-VAX DNA® (Pasteur Mérieux MSD), containing only the S protein, is produced in France.

Recombinant HB vaccines are produced in Belgium, China, Cuba, France, India, Israel, Japan, the Republic of Korea, Switzerland, the USA and Vietnam.²³

India has developed an indigenous yeast-derived, recombinant DNA vaccine, Shanvac-B® (Santha Biotechnics, 1997). At about US\$ 14 for three doses, Shanvac-B® is within the reach of the EPI.²

A licence application in both Europe and the USA has been filed in 1998 for Hepagene® (Medeva), the first recombinant hepatitis B vaccine to incorporate significant levels of HBV's pre-S1 and pre-S2 epitopes, and S protein. Further, like the surface of the virus itself, Hepagene®'s surface proteins are glycosylated. The result is that Hepagene® closely mimics the surface of HBV and produces a better immune response than that of other recombinant HB vaccines. Hepagene® has also been studied as an immunotherapy for the treatment of hepatitis B. Results are comparable with results reported after treatment with lamivudine.²⁹

Cross-protection by different serotype vaccines against different HBV subtypes has been observed in chimpanzees.¹⁵ Postexposure immunization after an HBV challenge has also been effective in chimpanzees.^{15, 28}

Vaccination of HBV carriers is safe but ineffective in eliminating HBsAg from chronically infected individuals.¹⁵ The HBV vaccine produces neither therapeutic nor adverse effects for individuals who possess antibodies against HBV from a previous infection. Passively acquired antibody will not interfere with active immunization.

Combination vaccines

The HBsAg vaccines (HB) can be combined with other vaccines such as Calmette-Guérin bacillus (BCG), measles, mumps, and rubella (MMR), Haemophilus influenzae b (Hib), and diphtheria, tetanus and pertussis combined with polio (DTP-polio). SmithKline Beecham offers a tetravalent DTP-HB vaccine, and a combined hepatitis A - hepatitis B vaccine.¹⁵

The combined hepatitis A and B vaccine (Twinrix®, SmithKline Beecham) has been introduced in Australia, Canada and some countries in Europe in 1997. In its adult formulation it contains 720 EL.U. of hepatitis A antigen (Havrix®) and 20 µg of hepatitis B surface antigen (Engerix®-B) adsorbed onto aluminium salts.^{15, 34}

Neonates born to mothers who are HBeAg-positive should be given a combination of passive and active immunization to provide immediate protection with HBIG in the first 6 h after delivery, followed by long-term immunity with the vaccine. At the currently recommended doses, HBIG does not interfere with the active immune response of the vaccine. When concurrent administration of HBIG and vaccine are contemplated, different sites should be used.¹⁵

The vaccines are to be administered by intramuscular injection in the anterolateral aspect of the thigh of newborns and infants or the deltoid (arm) muscle of children and adults in order to achieve optimal protection.^{3, 15}



The recommendation for universal infant vaccination neither precludes vaccinating adults identified to be at high risk of infection nor alters previous recommendations for postexposure prophylaxis for hepatitis B.³

Vaccine batches should be stored at 2-8°C but not frozen. Freezing destroys the potency of the vaccine since it dissociates the antigen from the adjuvant alum interfering with the immunogenicity of the preparation.¹⁵

The vaccine is thermostable and neither reactogenicity nor immunogenicity are altered after heating at 45°C for 1 week or 37°C for 1 month.²³

Factors that may reduce the immunogenicity of hepatitis vaccines include age (>40 years), gender, weight, genetics, haemodialysis, HIV infection, immunosuppression, tobacco smoking, subcutaneous injection, injection into the buttocks, freezing of vaccine, and accelerated schedule.^{15, 31}

An initial anti-HBs titre of >10 IU/l is regarded as being protective. Although the initial anti-HBs titre is followed by a decline of antibody, a rapid anamnestic response develops after exposure to the virus.^{23, 31}

The duration of vaccine-induced immunity is uncertain but it is definitely long term (>15 years). At present there is no recommendation for the administration of booster doses, although future studies could demonstrate a need for boosters.^{15, 23, 31}

A recent study designed to determine the safety and immunogenicity of a DNA vaccine consisting of a plasmid encoding hepatitis B surface antigen delivered into human skin suggests that this gene delivery system may induce a booster response, but that the vaccine at the dose used (0.25 µg) did not induce primary immune responses.³²



Hepatitis B vaccines available internationally

Manufacturer	Brand name*	Country	Type
Centro de Ingenieria Genetica Y Biotecnologia	Enivac-HB	Cuba	Recombinant DNA
Chiel Jedang	Hepaccine-B	South Korea	Plasma derived
Korea Green Cross	Hepavax B	South Korea	Plasma derived
Korea Green Cross	Hepavax-Gene	South Korea	Recombinant DNA
LG Chemical	Euvax B	South Korea	Recombinant DNA
Merck Sharp & Dohme	Recompivax H-B-Vax II	United States	Recombinant DNA
Merck Sharp & Dohme	Comvax	United States	Combined Hib and (recombinant)
Pasteur Mérieux Connaught	Genhevac B	France	Recombinant DNA (mammalian cell)
SmithKline Beecham	Engerix-B	Belgium	Recombinant DNA
SmithKline Beecham	Twinrix	Belgium	Combined hepatitis A and B (recombinant)
SmithKline Beecham	Tritanrix-HB	Belgium	Combined DTP and recombinant
SmithKline Beecham	Infanrix-HB	Belgium	Combined DTP (acellular P) and HB (recombinant)
Swiss Serum and Vaccines Institute	Heprecombe	Switzerland	Recombinant DNA (mammalian cell)

Numerous producers who sell only in country of production are not listed. Presence on this list does not imply endorsement of these products by the World Health Organization.

* Brand names may vary in different countries.

Abbreviations: DTP, diphtheria, tetanus and pertussis; HB, hepatitis B; Hib, *Haemophilus influenza* type b.

From: Mahoney FJ and Kane M. Hepatitis B vaccine. In: Plotkin SA and Orenstein WA, eds. *Vaccines*, 3rd ed. Philadelphia, W.B. Saunders Company, 1999:158-182.²³ with permission.

Recommendations for preexposure immunization with hepatitis B vaccine

Here is a list of groups for whom preexposure vaccination is recommended. If all members of these groups were immunized, the incidence of hepatitis B would decrease rapidly.^{3, 5, 15}

- Infants (universal immunization)
- Infants and adolescents not vaccinated previously (catch-up vaccination)
- Persons with occupational risk (exposure to blood or blood-contaminated environments) and students of health-care professions before they have blood contact



- Clients and staff of institutions for the developmentally disabled and susceptible contacts in day-care programs who are at increased risk from HBV carrier clients with aggressive behaviour or special medical problems that increase the risk of exposure
- Haemodialysis patients. Vaccination before dialysis treatment is recommended
- Recipients of frequent and/or large volumes of blood or blood components
- Susceptible injecting drug abusers
- Sexually active men or women (homosexual and bisexual men; persons with recently acquired sexually transmitted disease; prostitutes; promiscuous heterosexuals)
- Susceptible inmates of long-term correctional facilities who have a history of high risk behaviour
- Household contacts and sex partners of HBV carriers
- Populations with a high incidence of disease
- International travellers to areas of high HBV endemicity if specific at-risk circumstances exist.⁵⁰
- Transplant candidates before transplantation

In 1991 the WHO/EPI recommended that HB vaccine be included in national immunization programmes in all countries with an HBV carrier rate of 8% or over by 1995, and in all other countries (regardless of HBsAg prevalence) by 1997. Countries with a low prevalence may consider immunization of all adolescents (before age of 13) as an addition or alternative to infant immunization.

So far (March 2002) 151 countries (Albania, American Samoa, Andorra, Anguilla, Antigua and Barbuda, Argentina, Armenia, Australia, Austria, Azerbaijan, Bahamas, Bahrain, Barbados, Belarus, Belgium, Belize, Bermuda, Bhutan, Bolivia, Bosnia and Herzegovina, Botswana, Brazil, British Virgin Islands, Brunei Darussalam, Bulgaria, Cambodia, Canada, Cayman Islands, China, C.N. Mariana Islands, Colombia, Cook Islands, Costa Rica, Côte d'Ivoire, Cuba, Cyprus, D. People's R. of Korea, Dominica, Dominican Republic, Ecuador, Egypt, El Salvador, Eritrea, Estonia, Fiji, France, French Guiana, French Polynesia, Gambia, Georgia, Germany, Ghana, Greece, Grenada, Guadeloupe, Guam, Guyana, Honduras, Indonesia, Iran (Islamic Republic of), Iraq, Israel, Italy, Jamaica, Jordan, Kazakhstan, Kenya, Kiribati, Kuwait, Kyrgyzstan, Lao People's D. R., Latvia, Lebanon, Libyan Arab Jamahiriya, Lithuania, Luxembourg, Madagascar, Malawi, Malaysia, Maldives, Marshall Islands, Martinique, Mauritius, Mexico, Micronesia (Federated States of), Monaco, Mongolia, Montserrat, Morocco, Mozambique, Nauru, Netherlands Antilles, New Caledonia, New Zealand, Nicaragua, Niue, Oman, Pakistan, Palau, Panama, Papua New Guinea, Paraguay, Peru, Philippines, Poland, Portugal, Puerto Rico, Qatar, Republic of Korea, Romania, Rwanda, Saint Kitts and Nevis, Saint Vincent and the Grenadines, Samoa, San Marino, Saudi Arabia, Seychelles, Singapore, Slovakia, Slovenia, Solomon Islands, South Africa, Spain, Suriname, Swaziland, Syrian Arab Republic, Tajikistan, Thailand, The Republic of Moldova, Tokelau, Tonga, Trinidad and Tobago, Tunisia, Turkey, Turkmenistan, Turks and Caicos Islands, Tuvalu, Ukraine, United Arab Emirates, United Nations Relief and Works, United Republic of Tanzania, United States of America, Uruguay, U.S. Virgin Islands, Uzbekistan, Vanuatu, Venezuela, Viet Nam, Wallis and Futuna Islands, West Bank and Gaza, Yemen, Zimbabwe (from: WHO/V&B/VAM)) have introduced hepatitis B vaccine within their national immunization programmes.^{19, 37, 42}

In other countries, universal vaccination is still being postponed. The reasons for this are the weakness of a social commitment to preventive medicine and vaccines, the lack of medical and public awareness, the view of hepatitis B infection as a limited public health problem that does not justify the expense and other efforts of universal immunization, and the financial burden of national programmes.^{17, 37, 42}



Vaccine coverage in rural areas of many HBV high endemicity countries is a logistical and economic challenge. Since 1994 UNICEF, WHO, and several other international donor agencies have been helping developing countries to obtain HB vaccine and implement national programmes.

Recommended dosages and schedules for preexposure prophylaxis with hepatitis B vaccines licensed in the USA

Currently, two primary immunization doses given intramuscularly are followed using three injections at 0, 1, and 6 months or four injections at 0, 1, 2, and 12 months.^{3, 15, 31} For routine preexposure prophylaxis, the three-injections schedule is preferred, whereas the four-dose regimen is preferred for immunocompromised patients or in postexposure prophylaxis situations.

Group	Heptavax-HB® (0, 1, 6 months)	Recombivax HB® (0, 1, 6 months)	Engerix B® (0, 1, 6 months°)
Infants of HBsAg-positive mothers	10 µg	5.0 µg (US\$ 28.84)	10 µg
Children (≤10 years)	10 µg	5.0 µg (US\$ 28.84)	10 µg
Adolescents (11-19 years)	20 µg	5.0 µg (US\$ 28.84)	20 µg (US\$ 54.35)
Adults (≥20 years)	20 µg	10 µg (US\$ 59.50)	20 µg (US\$ 54.35)
Immunocompromised patients	40 µg	40 µg (US\$ 167.91)	40 µg

° Alternate 4-dose schedule of 0, 1, 2, and 12 months is standard for immunocompromised patients and when more rapid induction of antibody is desired.

Prices are the average wholesale costs per single-unit dose in the USA.

HB vaccines are packaged to contain 10-40 µg of HBsAg protein/ml after adsorption to aluminium hydroxide (0.5 mg/ml), thimerosal (1:20000 concentration) is added as a preservative.^{3, 49}

A four-dose schedule with a yeast-derived vaccine (2.5 µg of HBsAg at 0, 1, 2, and 12 months) provides a protective efficacy rate that is comparable with that found after combined HBIG plus vaccine therapy.

Vaccinees examined for anti-HBs concentration after completion of the basic immunization doses may show an inadequate level of protection if their anti-HBs values are below 10 mIU/ml. In these cases, booster doses consisting of one or two additional injections of vaccine are recommended.^{7, 12, 15}

The course of vaccination should never be started over when a scheduled dose is missed or postponed, but should be completed in due course.

The immune response when one or two doses of a vaccine produced by one manufacturer are followed by subsequent doses from a different manufacturer is comparable with that resulting from a full course of vaccination with a single vaccine.³

There is no evidence that nonresponders to plasma-derived vaccine will respond to genetically engineered vaccines.



Neither HBIG nor normal IG is recommended for preexposure prophylaxis because active immunization with HBsAg (vaccination) is more effective and gives long-term protection.¹⁵

Anti-HBs seroconversion rates after hepatitis B vaccination (%)

Neonates	>95%
Age (years)	
2-19	~99%
20-29	~95%
30-39	~90%
40-49	~85%
50-59	~70%
>59	~50%
Renal failure, HIV infection, other immunosuppression	50-70%
Liver disease	60-70%

Abbreviation: HIV, human immunodeficiency virus.

From Robinson WS. Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennett JE, and Dolin R, eds. *Principles and Practice of Infectious Diseases*, 4th ed. New York, Churchill Livingstone, 1995:1406-1439,³¹ with permission.

Recommendations for postexposure prophylaxis for perinatal or sexual exposure to HBV

Exposure	HBIG		Vaccine	
	dose (i.m.)	timing	dose (i.m.) ^o	timing (first dose)
perinatal	0.5 ml	<12 h of birth	0.5 ml	<12 h of birth
sexual	0.06 ml/kg	<14 days since last exposure	1.0 ml	Concurrent with HBIG (first dose)

^o Each ml contains 10 µg Recombivax HB[®] or 20 µg Engerix-B[®]; subsequent doses at 1 and 6 months for either vaccine or the alternate four-dose schedule (0, 1, 2, and 12 months) for Engerix-B[®]. HBIG and vaccine should be administered at different sites.

Abbreviations: ACIP, Advisory Committee on Immunization Practices;

From: Centers for Disease Control and Prevention. *Hepatitis B virus: A comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination: recommendations of the immunization practices advisory committee (ACIP)*.³

All pregnant women should be routinely tested for HBsAg before delivery, so that newborns of positive mothers can be appropriately immunized after birth. In developing countries, where funds and infrastructure to screen pregnant women may not be available, routine vaccination of infants at birth may be appropriate.³

Postexposure immunization should especially be considered for neonates born of HBsAg-positive mothers. Such infants are infected commonly, especially when mothers are HBeAg-positive, and the risk of becoming chronic carriers is extremely high (90%). When HBIG is given within the first hours after birth, the risk of infection can be reduced to 20%.^{3, 15, 31}



Ongoing clinical trials will determine whether HBIG alone, HBIG and vaccine, or vaccine alone is sufficient to prevent hepatitis B after percutaneous inoculation, oral ingestion, or direct mucous membrane contact with HBsAg-positive materials.¹⁵

No postexposure prophylaxis is indicated for contamination of unbroken skin, for staff members who provide routine care of patients with hepatitis B, or for people who inadvertently share food or utensils with a person who subsequently develops hepatitis B unless there are extenuating circumstances.¹⁵

Following sexual exposure to an infected person, it is currently recommended to use both HBIG and hepatitis B vaccine.^{15, 31}

For no reason should an HBIG be delayed until the results of HBV tests become available. There is no precedent for recommending HBIG prophylaxis if HBV exposure has occurred more than 7 days earlier. If a significant delay is anticipated in obtaining or dispensing the HBIG, conventional IG containing anti-HBs should be substituted for the HBIG until HBIG can be dispensed.^{15, 31}

Vaccine safety

Side effects are local, of low intensity and short duration, involving a generally clinically insignificant soreness at the injection site or a mild to moderate fever for 1-2 days following injection.^{5, 15, 23, 31}

Persons allergic to vaccine components should follow the recommendations for the use of HBIG.³¹

Neither pregnancy nor lactation should be considered a contraindication to vaccination of women.^{3, 23}

Vaccination does no harm to HBV-immune or HBV-carrier recipients.

Hypersensitivity reactions can be expected in some individuals who are allergic to yeast antigens. The yeast-derived vaccine is not recommended for such individuals.^{23, 31}

Both plasma-derived and yeast-derived hepatitis B vaccines are effective and safe for the prevention of HBV infection.^{15, 31}

A potential problem for hepatitis B vaccines may be the naturally occurring mutants that alter HBsAg specificity and permit mutant virus to escape the immune response to vaccination.²⁸ Such mutants are rare, but if they were to arise more frequently, they would require changes in HBV vaccines and in diagnostic testing procedures.³¹ As of today no such effect with public health implications has been observed (see section on "Hepatitis B virus mutants" (LINK TO PAGE 73)).

There is no scientific evidence that hepatitis B vaccine causes or exacerbates multiple sclerosis (MS) or other central nervous system demyelinating diseases.^{5, 23, 25, 31, 40, 46-48}

While any risk of MS following hepatitis B vaccination is hypothetical and so far unconfirmed, the risk of HBV infection and disease in non-immunized individuals is real. Hepatitis B causes about 4 million acute infections worldwide per year, and currently there are more than 350 million HBV carriers, about 25% of whom will die from cirrhosis or primary liver cancer.^{39, 40}

Hepatitis B vaccines are safe, more than 90% effective in preventing HBV infection, and particularly cost-effective. Unfortunately, unsubstantiated claims that HB vaccines might cause MS are reducing the uptake of this important vaccine in a few countries.⁵



WHO strongly recommends that all countries already using hepatitis B vaccine as a routine vaccine in their national immunization programmes continue to do so, and that all countries not yet using the vaccine begin as soon as possible.

Hepatitis B virus mutants

Antibodies to the antigenic determinant a mediate cross-protection against all subtypes.⁵²

The epitope a is located in the region of amino acids 124-148 of the major surface protein, and appears to have a double-loop conformation.⁵²

During a study on the immunogenicity and efficacy of hepatitis B vaccines in Italy, some patients who had mounted a successful immune response to the vaccine and become anti-HBs positive, later became infected with HBV. A characteristic for these cases was the coexistence of non-complexed anti-HBs and HBsAg, in the presence of other markers of HBV infection. Analysis of HBsAg using monoclonal antibodies suggested that the a epitope was either absent or masked. Sequencing of the HBV DNA isolated from these patients revealed a mutation in the sequence encoding the a epitope, showing a substitution of arginine for glycine at amino acid position 145 (G to A substitution, nt 587). This point mutation in the HBV genome has been found subsequently in viral isolates from Singapore, Japan, US, Germany, UK, Brunei and elsewhere.^{52, 53}

The region in which the mutation occurs is an important epitope to which vaccine-induced neutralizing antibodies bind, but the mutant virus is not neutralized by antibody to this specificity. The mutant virus replicates efficiently, implying that the amino acid substitution does not alter the binding of virions to the liver cell.^{52, 53}

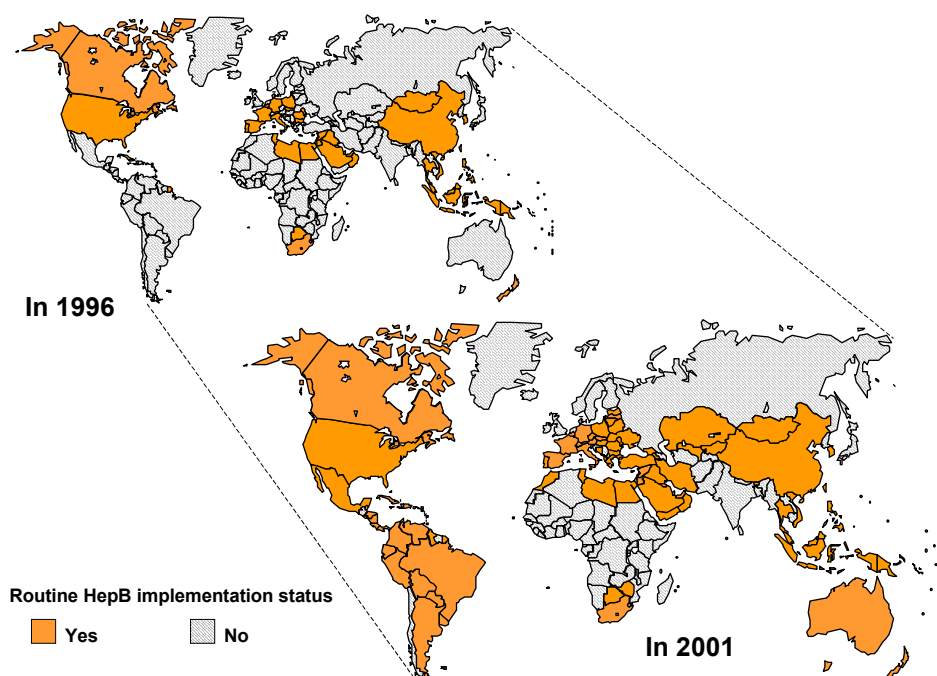
Variants of HBV with altered antigenicity of the envelope protein show that HBV is not as antigenically singular as thought previously.^{52, 53}

Two concerns arise from this finding: failure to detect HBsAg may lead to transmission through donated blood or organs, and mutant HBV may infect individuals who are anti-HBs positive after immunization.

Mutant strains of HBV are being sought and studied in many laboratories.^{28, 44, 52, 53}



Hepatitis B vaccine immunization policies



From: WHO/V&B/VAM

Progression of countries using hepatitis B vaccine in their national immunization system. (Note: The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.)

Prevention

The prevention of chronic HBV infection has become a high priority in the global community.²³

Immunization with hepatitis B vaccine is the most effective means of preventing HBV infection and its consequences.^{3, 11, 15, 30, 36}

HBIG protects by passive immunization if given shortly before or soon after exposure to HBV. It is also administered in combination with HBV vaccines to newborns of HBsAg positive mothers. The protection is immediate, but of short duration. HBIG is not recommended as a pre-exposure prophylaxis because of high cost, limited availability, and short-term effectiveness.^{11, 15}

Preventing HBV transmission during early childhood is important because of the substantial likelihood of chronic HBV infection and chronic liver disease that occurs when children less than 5 years of age become infected.³

Integrating HB vaccine into childhood vaccination schedules has been shown to interrupt HBV transmission.³

Routine screening of blood donors for HBsAg was mandated in 1972 (USA). The introduction of anti-HBc screening in 1986 (USA) has efficiently excluded those donors who were persistent, low-level carriers, and those in the window period of acute infection.¹⁵

The current overall risk of acquiring HBV after a transfusion is about one in 50 000 per recipient. Unfortunately, donors who are in the early incubation stage of their disease, capable of transmitting HBV, will remain unidentified with current techniques. The objective of no-risk blood supply is therefore not achievable.¹⁵

In order to avoid unnecessary risks of HBV infection, patients who depend on recurrent transfusion should be vaccinated.

Universal precautions should be used when handling human blood and body fluids. Specific precautions include the use of gloves, protective garments, and masks, when handling potentially infectious or contaminated materials.¹⁵

There is no substitute for good personal hygiene, strict surveillance, and appropriate environmental control measures to limit transmission.¹⁵

Autoclaving and the use of ethylene oxide gas are accepted methods for disinfecting metal objects, instruments, or heat-sensitive equipment.¹⁵

The expense and difficulty of treating hepatitis B medically and by hepatic transplantation is in contrast with the fact that the infection can be prevented by vaccination.

Vaccines against hepatitis B were introduced in the early 1980s. Recombinant vaccines became available in the mid 1980s. More than 110 countries have adopted a national policy of immunizing all infants with hepatitis B vaccine.



In endemic areas, mass immunization campaigns are under way mainly in East and South East Asia, the Pacific basin and the Middle East. Some regions in some countries in South America, and some countries in Africa, have started mass immunization.

There are plans to increase coverage in Africa.⁴²

Vaccination campaigns have shown that control of the disease is feasible, even in endemic areas. Some countries incorporate hepatitis B immunoglobulin (HBIG) in their vaccination strategies.

In endemic areas, procurement of low cost vaccine, education and acceptance, vaccine integration in the expanded program of immunization (EPI), prevention of vertical transmission, antibody escape mutations, protective efficacy, long term immunity and natural boosting are important questions and issues.

Since most HBV carriers are unaware of their condition, but pose a significant risk to health care workers and other people exposed to their blood, workers are advised to assume that all patients are potentially infectious, and should practice “universal precautions”.

Hepatitis B immunization

Introducing hepatitis B vaccine into national immunization services⁵¹

Immunization strategies

Routine infant immunization:

HB immunization of all infants as an integral part of the national immunization schedule should be the highest priority in all countries.

Additional immunization strategies that should be considered depending on the epidemiology of HBV transmission in a particular country are:

- Prevention of perinatal HBV transmission

In order to prevent HBV transmission from mother to infant, the first dose of HB vaccine needs to be given as soon as possible after birth (preferably within 24 hours). In countries where a high proportion of chronic infections is acquired perinatally (e.g. South-east Asia), a birth dose should be given to infants. It is usually most feasible to give HB vaccine at birth when infants are born in hospitals. Efforts should also be made in these countries to give HB vaccine as soon as possible after delivery to infants delivered at home. In countries where a lower population of chronic infections is acquired perinatally (e.g. Africa), the highest priority is to achieve high DTP3 and HB3 vaccine coverage among infants. In these countries, use of a birth dose may also be considered after disease burden, cost-effectiveness, and feasibility are evaluated

- Catch-up vaccination of older persons

In countries with a high prevalence of chronic HBV infection (HBsAg prevalence $\geq 8\%$), catch-up immunization is not usually recommended because most chronic infections are acquired among children <5 years of age, and thus, routine infant vaccination will rapidly reduce HBV transmission. In countries with lower endemicity of chronic HBV infection, a higher proportion of chronic infections may be acquired among older children, adolescents and adults; catch-up immunization for these groups may be considered.



Vaccine formulations

Hepatitis B vaccine is available in monovalent formulations that protect only against HBV infection and also in combination formulations that protect against HBV and other diseases.

- Monovalent hepatitis B vaccines *must be used* to give the birth dose of hepatitis B vaccine.
- Combination vaccines that include hepatitis B vaccine must not be used to give the birth dose of hepatitis B vaccine because DTP and Hib vaccines are not recommended to be given at birth.
- Either monovalent or combination vaccines may be used for later doses in the hepatitis B vaccine schedule. Combination vaccines can be given whenever all of the antigens in the vaccine are indicated.

Schedule

Hepatitis B vaccine schedules are very flexible; thus, there are multiple options for adding the vaccine to existing national immunization schedules without requiring additional visits for immunization.

Practically, it is usually easiest if the 3 doses of hepatitis B vaccine are given at the same time as the 3 doses of DTP (Option I). This schedule will prevent infections acquired during early childhood, which account for most of the HBV-related disease burden in high endemic countries, and also will prevent infections acquired later in life.

However, this schedule will not prevent perinatal HBV infections because it does not include a dose of hepatitis B vaccine at birth. Two schedule options can be used to prevent perinatal HBV infections: a 3-dose schedule of monovalent hepatitis B vaccine, with the 1st dose given at birth and the 2nd and 3rd doses given at the same time as the 1st and 3rd doses of DTP vaccine (Option II); or a 4-dose schedule in which a birth dose of monovalent HepB vaccine is followed by 3 doses of a combination vaccine, e.g. DTP hepatitis B (Option III). The 3-dose schedule (Option II) is less expensive, but may be more complicated to administer, because infants receive different vaccines at the 2nd immunization visit than at the 1st and 3rd visits. The 4-dose schedule (Option III) may be easier to administer in practice, but is more costly, and vaccine supply issues may make it unfeasible.

Administration

Hepatitis B vaccine is given by intramuscular injection in the anterolateral aspect of the thigh (infants) or deltoid muscle (older children). It can be given safely at the same time as other vaccines (e.g. DTP, Hib, measles, OPV, BCG, and yellow fever). If the hepatitis B vaccine is given on the same day as another injectable vaccine, it is preferable to give the two vaccines in different limbs.

Injection equipment

The injection equipment for hepatitis B vaccine is the same type as that for all other EPI vaccines (except for BCG vaccine):

- 0.5 ml auto-disable (AD) syringes are recommended.
- If AD syringes are not available, standard disposable syringes (1.0ml or 2.0ml) must be used ONCE ONLY, and safely disposed of after use.
- - A 25 mm, 22 or 23 gauge needle is recommended.

Dosage

The standard paediatric dose is 0.5 ml.



Vaccine procurement

In most countries, hepatitis B vaccine procured through The Vaccine Fund will be supplied through the UNICEF procurement mechanism. The number of hepatitis B vaccine doses required is estimated using the size of the birth cohort, the coverage rate for DTP and the number of doses in the immunization schedule. These calculations should also include wastage and the size of the reserve stock.

Presentation

Hepatitis B vaccines are available in liquid single-dose and multi-dose glass vials, and in pre-filled single-dose injection devices (e.g. Uniject™).

Storage and shipping volume

Storage volumes (vial plus packet containing vial plus other packaging) for hepatitis B vaccines supplied through UNICEF are as per the figure below: For comparison, the total storage volume for other EPI vaccines (BCG, DTP, measles, OPV, TT) is about 11.0 cm³ per dose.

Cold chain issues

The storage temperature for hepatitis B vaccine is the same as for DTP vaccine, from 2°C to 8°C. *Hepatitis B vaccine should never be frozen.* If frozen, hepatitis B vaccine loses its potency.

Adding hepatitis B vaccine to the national immunization schedule will require cold chain assessments at all administrative levels:

- to assure adequate storage capacity is available, and
- to assure policies and procedures are in place to prevent freezing of hepatitis B vaccine.

Reducing vaccine wastage

Since hepatitis B vaccines are more expensive than the traditional EPI vaccines, it is important to monitor vaccine wastage and to develop and implement strategies to reduce wastage.

Strategies to reduce wastage include:

- careful planning of vaccine ordering and distribution;
- implementation of WHO's multi-dose vial policy;
- appropriate use of single-dose and multi-dose vials;
- careful maintenance of the cold chain;
- attention to vaccine security; and
- reducing missed opportunities for immunization.

Injection safety

Hepatitis B vaccine should be supplied with AD syringes and safety boxes.

Managers at each level are responsible for ensuring that adequate supplies are available at all times so that each injection is given with a sterile injection device. Attention should also be given to proper use and disposal of safety boxes to collect these materials.



Revision of Immunization forms and materials

An important element of integrating hepatitis B vaccine into national immunization programmes is to revise training and informational materials, immunization cards and forms used to monitor and evaluate immunization services.

Training

Training for health care staff is essential because these staff are responsible for handling and administering hepatitis B vaccine and they are a major source of information for parents and others in the general public.

Advocacy and communication

Advocacy and communication efforts are important in order to generate support and commitment for the new vaccine. The primary target audiences are decision-makers/opinion leaders, health care staff, and the general public (including parents).

What information is needed to assess hepatitis B disease burden?

Adequate seroprevalence data needed to assess hepatitis B disease burden are generally available in all countries, or from adjacent countries with similar HBV endemicity. Thus, additional seroprevalence studies are usually not needed.

How should hepatitis B vaccine be phased into the existing infant immunization services?

A strategy in which hepatitis B vaccine is given to infants who have not yet completed the DTP vaccine series at the time hepatitis B vaccine is introduced is generally the most feasible to implement.

Are monovalent or combination vaccines most suitable?

Issues to consider in choosing a suitable hepatitis B vaccine for national immunization schedules include: flexibility in adding the vaccine to the national immunization schedule; impact on cold chain capacity; the number of injections per visit; vaccine security; impact on local vaccine production; and cost. Use of combination vaccines (e.g. DTP-HB vaccine) may offer certain programmatic advantages. These include:

- a decreased number of injections required per visit (and thus decrease the number of needles and syringes required); and
- a decrease in the amount of space required for cold chain storage and transport.

How can the addition of hepatitis B vaccine be used to strengthen national immunization services?

Hepatitis B vaccine introduction should be used as an opportunity to strengthen existing immunization services. Issues needing particular attention include stock management, reducing vaccine wastage, injection safety, and monitoring coverage.

Budgeting for the introduction of hepatitis B vaccine

Capital and recurrent costs related to the introduction of hepatitis B vaccine should be estimated and included in the annual immunization budget. Additional capital costs might include investment in cold chain equipment and information campaigns targeted to the general public. Additional recurrent costs include vaccines, AD syringes, training, safe disposal of waste, and evaluation of the impact of immunization.



Treatment

Currently, there is no treatment available for acute hepatitis B. Symptomatic treatment of nausea, anorexia, vomiting, and other symptoms may be indicated.^{15, 23}

Treatment of chronic hepatitis B is aimed at eliminating infectivity to prevent transmission and spread of HBV, at halting the progression of liver disease and improving the clinical and histologic picture, and at preventing HCC from developing, by losing markers of HBV replication in serum and liver like HBV DNA, HBeAg, and HBcAg. Normalization of ALT activity, resolution of hepatic inflammation and the improvement of a patients' symptoms usually accompany these virological changes.^{15, 23}

There are two main classes of treatment:

- antivirals: aimed at suppressing or destroying HBV by interfering with viral replication.²³
- immune modulators: aimed at helping the human immune system to mount a defence against the virus.

Neither corticosteroids, which induce an enhanced expression of virus and viral antigens, and a suppression of T-lymphocyte function, nor adenine arabinoside, acyclovir, or dideoxyinosine, have been shown to be beneficial for the treatment of chronic hepatitis B.^{15, 31}

Currently, chronic hepatitis B is treated with interferons.^{11, 15, 23, 31} The only approved ones are interferon- α -2a and interferon- α -2b. Interferons display a variety of properties that include antiviral, immunomodulatory, and antiproliferative effects. They enhance T-cell helper activity, cause maturation of B lymphocytes, inhibit T-cell suppressors, and enhance HLA type I expression. To be eligible for interferon therapy, patients should have infection documented for at least six months, elevated liver enzymes (AST and ALT) and an actively dividing virus in their blood (HBeAg, and/or HBV DNA positive tests). Patients with acute infection, end stage cirrhosis or other major medical problems should not be treated. Interferon- α -2b produces a long-term, sustained remission of the disease in 35% of those with chronic hepatitis B, with normalization of liver enzymes and loss of the three markers for an active infection (HBeAg, HBV DNA, and HBsAg). Complete elimination of the virus is achieved in some carefully selected patients.^{15, 23, 31, 33}

Interferon therapy for patients with HBV-related cirrhosis decreases significantly the HCC rate, particularly in patients with a larger amount of serum HBV DNA. In patients with HBeAg-positive compensated cirrhosis, virological and biochemical remission following interferon therapy is associated with improved survival. In patients with chronic HBV infection, the clearance of HBeAg after treatment with interferon- α is associated with improved clinical outcomes.^{9, 15, 16, 23, 27}

Interferon- α (Intron A (interferon- α -2b), Schering Plough, and Roferon (interferon- α -2a), Roche Labs) is the primary treatment for chronic hepatitis B. The standard duration of therapy is considered 16 weeks. Patients who exhibit a low level of viral replication at the end of the standard regimen benefit most from prolonged treatment.^{18, 33}

Permanent loss of HBV DNA and HBeAg are considered a response to antiviral treatment, as this result is associated with an improvement in necro-inflammatory damage, and reduced infectivity.

Interferon in high doses causes fever, fatigue, malaise, and suppression of white blood cell and platelet counts. These effects are reversible when the therapy is stopped.³¹

A new treatment introduced recently for chronic hepatitis B in adults with evidence of HBV viral replication and active liver inflammation is EPIVIR®-HBV (lamivudine, Glaxo Wellcome). The recommended 100 mg



once-daily oral dose in form of tablets is easy to take and generally well tolerated, although safety and effectiveness of treatment beyond 1 year have not been established.^{8, 11, 20, 23, 26}

Lamivudine is a 2',3'-dideoxy cytosine analogue that has strong inhibitory effects on the HBV polymerase and therefore on HBV replication in vitro and in vivo. Lamivudine is well tolerated and suppresses HBV replication in HBsAg carriers, but the effect is reversible, if therapy is stopped.^{8, 11, 21, 23, 26}

Combination therapy with interferon- α and lamivudine for patients who failed interferon- α monotherapy is under investigation.

Combination prophylaxis with lamivudine and HBIG prevents hepatitis B recurrence following liver transplantation.^{15, 24} Subjecting hepatitis B patients who develop end-stage liver disease to liver transplantation is very controversial because the graft is inevitably reinfected, especially if the patient is HBV DNA positive. To counteract this problem, the long-term iv administration of HBIG to these patients before the operation and continuously thereafter, helps maintain a minimal level of anti-HBs in the serum at all times. Some patients relapse however when therapy is interrupted.¹⁵

Adoptive transfer of immunity to hepatitis B has been a novel approach to terminating HBV infection in the carrier after bone marrow transplantation from a hepatitis B immune donor.^{11, 15}

Several new agents (e.g. Ritonavir, Adefovir, Dipivoxil, Lobucavir, Famvir, FTC, N-Acetyl-Cysteine (NAC), PC1323, Theradigm-HBV, Thymosin-alpha, Ganciclovir¹⁴) are in development, and some encouraging data are available.

Chronic hepatitis B: potential drug therapy

Agent	Effective	Ineffective	Toxic	Under evaluation
Interferon	Interferon-α	Interferon-γ		Interferon-β
Antiviral	lamivudine famciclovir	acyclovir dideoxyinosine azidothymidine foscarnet	fialuridine adenine arabinside	ribavirin lamivudine (long term) famciclovir (long term) adefovir entecavir
Immunomodulatory		prednisone interleukin-2 thymosin levamisole		adoptive immune transfer

Adapted from: Gitlin N. Hepatitis B: diagnosis, prevention, and treatment. *Clinical Chemistry*, 1997, 43(8(B)):1500-1506,¹¹ with permission.

Goals of interferon therapy

Goal	Implication
Loss of HBeAg	Significant decrease of infectious potential accompanied by clinical benefit
Loss of HBV DNA	Loss of ability of HBV to replicate
Return to normal ALT levels	Cessation of hepatic inflammation and interruption of progression of liver injury
Loss of HBsAg	Eradication of HBV



Contraindications for interferon therapy for chronic hepatitis B

Hepatic decompensation	albumin <3.0 g/l bilirubin >51.3 µ mol/l (30 mg/l) prolonged prothrombin time >3.0 s
Portal hypertension	variceal bleed ascites encephalopathy
Hypersplenism	leukopenia (<2 x 10 ⁹ /l) thrombocytopenia (<7 x 10 ⁷ /l)
Psychiatric depression	severe, suicide attempt
Autoimmune disease	polyarteritis nodosa, rheumatoid arthritis, thyroiditis
Major system impairment	cardiac failure obstructive airways disease uncontrolled diabetes
Pregnancy	
Current intravenous drug abuse	

From: Gitlin N. Hepatitis B: diagnosis, prevention, and treatment. *Clinical Chemistry*, 1997, 43(8(B)):1500-1506,¹¹ with permission.

Side-effects of interferon therapy

Constitutional	flu-like illness fever rigors arthralgia myalgia fatigue
Haematologic	leukopenia thrombocytopenia
Alopecia	
Neuropsychiatric	depression insomnia irritability
Weight loss	
Ocular	
Autoimmune	hypothyroidism diabetes

From: Gitlin N. Hepatitis B: diagnosis, prevention, and treatment. *Clinical Chemistry*, 1997, 43(8(B)):1500-1506,¹¹ with permission.



Guidelines for epidemic measures

1. When two or more cases occur in association with some common exposure, a search for additional cases should be conducted.
2. Introduction of strict aseptic techniques. If a plasma derivative like antihæmophilic factor, fibrinogen, pooled plasma or thrombin is implicated, the lot should be withdrawn from use.
3. Tracing of all recipients of the same lot in search for additional cases.
4. Relaxation of sterilization precautions and emergency use of unscreened blood for transfusions may result in increased number of cases.

Future considerations

Attaining global immunization coverage is a goal still unmet.

The development of a better and cheaper antiviral therapy should be pursued intensively for chronic HBV infections.³⁰

Strategies to activate appropriate immune responses during chronic virus infections may offer the best approach for terminating such infections.

Attempts at protecting the whole community by vaccinating only high-risk individuals have not been successful.³⁷ Universal vaccination is necessary to control and possibly eradicate hepatitis B. The next step is finding strategies for meeting that goal in countries with different health care structures and financial resources.

WHO goals

WHO aims at controlling HBV worldwide to decrease the incidence of HBV-related chronic liver disease, cirrhosis, and hepatocellular carcinoma, by integrating HB vaccination into routine infant (and possibly adolescent) immunization programmes.^{3, 23, 36}

Persons infected with HBV during infancy or early childhood are more likely to become infected chronically and to develop life-shortening chronic liver disease such as cirrhosis or even liver cancer than adults. This is one important reason why emphasis should be placed upon preventing HBV among the youngest age groups.

In 1991, the Global Advisory Group of EPI (Expanded Programme on Immunization) set 1997 as the target for integrating the hepatitis B vaccination into national immunization programmes worldwide. The group recommended strategies for implementation and delivery that vary according to epidemiology: advocating integration of the vaccine into immunization programmes by 1995 in countries with a HBV carrier prevalence of 8% or higher, and setting 1997 as the target date for all other countries. WHO endorsed the recommendation in May 1992, and the World Health Assembly added a disease reduction target for hepatitis B in 1994, calling for an 80% decrease in new HBV child carriers by 2001.



Commitment of public health resources to eliminate the spread of HBV requires recognition of the importance of hepatitis B, persistent efforts to ensure that populations are protected, and patience to achieve the goals of disease reduction.²³



Glossary

alopecia loss of hair occurring at any site and from any cause.

ALT alanine aminotransferase an enzyme that interconverts L-alanine and D-alanine. It is a highly sensitive indicator of hepatocellular damage. When such damage occurs, ALT is released from the liver cells into the bloodstream, resulting in abnormally high serum levels. Normal ALT levels range from 10 to 32 U/l; in women, from 9 to 24 U/l. The normal range for infants is twice that of adults.

amino acids the basic units of proteins, each amino acid has a NH-C(R)-COOH structure, with a variable R group. There are altogether 20 types of naturally occurring amino acids.

antibody a protein molecule formed by the immune system which reacts specifically with the antigen that induced its synthesis. All antibodies are immune globulins.¹

antigen any substance which can elicit in a vertebrate host the formation of specific antibodies or the generation of a specific population of lymphocytes reactive with the substance. Antigens may be protein or carbohydrate, lipid or nucleic acid, or contain elements of all or any of these as well as organic or inorganic chemical groups attached to protein or other macromolecule. Whether a material is an antigen in a particular host depends on whether the material is foreign to the host and also on the genetic makeup of the host, as well as on the dose and physical state of the antigen.¹

arthralgia joint pain with objective findings of heat, redness, tenderness to touch, loss of motion, or swelling.

AST aspartate aminotransferase the enzyme that catalyzes the reaction of aspartate with 2-oxoglutarate to give glutamate and oxaloacetate. Its concentration in blood may be raised in liver and heart diseases that are associated with damage to those tissues. Normal AST levels range from 8 to 20 U/l. AST levels fluctuate in response to the extent of cellular necrosis.¹

B-cells also known as B lymphocytes. A class of white blood cells which carry out humoral immune response. They mature in the bone marrow.

bilirubin is the chief pigment of bile, formed mainly from the breakdown of haemoglobin. After formation it is transported in the plasma to the liver to be then excreted in the bile. Elevation of bile in the blood (>30 mg/l) causes jaundice.⁴³

carcinoma a malignant epithelial tumour. This is the most frequent form of cancer.

carrier is a person who has HBV (HCV, HDV) in his or her blood for longer than 6 months even if all symptoms have disappeared. Because the virus is present in the blood, it can be transmitted to others. The HBV carrier can be recognized by a specific blood test.

cirrhosis a chronic disease of the liver characterized by nodular regeneration of hepatocytes and diffuse fibrosis. It is caused by parenchymal necrosis followed by nodular proliferation of the surviving hepatocytes. The regenerating nodules and accompanying fibrosis interfere with blood flow through the liver and result in portal hypertension, hepatic insufficiency, jaundice and ascites.

codon the smallest unit of genetic material that can specify an amino acid residue in the synthesis of a polypeptide chain. The codon consists of three adjacent nucleotides.



complete blood count chemical analysis of various substances in the blood performed with the aim of a) assessing the patient's status by establishing normal levels for each individual patient, b) preventing disease by alerting to potentially dangerous levels of blood constituents that could lead to more serious conditions, c) establishing a diagnosis for already present pathologic conditions, and d) assessing a patient's progress when a disturbance in blood chemistry already exists.

cytopathic that kills the cells.

cytoplasm the protoplasm of the cell which is outside of the nucleus. It consists of a continuous aqueous solution and the organelles and inclusions suspended in it. It is the site of most of the chemical activities of the cell.

endemic prevalent continuously in some degree in a community or region.⁴³

endoplasmic reticulum a network or system of folded membranes and interconnecting tubules distributed within the cytoplasm of eukaryotic cells. The membranes form enclosed or semienclosed spaces. The endoplasmic reticulum functions in storage and transport, and as a point of attachment of ribosomes during protein synthesis.

enzyme any protein catalyst, i.e. substance which accelerates chemical reactions without itself being used up in the process. Many enzymes are specific to the substance on which they can act, called substrate. Enzymes are present in all living matters and are involved in all the metabolic processes upon which life depends.

epidemic an outbreak of disease such that for a limited period a significantly greater number of persons in a community or region suffer from it than is normally the case. Thus an epidemic is a temporary increase in prevalence. Its extent and duration are determined by the interaction of such variables as the nature and infectivity of the casual agent, its mode of transmission and the degree of preexisting and newly acquired immunity.⁴³

epitope also known as antigenic determinant. A localized region on the surface of an antigen which antibody molecules can identify and bind.

fulminant describes pathological conditions that develop suddenly and are of great severity.¹

genome the total genetic information present in a cell. In diploid cells, the genetic information contained in one chromosome set.¹

Golgi apparatus a cytoplasmic organelle which is composed of flattened sacs resembling smooth endoplasmic reticulum. The sacs are often cup-shaped and located near the nucleus, the open side of the cup generally facing toward the cell surface. The function of the Golgi apparatus is to accept vesicles from the endoplasmic reticulum, to modify the contents, and to distribute the products to other parts of the cell or to the cellular environment.

hepadnavirus family of single stranded DNA viruses of which hepatitis B virus (HBV) and woodchuck hepatitis virus (WHV) are members.

hepatocytes are liver cells.¹

humoral pertaining to the humors, or certain fluids, of the body.¹

icterus see jaundice



IgG antibodies IgG is the most abundant of the circulating antibodies. It readily crosses the walls of blood vessels and enters tissue fluids. IgG also crosses the placenta and confers passive immunity from the mother to the fetus. IgG protects against bacteria, viruses, and toxins circulating in the blood and lymph.

IgM antibodies IgMs are the first circulating antibodies to appear in response to an antigen. However, their concentration in the blood declines rapidly. This is diagnostically useful, because the presence of IgM usually indicates a current infection by the pathogen causing its formation. IgM consists of five Y-shaped monomers arranged in a pentamer structure. The numerous antigen-binding sites make it very effective in agglutinating antigens. IgM is too large to cross the placenta and hence does not confer maternal immunity.

immune globulin (IG) is a sterile preparation of concentrated antibodies (immune globulins) recovered from pooled human plasma processed by cold ethanol fractionation. Only plasma that has tested negative for a) hepatitis B surface antigen (HBsAg), b) antibody to human immunodeficiency virus (HIV), and c) antibody to hepatitis C virus (HCV) is used to manufacture IG. IG is administered to protect against certain diseases through passive transfer of antibody. The IGs are broadly classified into five types on the basis of physical, antigenic and functional variations, labeled respectively IgM, IgG, IgA, IgE and IgD.

immune system our body's natural defence system, involving antibodies and a class of white blood cells called lymphocytes.

incidence the number of cases of a disease, abnormality, accident, etc., arising in a defined population during a stated period, expressed as a proportion, such as x cases per 1000 persons per year.¹

interferon a protein produced in organisms infected by viruses, and effective at protecting those organisms from other virus infections. Interferons exert virus-nonspecific but host-specific antiviral activity by inducing the transcription of cellular genes coding for antiviral proteins that selectively inhibit the synthesis of viral DNA and proteins. Interferons also have immunoregulatory functions. Production of interferon can be stimulated by viral infection, especially by the presence of double stranded RNA, by intracellular parasites, by protozoa, and by bacteria and bacterial products. Interferons have been divided into three distinct types (α , β , and γ) associated with specific producer cells and functions, but all animal cells are capable of producing interferons, and certain producer cells (leukocytes and fibroblasts) produce more than one type (both α and β).

jaundice is a yellow discolouration of the skin and mucous membranes due to excess of bilirubin in the blood, also known as icterus.⁴³

leukopenia an abnormal decrease in the number of leukocytes in the blood.

lumen the cavity or channel between a tube or tubular structure.

lymphocyte a leukocyte of blood, bone marrow and lymphatic tissue. Lymphocytes play a major role in both cellular and humoral immunity, and thus several different functional and morphologic types must be recognized, i.e. the small, large, B-, and T-lymphocytes, with further morphologic distinction being made among the B-lymphocytes and functional distinction among T-lymphocytes.¹

lymphoproliferative disease a neoplastic or systemic tumorlike proliferation of lymphocytes, as in lymphoid leukemia, malignant lymphomas, or in Waldenström's macroglobulinemia.¹

Major Histocompatibility Complex (MHC) originally defined as the genetic locus coding for those cell surface antigens presenting the major barrier to transplantation between individuals of the same species. Now known to be a cluster of genes on human chromosome 6 or mouse chromosome 17 that encodes the



MHC molecules. These are the MHC class I molecules or proteins that present peptides generated in cytosol to CD8 T cells, and the MHC class II molecules or proteins that present peptides degraded in cellular vesicles to CD4 T cells. The MHC also encodes proteins involved in antigen processing and host defense. The MHC is the most polymorphic gene cluster in the human genome, having large numbers of alleles at several different loci. Because this polymorphism is usually detected using antibodies or specific T cells, the MHC proteins are often called major histocompatibility antigens.

myalgia pain in the muscles.

nucleotide a molecule formed from the combination of one nitrogenous base (purine or pyrimidine), a sugar (ribose or deoxyribose) and a phosphate group. It is a hydrolysis product of nucleic acid.¹

nucleus a membrane-bounded compartment in an eukaryotic cell which contains the genetic material and the nucleoli. The nucleus represents the control center of the cell. Nuclei divide by mitosis or meiosis.

plasma the liquid matrix in which the blood cells and blood proteins are suspended in. It contains an extensive variety of solutes dissolved in water. Water accounts for about 90% of blood plasma.

plasmid a small, circular DNA molecule, separate from the bacterial chromosome, capable of independent replication.

polymerase an enzyme which catalyzes the replication of DNA (DNA polymerase) or RNA (RNA polymerase).

prevalence is the number of instances of infections or of persons ill, or of any other event such as accidents, in a specified population, without any distinction between new and old cases.⁴³

promoter a region of DNA usually occurring upstream from a gene coding region and acting as a controlling element in the expression of that gene. It serves as a recognition signal for an RNA polymerase and marks the site of initiation of transcription.

prophylaxis is the prevention of disease, or the preventive treatment of a recurrent disorder.⁴³

protein large molecule made up of many amino acids chemically linked together by amide linkages. Biologically important as enzymes, structural protein and connective tissue.

reverse transcriptase an enzyme that catalyzes the formation of DNA using an RNA template, and is thus an RNA-dependent DNA polymerase. The name refers to the fact that the enzyme transcribes nucleic acids in the reverse order from the usual DNA-to-RNA transcription.

rigors stiffness.

RT-PCR reverse transcriptase - polymerase chain reaction. A technique commonly employed in molecular genetics through which it is possible to produce copies of DNA sequences rapidly.

seroconversion the production in a host of specific antibodies as a result of infection or immunization. The antibodies can be detected in the host's blood serum following, but not preceding, infection or immunization.¹

serum is the clear, slightly yellow fluid which separates from blood when it clots. In composition it resembles blood plasma, but with fibrinogen removed. Sera containing antibodies and antitoxins against



infections and toxins of various kinds (antisera) have been used extensively in prevention or treatment of various diseases.⁴³

T-cells also known as T-lymphocytes. White blood cells which function in cell-mediated response. They originate from stem cells in the bone marrow but mature in the thymus.

thrombocytopenia a fewer than normal number of platelets per unit volume of blood, i.e. fewer than 130×10^9 platelets per liter.

titre a measure of the concentration or activity of an active substance.

transcription the process by which a strand of RNA is synthesized with its sequence specified by a complementary strand of DNA, which acts as a template. The enzymes involved are called DNA-dependent RNA polymerases.

translation the process of forming a specific protein having its amino acid sequence determined by the codons of messenger RNA. Ribosomes and transfer RNA are necessary for translation.¹

tumour a lump due to uncontrolled cell division, may be benign or malignant. Malignant tumours cause cancer. Tumours are able to spread to other parts of the body (metastasize) and begin secondary growths at these other sites.

vaccine an antigenic preparation used to produce active immunity to a disease to prevent or ameliorate the effects of infection with the natural or “wild” organism. Vaccines may be living, attenuated strains of viruses or bacteria which give rise to inapparent to trivial infections. Vaccines may also be killed or inactivated organisms or purified products derived from them. Formalin-inactivated toxins are used as vaccines against diphtheria and tetanus. Synthetically or genetically engineered antigens are currently being developed for use as vaccines. Some vaccines are effective by mouth, but most have to be given parenterally.^{1, 43}

vaccinee person receiving a vaccine

virion a structurally complete virus, a viral particle.¹

virus any of a number of small, obligatory intracellular parasites with a single type of nucleic acid, either DNA or RNA and no cell wall. The nucleic acid is enclosed in a structure called a capsid, which is composed of repeating protein subunits called capsomeres, with or without a lipid envelope. The complete infectious virus particle, called a virion, must rely on the metabolism of the cell it infects. Viruses are morphologically heterogeneous, occurring as spherical, filamentous, polyhedral, or pleomorphic particles. They are classified by the host infected, the type of nucleic acid, the symmetry of the capsid, and the presence or absence of an envelope.¹

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