Review of literature
Hepatitis B- the disease

History

The terms, hepatitis A and hepatitis B, were first introduced in order to categorize infectious (epidemic) and serum hepatitis (MacCallum et al., 1947). These terms were eventually adopted by the World Health Organization Committee on Viral Hepatitis (WHO report in 1973). Before the viruses causing hepatitis were isolated, transmission was differentiated on the basis of epidemiological observations. Type A hepatitis was considered to be predominantly transmitted via the fecal-oral route while type B hepatitis was believed to be primarily transmitted parenterally. In 1963, Blumberg discovered a previously unknown protein in the blood of an Australian aborigine (Blumberg et al., 1967). This protein was designated as Australia (Au) antigen and was shown to be related to type B hepatitis. Later, it was called hepatitis B surface antigen and was found only in the serum of type B hepatitis infected patients (Prince, 1968; Okochi et al., 1968). In 1973, virus-like particles, designated as hepatitis B virus, were observed in the serum of patients suffering from type B hepatitis (Dane et al., 1970). Although other un-related hepatitis viruses were discovered later, the hepatitis B virus retained its name. The viral nature of these particles was confirmed by the detection of an endogenous DNA-dependent DNA polymerase within its core (Kaplan et al., 1973). The discovery of this polymerase allowed detection and characterization of the HBV genome (Robinson et al., 1974). In the recent past, many related viruses have been found which are species specific. With human HBV as the archetype, the members of the hepadnaviridae family include, duck hepatitis B virus (DHBV), ground squirrel hepatitis virus (GSHV), snow goose hepatitis B virus (sgHBV), woodchuck hepatitis virus (WHV) and wooley monkey hepatitis virus. There is a likelihood of the prevalence of other viruses within hepadnaviridae which could be discovered in future.

Epidemiology

The prevalence of HBV is high in East Asia, sub-Saharan Africa and the Amazon basins (carrier rate ranges from 8% to 25%) and low in West Europe and North America (carrier rate < 2%). In India, the prevalence is estimated to be approximately 2-7% (Fig. 2). In high prevalence areas, the infection occurs mostly as neonatal transmission during infancy or horizontal transmission during childhood. In moderately endemic areas of infection, the
Fig. 2. World distribution map for hepatitis B prevalence.

Adapted from: www.cdc.org.
transmission of the virus is by non-sexual close contact besides vertical, sexual and parenteral transmission (Doganci et al., 2005). However, in low prevalence areas, infection spreads mostly horizontally in adult stage (Grob et al., 1998; Yarbough et al., 1999; Poland et al., 2004).

Transmission

Currently, there are four recognized modes of transmission:

1. Perinatal- from mother to child at birth.

2. Horizontal- by contact with an infected person, or exposure to blood or other infected fluids.

3. Sexual contact.

4. Intra-familial- frequent or household contact with the infected person.

There is a considerable variation between areas, countries and continents and the age at which most transmission takes place. The presence of the virus has been detected in a variety of body fluids such as saliva, nasopharyngael, semen and menstrual (Alter et al., 1977) and the virus has not been detected in feces, probably due to viral inactivation by enzymes within the intestinal mucosa or bacterial flora. One of the most effective routes, which allows virus to enter an unexposed individual, is through percutaneous introduction (i.e. needle-stick injuries, etc.). However, the sexual transmission could range between a mere 1%-3% for a single unprotected sexual encounter or increase to 15%-30% due to a regular infected partner. Homosexual men are reported to be at 10-20 times greater risk than the general population. The transmission efficiency through all other potential methods is not easily measured, but considered quite low (Mohoney et al., 1999; Hollinger et al., 2001). The hepatitis B virus, HBV, is stable on environmental surfaces for at least 7 days and an indirect inoculation could occur via inanimate objects like toothbrushes, baby bottles, toys, razors, eating utensils, hospital equipment and other objects or by contact with mucous membranes or bruised skin (Robinson et al., 1995). Infectious HBV can be present in blood without detectable HBsAg, and the failure to detect the antigen does not exclude the presence of the infectious virus (Tacket et al., 1999). It is reported that the
source of infection cannot be identified in about 35% of cases and HBV is about 100 times more infectious than HIV.

**Clinical types**

The course of hepatitis B could be extremely variable (Robinson et al., 1995) with different clinical manifestations depending on the patient’s age at infection, the immune status and the stage at which the disease is recognized. During the incubation phase of the disease (6 to 24 weeks), the patients feel unwell with possible nausea, vomiting, diarrhea, anorexia and headaches. Patients then become jaundiced, although low-grade fever and loss of appetite may improve. HBV infection sometimes produces neither jaundice nor obvious symptoms (Robinson et al., 1995; Hollinger et al., 2001). The asymptomatic cases are identified by detecting biochemical or virus-specific serologic alterations in their blood. It is quite likely that they 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 others (about 5 to 10%) do not clear the virus and progress to become asymptomatic carriers or develop chronic hepatitis, possibly resulting in cirrhosis and/or liver cancer (Robinson et al., 1995). Rarely, some patients develop fulminant hepatitis and die (Robinson et al., 1995). In general, the frequency of clinical disease increases with age and the percentage of carriers decreases. A small number of long-established chronic carriers apparently terminate their active infection and become HBsAg-negative (about 2% per year) (Robinson et al., 1995). The four major clinical types of hepatitis are:

**Acute Hepatitis**

The clinical course of HBV runs similar to that of Hepatitis A Virus (HAV) but tends to be more severe, at times associated with serum-sickness-like syndrome. The mildest attacks are asymptomatic and are detectable only by an increase in serum transaminase levels. Alternatively, the patient may be anicteric with gastrointestinal and influenza-like symptoms. These patients are likely to remain undiagnosed unless a clear history of exposure is available. The severity of infection varies from the symptomatic and icteric (from which recovery is typical) to fulminant and fatal viral hepatitis. Icteric attacks in adults are marked by a prodromal period (typically 3 - 4 days extending up to 2 - 3 weeks) during which a patient feels sick, suffering from digestive symptoms such as anorexia and
nausea and may, in the later stages, have mild pyrexia. Other common symptoms are mild pyrexia, rigors, loss of desire to drink alcohol or smoke, malaise, and occasionally severe headaches. The prodromal period is followed by the darkening of urine and lightening of feces, followed by the development of jaundice.

**Chronic hepatitis**

Although most adult patients recover completely from an acute episode of hepatitis B, in a significant proportion of 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 (Robinson *et al.*, 1995; Mahoney *et al.*, 1999). Surprisingly, some of the patients infected persistently may have no clinical or biochemical evidence of liver disease. 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 can last for decades and proceed to cirrhosis, and is associated with a 100-fold increase in the risk of developing a hepatocellular carcinoma (Robinson *et al.*, 1995; Mahoney *et al.*, 1999).

**Fulminant Hepatitis**

This is a rare form of the disease, which usually overwhelms the patient within 10 days. This form develops so quickly that the jaundice is inconspicuous and the disease may be confused with acute psychosis or meningo-encephalitis. On the other hand, the patient may become deeply jaundiced. Foreboding signs could be, repeated vomiting, fetor hepaticas, confusion and drowsiness. The 'flapping' tremor may only be transient, but rigidity is usual. These are then supervened by coma, indicating possible acute liver failure. The patient's temperature rises, jaundice deepens and liver shrinks, at times accompanied by widespread hemorrhage. Serum bilirubin and transaminase are poor prognostic indicators as transaminase levels may actually decrease as the patient's clinical condition worsens. Prothrombin is the best indicator of prognosis. Frequency of the fulminant course varies depending upon the type of viral hepatitis and prevalence of hepatitis B carriage (Gimson *et al.*, 1983).
Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma is the technical term for liver cancer. This form of the disease in chronic hepatitis B patients develops after a long time in individuals suffering from chronic hepatitis B infection. The events triggering the development of this disease form are currently unknown.

Diagnosis

Diagnosis of hepatitis is made by biochemical assessment of liver function. The liver function test markers include: total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, prothrombin time, total protein, albumin, serum globulin, complete blood count, and coagulation studies (Robinson et al., 1995; Hollinger et al., 2001).

The diagnosis is confirmed by demonstration in sera of specific antigens and/or antibodies. Serologic assays for HBV are the mainstay diagnostic tools for HBV infection. However, the advent of molecular biology-based techniques a new dimension has been added to the diagnosis and treatment of patients with chronic HBV infection. Over the past decade, improvements in molecular technology has permitted detection of as few as 10 copies/ml of HBV DNA in serum. This in turn has led to redefinitions of chronic HBV infection, as well as thresholds for antiviral treatment. As the sensitivity of these molecular techniques continues to improve, the challenge will be to standardize these assays and define clinically significant levels of HBV replication (Servoss et al., 2004).

Serological markers of HBV infection

During HBV infection, the serological markers vary depending on whether the infection is acute or chronic (Robinson et al., 1995; Gitlin et al., 1997; Mahoney et al., 1999) (Fig.3).

Hepatitis B surface antigen (HBsAg)

HBsAg can be detected in the serum several weeks before the onset of symptoms which can last for months after onset. HBsAg is present in serum during acute infections and persists in chronic infections (Fig. 3). In fact, in some chronic cases, HBsAg is spontaneously removed after long persistence (Fig. 3). The presence of HBsAg indicates
Fig. 3. Various serological patterns observed after HBV infection in different individuals. S+E+, S+E- and S-E- infections represent different profiles of the chronic hepatitis B infection on the basis of clearance or persistence of HBsAg and HBeAg
that the person is potentially infectious. It is useful for the diagnosis of HBV infection and for screening of blood. Recently, an M13 phage, PHH2, was isolated which had the ability to bind HBsAg. The HBsAg binding phage was used in an assay referred to as "PHALISA", an abbreviation for Phage-Linked Immune-Sorbent assay. This assay was at least 20-100 times more sensitive in the detection of HBV antigen than conventional enzyme-linked immune-sorbent assay (ELISA) (Lu et al., 2004).

**Anti-HBsAg**

This is the specific antibody to hepatitis B surface antigen. Its appearance in 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. Anti-HBs replaces HBsAg as the acute HBV infection is resolving (Fig. 3). Anti-HBs generally persists for a lifetime in over 80% of patients and indicates immunity (Robinson et al., 1995; Mahoney et al., 1999; Hollinger et al., 2001).

**Hepatitis B core antigen (HBeAg)**

Hepatitis B core antigen is derived from the protein envelope that encloses the viral DNA, and it is not detectable in the bloodstream. When HBeAg peptides are expressed on the surface of hepatocytes, they induce an immune response that is crucial for killing infected cells. The HBeAg is a marker of the infectious viral material and it is the most accurate index of viral replication.

**Anti-HBeAg**

It is the first antibody to appear. This is the specific antibody to hepatitis B core antigen. Antibodies to HBeAg are of IgM and IgG class. They do not neutralize the virus. The presence of IgM identifies an early acute infection. IgM anti-HBc is present in high titer during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis. An absence of HBsAg and anti-HBs suggests a recent infection. IgG with no IgM type antibodies may be present in chronic as well as resolved type of hepatitis and generally remains detectable for a lifetime (Robinson et al., 1995; Mahoney et al., 1999; Hollinger et al., 2001) (Fig. 3).
Hepatitis B precore antigen (HBeAg)

HBeAg appearing during weeks 3 to 6 indicates an acute active disease, and indicates that the patient is infectious (Fig. 3). Persistence of this virological marker beyond 10 weeks shows progression to chronic infection and infectiousness (Fig. 3). Continuous presence of anti-HBe indicates chronic 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. Function of HBeAg is uncertain.

Anti-HBeAg

This is the specific antibody to HBeAg. During the acute stage of infection the seroconversion from e antigen to e antibody is prognostic for resolution of infection (Fig. 3). Its presence in the patient's blood along with anti-HBc and in the absence of HBsAg, anti-HBs and core HBV mutants indicates low contagiousness and convalescence. Anti-HBe appears after anti-HBc and its presence correlates to a decreased infectivity (Robinson et al., 1995; Mahoney et al., 1999; Hollinger et al., 2001).

Hepatitis B x antigen (HBxAg)

Hepatitis B x antigen is detected in HBeAg positive blood in patients with both acute and chronic hepatitis. HBxAg is reportedly involved in transcriptional regulation.

Anti-HBxAg

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 hybridization assays or Polymerase chain reaction (PCR) one week after initial infection. The tests are generally performed for monitoring of antiviral treatment or to detect mutants that escape detection by current methods.

HBV DNA polymerase
Review of literature

Tests for the presence of HBV DNA polymerase, detectable within a week of initial infection, are only performed for research purposes.

**The liver as the target organ**

The liver plays an essential role in energy storage and conversion, blood homeostasis, chemical detoxification and immunity to microbial infections. Although the liver is composed of many different types of cells, much of the functional activity resides in Hepatocytes (which constitute 70% of the liver), bile ductile epithelium, and Kupffer cells (macrophages) (Gartner et al., 1997).

Liver is considered to be divided into small compartments called lobules. Blood enters the lobule through portal veins and hepatic arteries and is distributed by smaller vessels to enter the sinusoidal spaces, created by plates of Hepatocytes. Blood passes through these spaces which are lined by fenestrated endothelial cells and fixed macrophages (Kupffer cells), where the various functional interactions take place. The blood is collected in central veins and exits the liver. During catabolism of heme produced by red blood cell breakdown in the liver, bile is formed and secreted into the bile canaliculi, the small channels formed at the junctions of Hepatocytes. Bile flows in the opposite direction from blood, passing through a region known as the Canal of Herring to enter bile ducts. From there it flows into larger ducts and is eventually transported to the gall bladder and intestine (Seegar, 2000).

Liver has a tremendous capacity to regenerate as seen in rats where the full liver mass is restored after 90% partial hepatectomy. It is important to know how components of the virus replication machinery, including transcriptionally active viral DNAs, behave as the infected cells begin to divide. Hence, an understanding of liver regeneration is required for an understanding of the transient and chronic infections.

**HBV structure**

HBV or Dane particle is an enveloped partially double-stranded circular DNA virus with a diameter of 42 nm. The envelope is made up of phospholipids and HBsAg and is 7nm thick. It contains a 27 nm inner nucleocapsid core of viral DNA and a tightly bound 19 kilodalton hepatitis B core antigen (HBcAg). The viral core also contains DNA
polymerase and protein kinase activity (Meyer et al., 1997). The genome of HBV is partially double-stranded relaxed circular (RC) DNA molecule with a single stranded region of variable length. The antisense strand is the longer strand designated as L(-) and the sense strand is the shorter strand designated as S(+). The S (+) strand varies from 50 to 70 % of the L (-) strand, which consists of 3200 bases (Howard, 1995). The positions of the 5'-end of both the strands are fixed whereas the position of the 3'-end of the plus strand (short strand) is not fixed. A 224 base pair 5'-cohesive terminus maintains the circularization of the genome (Sattler et al., 1979). There is an 11 bp direct repeat (DR1 and DR2) (5' TTCACCCTCTGT-3') at both sites of this cohesive terminus, which is involved in the initiation of viral DNA synthesis. The L (-) DNA contains a protein linked to the 5' terminus while S (+) strand DNA contains an oligoribonucleotide at its 5' end (Sattler et al., 1979) (Fig.4).

The minus strand contains four open reading frames (ORFs) and carries all the protein-coding capacity of the virus (Ganem et al., 2001). Importantly, these overlap in frame-shifted manner with one another so that the minus strand is read one and a half times (Ganem et al., 2001). The longest ORF encodes the viral polymerase (Pol). The ORF for the envelope (Pre-S/S) genes is completely located within the Pol ORF, and the ORF for the core (Pre-C/C) and X genes partially overlap with Pol ORF. The HBV encodes more than one protein from one ORF by using multiple internal AUG codons within an ORF, creating additional start sites for protein biosynthesis (Will et al., 1981).

**Pre-S/S ORF**

The gene for the HBV surface antigen consists of a single ORF divided into three coding regions, preS1, preS2 and S, each starting with an in-frame ATG codon. By translation initiation at the first AUG, the large hepatitis surface antigen (LHBs) encompassing PreS1, PreS2 and S is synthesized; by initiation at the second AUG the middle surface protein (MHBs, encompassing PreS2+ S) and is synthesized by initiation at the third, the small surface protein (SHBs) is synthesized (Ostapchuk et al., 1994; Prange et al., 1995). The HBsAg contains small (SHBs), medium (MHBs), and large (LHBs) proteins, all of which are glycosylated, type II transmembrane proteins that can form multimers stabilized by disulfide bridges formed by cysteine residues present in the S domain (Seeger, 2000). All these proteins exist in two forms differing in the extent of glycosylation. N-linked
Fig. 4. Transcriptional and translational map of HBV. The minus strand is complete and the RT is attached to its 5' end and a capped RNA oligomer is attached to the 5' end of the incomplete plus-strand DNA. The positions of the direct repeats, DR1 and DR2 and the two enhancers, EN1 and EN2, are indicated. The outer circle depicts the three major viral RNAs, the core (C) or pgRNA, the pre-S (L) mRNA, and the S mRNA. (Fig. adapted from Seeger and Mason, 2000)
glycosylation and glucosidase processing are necessary for virion, but not subviral particle and secretion (Kann et al., 1997) (Fig. 4).

**Small surface protein**

The SHBs domain is 226 amino acids long and is the most abundant protein of all three HBV-associated particles. The SHBs is found in a glycosylated and a nonglycosylated form.

**Middle surface protein**

The MHBs Pre-S2 domain is a minor component of the virion or HBs particle and consists of the S and a 55-amino-acid N-terminal extension (Ganem et al., 2001). It is either single or double glycosylated (Fig. 4).

**Large surface protein**

The LHBs contains a further N-terminal extension to the M protein of 108 or 119 amino acids (depending on the subtype/genotype) and is more prevalent than MHBs in virions and filaments but less prevalent in HBs spheres. The LHBs contains three domains, Pre-S1, Pre-S2, and S, and is glycosylated (Fig. 4).

**Pre-C/C ORF**

The Pre-C/C ORF encodes the core protein P21, which is the major polypeptide of the nucleocapsid and expresses the HBcAg. The HBc protein is either 183, 185, or 195 amino acids long, depending on the genotype of the virus. ORF C is preceded upstream by a short, in-phase ORF called the Pre-core region from which the soluble hepatitis B early antigen (HBeAg) is made (Ganem et al., 2001) (Fig. 4).

**Hepatitis B core Protein**

The HBc protein is translated from pregenomic RNA. The expression of the core protein ORF results in the production of core protein in the cytoplasm and nucleus (Guidotti et al., 1994 b). The core protein expressed this way assembles itself into core particles that display core antigenicity.
**Hepatitis B precore Protein**

The core protein ORF is preceded by an in-frame initiation codon positioned 29 codons upstream. The sequence encompassing these 29 codons has been termed the ‘precore’ sequence and the protein translated from the upstream initiation codon has been termed the ‘precore’ protein (Ou et al., 1986). Although the precore protein contains the entire sequence of the core protein and the amino terminal extension of 29 amino acids, it is not precursor of the core protein (Ou et al., 1986; Roosenick et al., 1986). HBeAg is a soluble secretory protein and is regarded as an accessory protein of the virus. The first 19 amino acids of the Precore protein form a secretion signal that allows the translocation of the Precore protein into the lumen of the endoplasmic reticulum (ER). These 19 amino acids are cleaved off by a host cell signal peptidase, leaving the Precore protein derivative P22. The P22 is then secreted through the ER and Golgi apparatus and further modified by C-terminal cleavage of up to 34 amino acids, resulting in the secretion of a heterogeneous population of proteins of 15 to 18 kDa, serologically defined as HBeAg. Thus, HBeAg protein differs in almost all aspects from HBc protein, although the primary sequences of these two molecules are almost identical (Kann, 1998) (Fig. 4).

**Polymerase ORF**

The Pol gene is the longest ORF, spanning almost 80% of the genome and overlapping the other three ORFs. The Pol protein is translated from pregenomic RNA. The 834 to 845 codons found in the Pol ORF have sequence homology to reverse transcriptases (Ganem et al., 2001) and most parts of the ORF are essential for viral replication. The 90-kDa product of the Pol ORF is a multifunctional protein that has at least four domains (Ganem et al., 2001). The N-terminal domain encodes the terminal protein that is covalently linked to the 5' end of the minus strand of virion DNA. This part of Pol ORF is necessary for priming of minus-strand synthesis. An intervening domain with no specific recognized function is referred to as the spacer or tether region. The third domain encodes the RNA- and DNA-dependent DNA polymerase, that is, the reverse transcriptase. The C-terminal domain encodes ribonuclease (RNase) H that cleaves the RNA in the RNA-DNA hybrids during reverse transcription. The terminal protein's role in protein priming of reverse transcription includes the provision of the substrate tyrosine at amino acid 63 of the HBV Pol for the formation of the covalent bond between the enzyme and the first nucleotide (G) of the minus-strand DNA (Zoulim et al., 1994 a) (Fig.5).
Fig. 5. Biosynthesis of the pre-core/core, polymerase, envelope, and X proteins from the various HBV transcripts.
Hepatitis B X ORF

The X ORF encodes a 154 amino acids long polypeptide in length (HBx) with a molecular weight of 17 kDa. This is the second accessory protein of HBV and is conserved in a similar form across all the mammalian hepadnaviruses. The expression of full-length HBx protein is dispensable for virus production in vitro but is a critical component of the infectivity process in vivo (Zoulim et al., 1994b). HBx behaves as a transcriptional transactivator of a number of viral and cellular gene promoters through direct interaction with transcription factors (Kann, 1998) (Fig. 5).

The hepatitis B virus life cycle

The HBV virion binds to a receptor at the surface of the hepatocyte (Ganem et al., 2001). 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 (Guidotti et al., 1994b; Chisari, 1997; Ganem et al., 2001) (Fig. 6). 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 (Mahoney et al., 1999; Ganem et al., 2001) (Fig. 6). 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) (Chisari, 1997; Mahoney et al., 1999; Ganem et al., 2001). The envelope proteins insert themselves as integral membrane proteins into the lipid membrane of the endoplasmic reticulum (ER) (Fig. 6).

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 (Mahoney et al., 1999; Ganem et al., 2001). 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 (Mahoney et al., 1999; Ganem...
Fig. 6. HBV life cycle (Adapted from Seeger et al., 2000).
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 (Mahoney et al., 1999; Ganem et al., 2001). In the genome amplification pathway, the nucleocapsids deliver their genome to amplify the intranuclear pool of covalently closed circular DNA (cccDNA) (Mahoney et al., 1999; Ganem et al., 2001). 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 (HBeAg).

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 (Mahoney et al., 1999; Ganem et al., 2001). 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 (Mahoney et al., 1999; Ganem et al., 2001). Nuclear localisation signals on the polymerase mediate the transport of covalently linked viral genome through the nuclear pore (Chisari, 1997; Ganem et al., 2001).

**Immune responses in hepatitis B infection:**

HBV infects hepatocytes and the most common disease manifestations are seen in the liver. Acute infection may result in mild disease or in clinically apparent acute hepatitis B of varying severity of mild to fulminant hepatitis with extensive liver necrosis. Persistent infection could be associated with very mild changes in liver, chronic persistent hepatitis or chronic active hepatitis with chronic liver cell necrosis, an inflammatory response, lymphocytic infiltration and liver regeneration. Chronic hepatitis B can progress to cirrhosis characterized by liver cell necrosis, fibrosis and regeneration.

The existence of a period of viremia without liver damage in the prodromal phase of acute infection and of carriers with high level of HBV replication without liver damage suggests that HBV itself is not cytopathic. Host immune system plays an important role in causing liver injury. A delicate balance between host immune response and the viral factors decides the outcome of the infection i.e. disease pathogenesis and viral clearance.
Non-specific immune response

The intrahepatic innate immune response contributes to early control of HBV replication via secreted cytokines and lysis of infected cells. This response activates intrahepatic immune cells, influences the priming of antigen-specific cells by the created cytokine milieu, induces the cytokine- and chemokine-mediated recruitment of lymphomononuclear inflammatory cells, and enhances MHC class I expression and antigen recognition by HBV-specific T cells. It is known that type 1 interferons-α and -β can effectively inhibit HBV replication (Caselman et al., 1992).

In acute HBV infection, the number of NK cells increase very early in the incubation phase and prior to development of the clinical signs of liver injury (Webster et al., 2000). This early response is facilitated by the NK cells' capability to recognize target cells prior to upregulation of major histocompatibility complex (MHC) class I molecules (Lanier, 1998). Upon recognition of target cells, NK cells may directly lyse HBV-infected cells and/or downregulate HBV replication by production of interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNF-α). In addition, they produce CCL3, CCL4 (formerly called MIP-1β) and CCL5 (formerly called RANTES-regulated upon activation, normal T cell expressed and secreted), which modify distribution and migration of polymorphonuclear leukocytes. Direct activation of intrahepatic NKT cells is sufficient to control viral replication (Kakimi et al., 2001). Thus, the innate immune response has the potential to control viral replication during natural HBV infection.

Non-specific activation of Kupffer cells: a special liver-resident macrophage population in liver may also affect viral replication and transcription by producing TNF-α (Koziel et al., 1998). In fact, activation of macrophages by non-HBV specific stimuli can be sufficient to downregulate HBV replication in the transgenic mouse model. Malaria infection of mice that replicate full-length HBV, for instance, triggers a cytokine response sufficient to clear HBV from Hepatocytes (Pasquetto et al., 2000) (Fig. 7).

Specific immune response

CD4+ T-Helper cells
Fig. 7. Schematic presentation of the immune responses during HBV infection
Review of literature

The CD4+ Th cells recognize viral peptides in the context of MHC class II molecules on antigen-presenting cells. These viral peptides are generally derived from phagocytosed and proteolytically cleaved HBV proteins. Thus, the antigen-presenting cell does not need to be HBV infected to be recognized by HBV-specific CD4+ T cells. Earlier it was considered that hepatocytes do not express MHC class II molecules on their surface and during viral hepatitis the resident APCs could probably be the Kupffer cells, although circulating B cells were also suggested to function as APCs (Huang et al., 1997). But, recently it has been suggested that MHC II-expressing hepatocytes, as found in clinical hepatitis, can present antigen and activate CD4+ T cells (Herkel et al., 2003). CD4+ Th cells provide help for activation and differentiation of B cells. These also contribute to induction (Ridge et al., 1998) and maintenance of HBV-specific CD8+ T cells, and license dendritic cells to activate CD8+ effector T cells (Ridge et al., 1998; Sigal et al., 1999). Together with CD8+ T cells, CD4+ T cells may also secrete IFN-γ and TNF-α that inhibit replication and gene expression of HBV (Guidotti et al., 1999 b). Finally, CD4+ T cells may lyse HBV-infected cells directly (Franco et al., 1997). CD4+ T cell help has also been suggested for an effective non-cytolytic antiviral response generated by CD8+ T cells (Maini et al., 2000). In fact, envelope-specific CD4+ T cells may control HBV replication directly by producing anti-viral cytokines rather than providing help for cytotoxic T cells in therapeutic vaccination against chronic HBV infection (Ren et al., 2003). Th1 cytokines are suggested to be positively correlated with hepatic inflammatory activity in chronic hepatitis B, whereas Th2 cells may be associated with the persistence of HBV infection (Jiang et al., 2002). An activation of Th1 immunity has been associated with responsiveness to recombinant HBsAg (Kardar et al., 2002; Jafarzadeh et al., 2003) and for a successful treatment of hepatitis B (Tsai et al., 2003). Interferon-α therapy can modulate the balance of Th1/Th2 type cytokines, and this is related to its clinical effect, thus the levels of Th1/Th2 type cytokines could be a predictor of clinical response during Interferon-alpha treatment (Xing et al., 2001).

Cytotoxic T Lymphocytes

Viral peptides associated with MHC class I molecules and presented on cell surface make a cell target for the cytotoxic cell lysis by HBV-specific CD8+ T cells. The peptides recognized are [with the exception of peptides derived from the hydrophobic HbsAg (Schirmbeck et al., 1994)] exclusively derived from endogenously synthesized proteins.
Uninfected hepatocytes usually express very little MHC class I glycoproteins. But, in early stage of acute HBV infection, after the production of interferon-α, MHC expression on hepatocytes increases and coincidentally transaminase levels rise, presumably as a result of hepatocyte lysis (Thomas et al., 1996). The CTL response to HBV is vigorous, polyclonal and multispecific in patients with acute hepatitis who ultimately clear virus (Rehermann et al., 1995). Whereas, this response is weak or barely detectable in patients with chronic hepatitis (Bertoletti et al., 1991; Chisari, 1997). Studies in transgenic mice have further strengthened the importance of CTLs. The transgenic mice, which expressed and replicated HBV in their hepatocytes, developed an acute necroinflammatory liver disease after adoptive transfer of hepatitis B surface antigen specific CTL lines and clones (Moriyama et al., 1990; Ando et al., 1993). The CTLs have been shown to be directly cytopathic for their target cells. In addition to cytolytic killing of hepatocytes, these inhibit HBV gene expression and replication in the liver of transgenic mice non-cytopathically by secreting antiviral cytokines that interrupt the HBV life cycle (Guidotti et al., 1994 a; Lisa et al., 1995; Chisari, 1997). Following antigen recognition, the CTLs also produce cytokines that lead to the recruitment of antigen non-specific inflammatory cells, such as macrophages and NK cells that contribute to amplify the liver disease initiated by CTLs (Guidotti et al., 2002). Although the CD4+ and CD8+T-cell responses to the hepatitis B virus (HBV) are thought to be crucial for the control of HBV infection, the relative contribution of each T-cell subset as an effector of viral clearance was examined in a recent study where the course of HBV infection in control, CD4-depleted, and CD8-depleted chimpanzees was monitored. The results demonstrated that CD8+T cells were the main effector cells responsible for viral clearance and disease pathogenesis during acute HBV infection, and they suggested that viral clearance is mediated by both noncytolytic and cytolytic effector functions of the CD8+ T cell response (Thimme et al., 2003). The observation in animals is supported by the studies in acute patients where a high frequency of circulating HBV-specific CD8+ T cells has been shown to coincide with the clinically acute phase of hepatitis B (Maini et al., 1999).

The relationship between virus-specific CTL responses, liver damage and viral replication have been investigated in the recent past. In the presence of an efficient virus-specific CTL response, a scenario is emerging where inhibition of viral replication can be independent of liver pathology. Thus, there could be a possibility that an inadequate CTL response ‘unable to control viral replication’ may contribute to liver pathology not only directly but
also via the recruitment of non-virus-specific T cells (Maini et al., 2000; Bertoletti et al., 2000) (Fig. 7).

*Humoral immune response*

Antibody responses are often directed against several proteins, although usually it is antibody responses against viral envelope proteins, which serve as neutralizing antibodies. The antibody response to HBV is a T-cell dependent process. These anti-envelope antibodies are readily detectable in patients who clear the virus and recover from hepatitis and they are usually undetectable in patients with chronic HBV infection. They also contribute to the pathogenesis of the extrahepatic syndromes associated with HBV infection and to the prodromal syndromes of urticaria and arthralgias, by forming antigen-antibody complexes (Chisari et al., 1995). Antibodies against nucleocapsid antigens are non-neutralizing and are present in high titres during acute as well as chronic infection. Their role in pathogenesis is not clear; though passively administered anti-HBe antibodies are reported to protect chimpanzees against HBV infection (Chisari et al., 1995).

*Genetics of infectious diseases*

Susceptibility to infectious diseases is mainly influenced by a complex interaction of environmental, pathogenic and host genetic factors. During the past few years there has been a substantial progress in identifying the genetic basis of infectious diseases. An increasing identification of individual-to-individual genetic variations has further led to an enhanced association of the genetic variations with disease susceptibility. The knowledge of genetic components not only provides clues to the ethnic diversity of infection but also to the issue of disparity in therapeutic response.

*Evidence and extent of role of host genetic components*

Evidences indicate the importance of host genetic background in disease susceptibility and these are:

*Familial studies*

Familial studies have been used in the past to assess the extent of host genetic contribution by studying familial clustering. The increase in the risk of a disease found in a relative of a
case is compared to the general population risk. However, one of the major limitations in using this in infectious diseases is that it somewhat overestimates the importance of the host genetics because of the increased risk of exposure to infection in family members as compared to the general population (Todd et al., 1996).

**Adoptee or twin studies**

These studies are used to estimate the host genetic contribution in the diseases, which are relatively independent of environmental effects. However, it has to be borne in mind that the risk of adoptees getting disease if the parents are infected will be much less than the twins getting the disease (Sorensen et al., 1988).

**Identical and non identical twin studies**

These studies are used to establish the role of genetic component if the concordance rate of the disease is higher in monozygotic twins than dizygotic twins. There have been reports of this phenomenon in tuberculosis (Comstock et al., 1978), leprosy (Fine, 1981) and hepatitis B (Lin et al., 1992).

**Strategies for identifying disease susceptibility genes:**

A disease is considered complex if the outcome of the disease is influenced by environment and/or viral and host genetic factors with no clear pattern of inheritance. Strategies developed to identify host genetic factors in complex diseases are:

**Genetic linkage studies**

These studies analyze the segregation of genetic markers in families with more than one individual affected by the disease. The basis for genetic linkage studies is a phenomenon called linkage disequilibrium, which refers to the non-random association of alleles such that a particular allelic combination occurs more often than it is expected by chance. It can occur due to genetic proximity or from selection pressure favoring particular combination of alleles at two distant loci (Thio et al., 1999). It is assumed that if the marker is linked with a disease-associated polymorphism it will be found more frequently in the patients.
Affected sibling pair studies

Classical genetic linkage studies analyze the segregation of genetic markers in large multigenerational pedigrees. For infectious and other multifactorial diseases, simpler pedigrees consisting of two or more affected siblings and their parents are preferred. (de Vries et al., 1976). The principle of sib-pair studies is based on Mendelian inheritance. It is assumed that a genetic marker would be transmitted from a parent to one of the siblings 50% of the time, to neither of them 25% of the time and to both of the siblings 25% of the time. However, if the marker is in linkage disequilibrium to a disease susceptibility gene, then the Mendelian pattern of inheritance will be skewed. Therefore, a much higher proportion of siblings with disease will share the marker. By analyzing allele sharing only in affected-sibling pairs, the problem of classifying healthy family members as either resistant or simply unexposed to the infectious agent is avoided. Moreover, novel genes and important pathways of disease development could also be identified using this technique (Thurz, 2001). But, the problem in such studies remains to recruit a large number of families with such features. Main limitations of the genetic linkage approach are its lower power than association studies and the requirement for multicase families, which are often difficult to recruit (Merikangas et al., 1996).

The transmission test for Linkage Disequilibrium (TDT)

The TDT considers parents who are heterozygous for a marker associated with disease and evaluates the frequency with which that allele or its alternate is transmitted to affected offspring. The advantage is that it does not require data either on multiple affected family members or on unaffected sibs (Spielman et al., 1993). TDT can also be used to map more finely the regions of linkage. Once a positive TDT is found, a detailed analysis of neighboring genes can be undertaken.

Genome-wide scan

Linkage studies can be performed with a few markers as well as whole genome-wide scan with a large number of markers, but the principle remains the same. It uses densely distributed markers, either microsatellites or single nucleotide polymorphisms (SNPs) to identify the areas of interest in the genome. Genome wide scan can be based on either population or family studies. In population based genome wide scan, frequencies of the
markers in a cohort of patients with a specified outcome are compared with those without the outcome (Dib et al., 1996; Liu et al., 1998). The problem in population studies is that an association can occur even in absence of linkage, for example, as a result of population stratification. This can be overcome in AFBAC (Affected family-based controls) studies in which control and disease samples are obtained from the same family (Spielman et al., 1993).

**Candidate gene approach and disease association studies**

In complex genetic diseases, linkage studies provide less information as the contribution of individual marker is less. Here, unaffected family members usually provide much less information than affected members. The association, therefore, is demonstrated by comparing allele frequencies at marker locus in random samples of unrelated patients and controls. It is much simpler since only a single affected member from each family is studied and there is no need to recruit families with multiple affected members. A candidate gene is a gene whose products are known to influence the course of a particular disease (pathogenesis or protection) and which can be hypothesized to influence the susceptibility to a particular disease. To confirm the hypothesis, the polymorphisms in the gene are examined in disease association or family studies. Candidate genes are suggested based on the understanding and the knowledge of the immune mechanisms in infectious diseases. Genes identified in an animal model or implicated in a similar disease could also be proposed as candidate genes. Many of the candidate genes relevant for the present study, could fall into the following categories: (1) genes that mediate the processes of viral entry into hepatocytes, including genes involved in viral binding, fusion with cellular membrane and transportation in target cells; (2) genes that modulate or control the immune response to HBV infection influencing viral persistence and removal; (3) genes that participate in disease pathogenesis and development of disease outcome such as liver cirrhosis and hepatocellular carcinoma associated with chronic HBV infection; and (4) those that contribute to resistance to antiviral therapies (Wang et al., 2003). Since genetic interactions are complex, it is unlikely to find a single allelic variant responsible for HBV resistance or susceptibility. And, the collective influence of several single nucleotide polymorphisms (SNPs) in different candidate genes may underlie the natural combinational or synergistic protection against HBV. Another approach to identify the candidate genes is through linkage analysis, which can be used to map the genes affecting
susceptibility. The limitations of candidate gene approach are: prior identification of a gene, its functional significance and its polymorphisms (Hill, 1998; Thio et al., 2000). Thus, major genes, which are not characterized but affect susceptibility to infectious diseases, may be missed. General problems in interpretation of the results are:

- Inconsistencies between different populations
- If sample size is small it is difficult to detect convincing allele associations
- Lack of power of association in multiple alleles.
- Lack of a matching control group to the cases.
- Population stratification.

Demonstration of an allele association with the disease does not mean that the allele is responsible for the disease. Rather, the allele may simply be linked to some other disease-causing allele.

**Genetic Susceptibility and Hepatitis B**

HBV exposure can result in broad spectrum of no infection to different clinical conditions and the reasons for the individual variation in infection, severity and outcome of the disease are not fully understood. But, environmental and virological factors such as viral load, genotype and genetic divergence due to viral gene mutations and host genetic factors, which affect host immunological response against virus, are believed to play important role in disease development (Wang, 2003).

The virological and immunological factors of HBV have been extensively studied, but the study of host genetic factors and the relationship between host genetics and HBV resistance is still in its infancy (Hill, 1998; Dean et al., 2002). However, in the recent past, progress has been made in the study of human genetic backrounds at certain loci associated with susceptibility to HBV infection (Fig. 8).

**Indirect evidence of the importance of genetic parameters in Hepatitis B**

A variety of indirect evidences have been reported in literature in support of host genetic background as a risk factor in suffering from hepatitis after HBV infection. Initial studies have suggested that persistent HBV segregated within families in a manner suggestive of an autosomal recessive trait (Blumberg et al., 1969). But, since then knowledge of the
Fig. 8. The candidate genes implicated in association with hepatitis B infection
vertical transmission has complicated this sort of analysis. Later, twin studies conducted in Taiwan strengthened the role of genetic factors. In these studies it was demonstrated that monozygotic twins were concordant for HBsAg carriage in 50% of cases whereas dizygotic twins (and non-twinned siblings) were concordant in only 20% of families (Lim et al., 1989). The role of genetic factors was also highlighted with the observation of various clinical outcomes in patients infected with the same HBV virus (Hohler et al., 1997). This observation was supported by another study where the severity and outcome of infection in fulminant cases infected by a precore variant was been found to be unrelated to any additional variation in entire HBV genome and hence the role of host factors was highlighted (Karayiannis et al., 1995). Occurrence of variable phenotype infection in different individuals infected by HBV also supports the role of host genetic factors. The long-term follow-up studies have indicated that some individuals in high-risk groups (e.g. spouses in HBV-infected families) never develop the disease. This suggests the existence of an individual-specific resistance to HBV infection (Luo, 2001). Other factors supporting the role of host genetic factors are: difference in the incidence and infection rate among global ethnic populations (Hoffmann et al., 2002) and differential response to interferon-alpha or Lamivudine antiviral therapy (Marcellin et al., 1994) and HBV vaccination (Kubba et al., 2003).

**Candidate genes shown to be associated with genetic susceptibility to HBV infection**

There have been some reports of associations of some candidate genes with hepatitis B outcome (Fig. 8) as discussed below:

**HLA class I and II alleles**

The HLA complex consists of class I (HLA-A, HLA-B and –C) and class II (HLA-DRB1, -DQA1, -DQB1, -DPA1, and -DPB1) genes. CD8+ T cytotoxic cells recognize antigenic peptides in association with class I molecules and CD4+ T helper cells recognize antigen presented by class II HLA molecules leading to activation of both humoral and cell-mediated response. HLA genes are highly polymorphic and the HLA-type of an individual determines the repertoire of peptides bound on HLA molecules. Therefore HLA-type influences the activation of T cells. Results of the associations of HLA class I with chronic hepatitis B, asymptomatic HBsAg carriage, and controls have largely been inconsistent.
Increased frequency of HLA-B35 and HLA-B15 have been associated with moderate to severe chronic hepatitis (Mota et al., 1987) and asymptomatic HBsAg carriage (Giani et al., 1979). But, later studies did not find any significant association of class I alleles with HBV persistence (Thio et al., 1999). However, in Chinese population, HLA-DRB1*0301, HLA-DQA1*0501 and HLA-DQB1*0301 were shown to be related with susceptibility to chronic hepatitis B, and HLA-DRB1*1101/1104 and HLA-DQA1*0301 related with resistance to chronic hepatitis B (Jiang et al., 2003). HLA-DR, -DP, and -DQ genes were shown to determine the immune response to hepatitis B vaccine (Desombere et al., 1998). It was shown that HLA DQB1*0603 was associated with hepatitis B virus associated membranous nephropathy (Bhimma et al., 2002) and HLA class II allele DRB1*1302 was associated with a self-limiting course of acute hepatitis B in African population (Thursz et al., 1995). The effect of DBR1*1302 in clearing the HBV infection in vivo was confirmed in Caucasian population (Hohler et al., 1997). Heterozygosity at HLA class II loci which leads to increased HLA diversity and an increase in number of MHC class II restricted epitopes has been shown to confer a selective advantage in Hepatitis B viral infection clearance (Thurz et al., 1998). An association of HLA class II homozygosity with HBV persistence was further confirmed (Thio et al., 1999). They found that haplotype cluster of DQA1*0501-DQB1*0301-DRB1*1302 had a significant association with viral persistence. However, it was later reported that no correlation could be observed between the clearance of HBV or HCV virus and HLA phenotypes (Zavaglia et al. 1996). HLA-DR13 alleles were also shown to have a positive influence on the clearance of HBV infection. This was explained by a more vigorous HBV core-specific CD4+ T cell response and due to a more proficient antigen presentation by the HLA-DR13 molecules (Diepolder et al., 1998). A later study also showed a strong association of HLA-DR13 genotype with the clearance of HBV in Koreans (Ahn et al., 2000).

**Tumor necrosis factor-alpha**

Tumor necrosis factor-alpha (TNF-α) is an important cytokine involved in noncytotoxic antiviral mechanisms (Knight et al., 1999). TNF-α, secreted by CTLs and antigen-non-specific macrophages, has been shown to reduce HBV gene expression and replication by non-cytolytic mechanisms (Guidotti et al., 1994 a; Guidotti et al., 1996; Romero et al., 1996). This gene is located within the class III region of the MHC complex. The A allele of TNF-α with G>A polymorphism at -488 position (according to HUGO nomenclature),
has been shown to be associated with cerebral malaria in African population (McGuire et al., 1994). Also, the hepatitis B surface antigen has been shown to associate with severe malaria in Gambian population (Thurz et al., 1995). These observations pointed to an indirect association of polymorphism of TNF-α to hepatitis B. Also, a significant association of G/G genotype at -418 position was found with hepatitis B persistence in chronic hepatitis B patients as compared to healthy controls and spontaneously recovered or acute patients (Hohler et al., 1998a) in German population. Later, studies in Japanese population (Miyazoe et al., 2002) and Caucasian population (Ben-Ari et al., 2003) could not find any association between TNF-α gene promoter polymorphisms and disease progression in HBV infection. However, a study in Korean population showed association of TNF-α haplotypes for variation at six loci with the clearance of hepatitis B virus infection (Kim et al., 2003). Recently, a case control study in Chinese population also revealed that the frequency of G allele at -418 position was significantly higher in patients with chronic hepatitis B than in individuals with self-limited HBV infection (Lu et al., 2004). The reasons for the discrepancy in associations could be due to genetic heterogeneity between different population groups. Also, it is important to note that the mode of infection could be different, which can also account for a presence of correlation in one and a lack of correlation in another.

**Interferon-gamma**

IFN-γ clears HBV *in vivo* by a noncytolytic effect (Guidotti et al., 1996) and is involved in liver injury (Ohta et al., 2000). Asian population has been shown to possess those IFN-γ genotypes that result in low expression than do Caucasian populations (Hoffmann et al. 2002), which suggests the possibility of an association between low IFN-γ expression in the highly HBV-susceptible Asian population. A significant difference in the distribution of IFN-γ polymorphism has been found between patients with chronic HBV infection and controls in Asian population (Ben-Ari et al., 2003).

**Interleukin-1 B and interleukin-1 receptor antagonist gene**

An association between polymorphisms of the promoter region of IL-1B and IL-1RN intron 2 and chronic hepatitis B virus infection and HBV-DNA relication was found in Chinese population (Zhang et al., 2004).
**Interleukin-6 (IL-6)**

IL-6 plays an essential role in the regulation of immune response to chronic diseases. In a study conducted in Korean population, three known single nucleotide polymorphisms (SNPs) in IL-6 promoter region were genotyped in a large chronic hepatitis B cohort. However, no significant associations were detected between -572 C>G transversion and chronic hepatitis B outcome (Park et al., 2001).

**Interleukin-10 (IL-10)**

IL-10 is a Th2 cytokine, which acts as a potent inhibitor of Th1 effector mechanisms (Mosmann, 1994). An analysis of the distributions of IL-10 promoter SNPs in Japanese HBV-infected patients suggested that the -819T and -592A wild-type alleles were more common in asymptomatic carriers than in patients with chronic progressive liver diseases (Miyazoe et al., 2002), suggesting that the IL-10 gene promoter polymorphisms do have some relevance in the progression of chronic hepatitis B disease, perhaps due to a decreased IL-10 production induced by -819T and -592A haplotype allele.

**Mannose Binding Protein (MBP)**

MBP is a calcium-dependent opsonin that plays an important role in innate immunity by activating the classical complement pathway and phagocytosis. There are three identified polymorphisms in the MBP coding region (codons 52, 54 and 57), leading to a low serum concentration and abolishing its ability to affect host immunity because of an opsonic defect. The middle surface protein of HBV viral envelope contains a mannose-rich oligosaccharide to which MBP could potentially bind. Codon 52 mutant allele was shown to be associated with persistent HBV infection in Caucasian population (Thomas et al., 1996). But, the authors could not find any association of codon 52 mutations with Asian population. The higher frequency of the codon 52 mutation among the HBV patients than among controls is probably consistent with the fact that the mutation leads to the failure of opsonisation and phagocytosis of HBV. Codon 54 mutation was associated with viral infection in Chinese (Yuen et al., 1999), Japanese (Hakozaki et al., 2002) and Vietnamese (Song et al., 2003) patients. In German Caucasians and Gambians, these MBP polymorphisms were not associated with the chronic disease in hepatitis B (Hohler et al., 1998 b).
**Vitamin D receptor gene**

The active form of vitamin D is an immunomodulatory hormone that inhibits the Th1 response and activates the Th2 immune reaction. Bellamy et al. studied two known Vitamin D receptor gene polymorphisms in Gambian HBV-infected patients and found that the tt genotype of one polymorphism was associated with viral clearance (Bellamy et al., 1999).

**Estrogen receptor alpha gene polymorphisms**

An analysis of two haplotype-tagged SNPs (htSNP), T29C in exon 1 and A252966G in intron 5 in estrogen receptor gene was carried out in 1,277 persistent HBV-infected cases, 748 spontaneously recovered controls, and 293 nuclear families. It was observed that the subjects bearing ESR1 29T/T genotype had an increased susceptibility to persistent HBV infection compared to those bearing at least one 29C allele. Consistent with the results of population-based association study, a significantly greater than expected transmission of the 29T allele (56.4%) from heterozygous parents to offspring with persistent HBV infection was observed using the transmission-disequilibrium test (TDT) in 293 nuclear families. It was, therefore suggested that the genetic variation at the ESR1 locus influences susceptibility to persistent HBV infection in Chinese population (Deng et al., 2004).

**Conclusions and prospects for study of HBV resistance alleles**

Thus far, reports from various laboratories have been inconsistent in showing the effect of host genetic factors on HBV clearance and persistence. This ambiguity may be attributed to one or more of the following reasons: (1) a complex interaction between the virus and host multiple alleles; (2) the ethnic differences in the studied groups; (3) an association with a gene in linkage disequilibrium. Therefore, global multicohort studies are needed to integrate the genetic data and the clinical data for a complete understanding of underlying immunogenetics resulting in the pathogenesis of HBV infection. Alternatively, involvement of certain candidate genes need to be identified and screened for polymorphisms in association with the disease phenotype i.e. hepatitis. It is well known that genetic interactions are complex, therefore, it is unlikely that a single allelic variant would be responsible for HBV resistance or susceptibility (Griffiths, 2002). However, the collective influence of several SNPs or haplotypes may provide in
combination a synergistic protection against either the infection or its resultant pathogenesis. Recently developed genetic epidemiology strategies and dense genome-wide searches, together with the growing numbers of candidate genes and sequence information, provide a repository of information to identify genes associated with chronic HBV infection.

**Candidate genes chosen in the present study**

**Fas/Fas Ligand**

Fas, located on 10q24.1 is a type 1 membrane protein of 45 KDa belonging to Tumor necrosis factor and Neural growth factor receptor family. Fas/FasL system plays a role in B and T lymphocyte development and maturation. Fas is constitutively expressed in heart, liver, lung and kidney (Nagao et al., 1999). Fas ligand is expressed by activated T-cells (Rouvier et al., 1993; Suda et al., 1993) and its binding to Fas receptor on hepatocytes may lead to destruction of hepatocytes (Ando et al., 1994; Rouquet et al., 1995). Agonistic anti-Fas antibody also transduces death signal in Fas bearing cells.

The role of Fas in inducing apoptosis in liver has been supported by the observation that anti-Fas antibody administration in mice induced rapid massive liver damage to hepatocytes showing apoptotic characters, fulminant hepatic failure and death (Ogasawara et al., 1993). Anti-Fas antibody also induced primary hepatocyte cultures to apoptosize (Hayashi et al., 1997).

The cytotoxic T lymphocyte (CTL) clones specific for hepatitis B surface antigen (HBsAg) caused acute liver disease in HBsAg transgenic mice and killed hepatocytes expressing HBsAg in a Fas-dependent manner (Kondo et al., 1997). Fas-L-positive CD8 T-cells were shown to play a major role in the hepatocellular injury in chronic hepatitis (Ibuki et al., 2002). In hepatitis B virus-related cirrhosis and in acute liver failure, Fas expression was highly elevated in hepatocytes, which closely correlated with the activity of the viral hepatitis whereas normal cases did not show Fas expression (Mochizuki et al., 1996). It was also observed that Fas ligand messenger RNA expression was absent in normal liver but was detected at high levels in livers with ongoing liver damage. In cases of liver cirrhosis due to hepatitis B virus and in the acute hepatic failures, the ligand expression was found primarily in areas with lymphocytic infiltration. These findings
suggested that liver destruction in hepatitis B might primarily involve killing of hepatocytes by T lymphocytes using the Fas receptor-ligand system. In contrast, in patients with alcoholic liver damage, a high Fas ligand messenger RNA expression was found in hepatocytes which suggested that the death of hepatocytes might occur by fratricide and paracrine or autocrine mechanisms mediated by the hepatocytes themselves (Galle et al., 1995) (Fig. 9). Fas system has been shown to be involved in liver injury in fulminant hepatitis B (Rivero et al., 2002) and chronic liver disases (Watanabe-Fukunage et al., 1992; Galle et al., 1995; Mochizuki et al., 1996). Fas positive lymphocytes were found in HBsAg positive patients, which made them and the hepatocytes sensitive to FasL positive lymphocytes and the hepatocytes and resulted in lowered viability of these lymphocytes. This may explain, at least in part, the defective removal of virus-infected cells in chronic hepatitis (Ehmann et al., 2000). Amplified FasL expression during HBV infection due to HBx induced IL-18 expression in liver, is suggested to be associated with hepatic injury (Lee et al., 2002).

Fas/Fas L were shown to be increased in liver followed by partial heatectomy in rats (Taira et al., 2001). Liver regeneration kinetics were reported to be delayed in mutant (lpr) mice with decreased cell surface Fas expression. In contrast, regeneration was not delayed in lpr-cg mutant mice, which had a Fas mutation that prevented Fas-induced death but not Fas-dependent proliferative stimulation. Fas engagement on cells in regenerating or healing tissues has been suggested to promote cell growth. (Desbarats et al., 2002) (Fig. 9). Fas has also been reported to transduce growth-promoting signals in proliferating T cells, fibroblasts and some tumor cells.

In HBV-associated hepatocellular carcinoma (HCC), hepatoma cells are known to eliminate Fas expression on themselves and let hepatocytes and infiltrating mononuclear cells generate sFas and thus escape from the host immune system and metastasize by modulating Fas expression as the infiltrating cells express FasL (Strand et al., 1996; Shin et al., 1998; Nagao et al., 1999). Additionally, common defect in the expression of proteins interacting with Fas like upregulation of FAP and downregulation of downstream molecules in Fas/LasL pathway such as FADD and FLICE was also reported in HCC (Shin et al., 1998).
DNA fragmentation and Apoptosis/necrosis

FasL expressed by immune privileged sites

HBx induces FasL expression

Fas constitutively expressed by liver, heart and kidney

Controls B and T lymphocyte development & maturation

Liver regeneration

Fas Ag correlates with chronic liver disease in HBV carriers

Involved in Ag-sp killing by CTLs

Tumor cells exp FasL and eliminate Fas exp'n

Fig. 9. Major roles played by Fas/FasL system in liver and during hepatitis B infection.
TGF-β1 has been shown as an enhancer of Fas-mediated apoptosis and plays a role in the pathophysiology of pulmonary fibrosis (Hagimoto et al., 2002).

**Transforming growth factor beta1 (TGF-β1)**

Transforming growth factor-beta1 (TGF-β1) is a multifunctional peptide that controls proliferation, differentiation, and other functions in many cell types. Many cells synthesize TGF-β1 and essentially all of them have specific receptors for this peptide. TGF-β1 regulates the actions of many other peptide growth factors and determines a positive or negative direction of their effects (Sporn et al., 1986).

In liver, it is synthesized by mesenchymal cells i.e. Kupffer and hepatic stellate cells. TGF-β1 is secreted from cells in a biologically latent form, non-covalently bound to 80kDa latency associated peptide (LAP). Latent TGF-β1 is converted to active TGF-β1 in extracellular space. Thus, latent and active TGF-β1 may diffuse easily into blood in a well-perfused organ.

TGF-β1 has a critical role in homeostatic regulation of the hepatic immune system, inhibiting the development or expansion of hepatic cytolytic CD4+ T cells (Rudner et al., 2003). Hepatocellular carcinoma cells producing TGF-β1 may reduce the generation or activation of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, and thus could enhance their ability to escape immune-mediated surveillance (Mouri et al., 2002; Lee et al., 2004). TGF-β1 secreted from apoptotic T cells has been shown to inhibit proinflammatory cytokine production by activated macrophages to foster immune suppression (Chen et al., 2001) (Fig. 10). In autoimmune hepatitis, strong TGF-β1 expression is found in the inflamed liver. TGF-β1 overexpression may be part of a regulatory immune response attempting to suppress autoreactive T cells. TGF-β1 has an important role in immune homeostasis in the liver and may teleologically explain TGF-β1 upregulation in response to T cell-mediated liver injury (Schramm et al., 2003). TGF-β has been shown to inhibit HLA class I-restricted HBV Ag-specific CTL activity in patients with chronic hepatitis B (Kakumu et al., 1993) (Fig. 10).

TGF-β1 −/− knockout mice die of multifocal inflammatory syndrome soon after weaning whereas TGF-β1 +/− mice have diminished levels of TGF-β1 and show increased
DNA fragmentation and Apoptosis/necrosis

Inflammation

HBx induces TGFβ1 expression

Levels increased during chronic & acute hepatitis

Carcinogenic role

Controls cell proliferation and differentiation

Suppresses Liver regeneration

Released by liver mononuclear & mesenchymal cells

Tissue fibrosis & extracellular matrix production

Immunosuppression

Fig. 10. Major roles played by TGF-β1 in liver and during hepatitis B infection.
tumorigenesis in liver and lung in response to chemical carcinogens. The heterozygtes TGF-β1 +/- show no effect on initiation, but do show enhanced promotion and progression. Thus, TGF-β1 has been assigned a role of a tumor suppressor with true haploid insufficiency as a cause for carcinogenesis. Paradoxically, TGF-β1 has an oncogenic role also, reflected in the suppression of immune surveillance and induction of angiogenesis (Tang et al., 1998) (Fig. 10). TGF-β1 loss is not quantitatively compensated for by the upregulation of TGF-β2 or TGF-β3.

TGF-β1 stimulates production of extracellular matrix proteins, like collagens, non-collagenous glycoproteins and proteoglycans. It has matrix degradation inhibitory action as it decreases synthesis of matrix degrading enzymes and stimulates gene expression of plasminogen activator inhibitor and tissue inhibitor of metalloproteinase. It also modulates expression of integrins. Thus, it is involved in pathobiology of tissue fibrosis. Detection of TGF-β1 by means of immunohistochemical reaction in liver biopsy specimens taken from patients having different chronic active liver diseases at the site of fibrosis suggests that TGF-β1 might have a role in the process and progression of fibrosis during the development of the disease (Nagy, 1991). To support this, plasma TGF-β1 levels were found to be increased in parallel with the histological degree of necroinflammation and of liver fibrosis (Murawaki, 1998). Continuous presence of both severe liver damage and upregulation of TGF-β1 synthesis is necessary to induce hepatic fibrosis in chronic hepatitis model of chronic liver injury induced by repeated administration of con A. (Kimura et al., 1999).

An involvement of TGF-β1 in association with the arrest of liver cell regeneration and induction of apoptosis in liver diseases has also been shown (Takiya et al., 1995; Hayashi et al., 2004). TGF-β1 causes apoptosis in hepatocytes through similar pathways to those activated by Fas ligation, that is, through generation of active caspase-8, cytochrome c release by mitochondria, and activation of various execution caspases, including caspase-3 and caspase-7 (Cain et al., 2001). TGF-β1 induced apoptosis also occurs via the release of cytochrome c and the subsequent oligomerisation of Apaf-1 into an approximately 700 kDa apoptosome complex. Even though TGF-β1 induction of apoptosis is a receptor-mediated event, it operates through the mitochondrial/Apaf-1 caspase activation pathway that appears to act as a common execution pathway for many diverse apoptotic stimuli.
Cain et al., 2000). TGF-β1 has been shown to be associated with apoptosis of human hepatoma cells, cell lines (Lin et al., 1992), primary rat hepatocytes (Oberhammer et al., 1992) and is involved in controlling liver size, by inducing apoptotic cell death in hepatocytes. Interestingly, TGF-β1 has anti-apoptotic roles also as it has been reported to protect from apoptosis by Fas or by corticosteroids (Lanvin et al., 2003; Undevia et al., 2004; Jung et al., 2004; Kawakami et al., 2004). TGF-β1 plays a role in the arrest of liver cell regeneration and atypical bile duct proliferation as in case of fulminant hepatitis. This phenomenon may account for the inadequate hepatic regeneration that occurs with liver disease (Takiya et al., 1995).

**Tumour necrosis factor (TNF-α)**

TNF-α, the gene for which is located on chromosome 6p21.3, is released upon various stimuli from resident liver macrophages (Dieter et al., 1997; Dieter et al., 1999), bile duct epithelia, intrahepatic vascular endothelial cells or immigrating leukocytes (Loffreda et al., 1997). It is a multifunctional proinflammatory cytokine, with effects on lipid metabolism, coagulation, insulin resistance, and endothelial function (Dieter et al., 1999) (Fig. 11). It is also the principal mediator of inflammation and cellular immune responses with multiple biological functions, including cytotoxicity towards tumor cells and virus-infected cells, several immunomodulatory actions, direct antiviral activity and initation of the inflammatory response (Kumar et al., 2001) (Fig. 11).

TNF-α was shown to trigger apoptosis or necrosis of hepatocytes and anti-TNF-α antibodies were shown to reduce liver cell damage (Iimuro et al., 1997). It induced apoptosis in hepatocyte cell lines expressing HBV (Guilhot et al., 1996) and was implicated in hepatocyte apoptosis during HBV infection (Kagi et al., 1994). TNF-α is an important mediator of liver cell injury caused by HBV infection. It was observed that HBx increased the expression of TNF-α in human hepatoma cell line, HepG2 (Yia et al., 2003; Lara-Pezzi et al., 1998) and sensitized hepatocytes to apoptosis in the presence of TNF-α (Su et al., 1997; Wang et al., 2004). Patients with chronic and fulminant hepatitis B show an elevated plasma TNF-α levels, which are related to the inflammation of liver cells. The peripheral mononuclear blood cells of such patients show an enhanced TNF-α production in vitro (Sheron et al., 1991; Wang et al., 1999; Zhang et al., 2002). Furthermore, a massive increase in spontaneous TNF-α production by peripheral blood mononuclear cells
Fig. 11. Major roles played by TNF-α in liver and during hepatitis B infection.
in vitro was observed in chronic HBV infected patients undergoing interferon alpha (IFN-α) treatment at the time of successful seroconversion i.e., removal of hepatitis B e antigen (HBeAg) removal (Sheron et al., 1990). An upregulation of TNF receptor system was found was correlated with hepatic inflammation (Marinos et al., 1995; Fang et al., 1996) in chronic hepatitis B infection. Transmembrane and soluble TNF were shown to be involved in the development of hepatic injury in experimental hepatitis (Küsters et al., 1997) (Fig. 11).

Paradoxically, TNF-α was also shown to mediate non-cytolytic control of viral replication and viral gene expression by viral antigen specific cytotoxic CD8+ T lymphocytes in transgenic mouse (Guidotti et al., 1994 a; Guidotti et al., 1996). Envelope-specific CD4+ T cells and activated APCs were shown to directly control HBV replication by producing TNF-α and other antiviral cytokines (Kimura et al., 2002; Ren et al., 2003). TNF-α was reported to act synergistically with IFN-gamma for inhibiting HBV gene expression (Pasquetto et al., 2002).

TNF-α has been shown to play a critical role in not only viral clearance but also in lymphoid tissue development and stem cell differentiation. TNF-α was shown to be essential for the proliferation of HBV-specific CTLs both in vivo and in vitro (Kasahara et al., 2003) (Fig. 11). Thus, TNF-α plays a role both in contributing to apoptosis of hepatocytes and inhibition of HBV replication. Interestingly, it confers two opposing effects in liver, hepatocyte apoptosis and regeneration, depending on the general setting (Diehl et al., 1996; Diehl et al., 2000; Fausto et al., 2000). It signals through two receptors, TNF-R1 (55 kDa) and TNF-R2 (75 kDa) (Bradham et al., 1998), both of which have been shown experimentally to be expressed on mouse hepatocytes (Adamson et al., 1992). Activation of TNF-R1 leads to apoptosis via the adaptor molecules TNF-R1-associated death domain (TRADD) and Fas-associated death domain (FADD) leading to the activation of procaspase-8, terminally resulting in an activation of the effector caspase-3 (Bradham et al., 1998). In hepatocytes this pro-apoptotic stimulus is counterbalanced by signaling through TRADD, receptor interacting protein 1 and TNF receptor-associated factor (TRAF)-2, which lead to an activation of nuclear factor κB (NF κB) and Jun-N-terminal kinase. This results in the de-novo synthesis of anti-apoptotic proteins and the synthesis of proteins that allow the hepatocyte to leave the G₀ phase and reenter the cell cycle (Bradham et al., 1998). The latter anti-apoptotic signal chain could also be elicited
Review of literature

via the TNF-R2 and the adaptor molecules TRAF1 and TRAF2. In case NFκB activation or NFκB-dependent synthesis of mRNAs or anti-apoptotic proteins are inhibited, TNF α could cause hepatocyte apoptosis because the TNF-dependent pro-apoptotic signal cascade is still active. Interestingly, IL-6 has been observed to enhance TNF α-induced apoptosis in hepatocytes, probably by increasing cell surface expression of the TNF receptor(s) (Boer et al., 2003).

**Interleukin-6 (IL-6)**

IL-6, located on chromosome 7p21 is an immunoregulatory cytokine commonly produced at local tissue sites and is released in almost all situations of homeostatic perturbations.

Although the source of IL-6 within the liver has not been unequivocally established, studies with bone marrow transplantation provide evidence that hepatic Kupffer cells (liver macrophages) are responsible for production of IL-6 in response to liposaccharide or TNF-α. IL-6 exerts diverse effects on a variety of different organs or cell systems. Together with TNF-α and IL-1, it is required for induction of acute phase response. IL-6 plays a central role in the differentiation and growth of haematopoietic precursor cells, B-cells, T-cells, keratinocytes, neuronal cells, endothelial cells, osteoclasts and osteoblasts (Kallen, 2002). It is involved in regulating inflammatory and immunological responses in the liver through cytokines and cellular factors (Andus et al., 1991). Secreted IL-6 acts on neighboring hepatocytes in a paracrine fashion to stimulate liver regeneration and repair. IL-6 bound to the soluble IL-6 receptor signals via gp130 and Janus kinase-1 (JAK-1), leads to activation of the Stat3 transcription factor and the MAPK signal transduction cascade (Taub et al., 2003). It has hepatoprotective and anti-apoptotic role as it confers resistance to liver injury by hepatic toxins and ischemia (Taub, 2003). Pretreatment of hepatocytes with IL-6 was shown to sensitize hepatocytes to TNF-α-induced apoptosis (Boer et al., 2003) and in another study, the Apo1/Fas/CD95-induced apoptosis was shown to be inhibited by IL-6 (Kovalovich et al., 2001). Since, TNF-α and Fas share a common intracellular signal chain distal of the adaptor protein FADD, the sensitization of hepatocytes to a TNF-induced apoptosis most likely occurred at a site proximal to FADD, and by increasing the TNF receptor number on the hepatocyte surface (Boer et al., 2003). IL-6 has also been reported to inhibit TGF-β-induced apoptosis in liver cells (Chen et al., 1999) (Fig. 12).
Fig. 12. Major roles played by Interleukin 6 in liver and during hepatitis B infection.
It was shown that human IL-6 facilitates HBV infection in vitro and in vivo (Galun et al., 2000). IL-6 levels are reported to increase during chronic hepatitis B infection (Tangkijvanich et al., 2000; Kakumu et al., 1991). A positive correlation was shown between serum IL-6 and clinical severity of chronic HBV infection (Tangkijvanich et al., 2000). IL-6 was also shown to participate in the pathological process of Chronic Hepatitis, cirrhosis (Napoli et al., 1994) and ascites formation (Zhang et al., 2002) (Fig. 12) and regulated the enhancer activity of HBV Enh 1 (Ohno et al., 1997). It is speculated that the elevated levels of IL-6 seen in hepatitis increase HBx expression by a mechanism involving NF-κB (Lee et al., 1998) (Fig. 12).

Intrahepatic IL-6 production is enhanced in liver regeneration (Iwai et al., 2001; Fausto et al., 1995), thus implicating it in the regulation of liver regeneration. This finds support in the observation that the IL-6 knockout mice was shown to be defective in regeneration (Cressman et al., 1996). In rats, partial hepatectomy strongly increases serum concentrations of IL-6 after 12 h and concentrations of the sIL-6R after 24 h (Fulop et al., 2001). IL-6 has a major role in the induction of an adaptive response to partial hepatectomy and ensures body homeostasis and survival (Blindenbach et al., 2003). Since, in TNF-R1 knockout mice, the defect in hepatocyte proliferation could be rescued by treating the animals with IL-6, it was speculated that the increase in TNFα levels were important to induce IL-6, which is ultimately involved in triggering liver regeneration (Streetz et al., 2000). Recent reports show that IL-6 functions as the direct hepatic mitogen in vivo and could be a potent liver growth factor with potential clinical utility for increasing liver mass following injury (Zimmers et al., 2003) (Fig. 12). Transfection of IL-6 into non-metastatic HCC cells has been shown to cause metastasis of these cells (Reichner et al., 1998). It is also reported to have a protective role in pathogenesis of liver diseases and fibrosis (Kovalovich et al., 2000; Wuestefeld et al., 2003).
Liver damage, viral control and candidate genes for hepatitis B infection

Liver and apoptosis

Loss of cell viability and the resulting cell death are the key events in many clinical settings. Various mechanisms of cell death in liver include immunologically mediated injury, direct injury by drugs or pathogens, cell death as a result of reversal of hyperplasia or vascular causes. Viral hepatitis, which is characterized by a diffuse inflammatory reaction in the liver infected by hepatotrophic viruses, is known to be associated with liver damage and cell loss (Patel et al., 1999). Cytotoxic lymphocytes attack antigens exposed on the surface of cells in the liver as seen in acute hepatitis (viral and drug induced), chronic active hepatitis, primary biliary cirrhosis, and possibly alcoholic liver disease (Guidotti, 2002). Lymphocyte-mediated liver damage plays a central role in the pathogenesis of viral hepatitis and many drug reactions as well as acute presentations of autoimmune hepatitis. Besides causing immune mediated liver injury, some viruses are intrinsically cytotoxic, for example hepatitis D virus (Cole et al., 1991). Virally infected hepatocytes may also be sensitive to the lethal effects of inflammatory cytokines (Gilles et al., 1992). Various hepatotoxic chemicals like chloroform, paracetamol and galactosamine can also cause liver injury. However, a wide range of drugs and environmental pollutants might induce hyperplasia or hypertrophy of hepatocytes. Once the stimulus for hyperplasia has been removed, however, the liver rapidly reverts to its normal size through apoptosis (Alison et al., 1994). The cells of the liver, like all other cells, may die by one of the two distinct processes, necrosis or apoptosis. Necrosis is characterized by the simultaneous activation of multiple-deregulated processes that culminate in the loss of cell membrane integrity. Membrane rupture causes leakage of cellular constituents, which stimulates a secondary inflammatory response. Apoptosis involves an ordered sequence of events culminating in a controlled shutdown of cellular functions. It may be viewed as a physiological response, which allows the cell to die, and its remnant particles to be swiftly cleared, with minimal disturbance to the neighboring cells and tissues (Lau et al., 1998; Thomson et al., 1999). During viral hepatitis, formation of acidophilic hepatocytes is seen. These hepatocytes are composed of deeply acidophilic cytoplasm in the initial phase and acidophilic (Councilman) bodies in the later phase. These acidophilic cells eventually undergo fragmentation, features that are compatible with apoptosis (Lau et al., 1998).
proapoptotic molecules like Fas ligand (Shin et al., 1999; Wang et al., 2004), TNF-alpha (Wang et al., 2004) and TGF-β1 (Yoo et al., 1996) in the cell.

It is interesting to note that HBx has also been shown to have anti-apoptotic effects as it has been shown to mediate protection against anti-Fas mediated apoptosis by up regulating NF-kB (Pan et al., 2001). HBx has also been shown to inhibit p53-mediated apoptosis (Wang et al., 1995).

Non-cytolytic removal of HBV

HBV specific CTLs play a critical role in viral clearance, which can be achieved through recognition of infected cells and killing of viral-infected cells as well as by non-cytolytic control of HBV replication. Non-cytolytic removal of HBV gene expression by HBV specific cytotoxic T lymphocytes has been shown in transgenic mice (Guidotti et al., 1996) and later on more convincingly in chimpanzee infected with hepatitis B virus (Guidotti et al., 1999 a). Thus, it avoids killing of the vital organ liver by CTLs and is strategic for host survival. In addition, it also acts as viral evasion strategy because it contributes to viral persistence by down regulating viral antigen and contributing to viral persistence. This hypothesis is supported by the fact that traces of HBV have been shown to persist for several decades after complete serological and clinical recovery from acute hepatitis (Rehermann et al., 1996). Evidences for the control of other infections such as, cytomegalovirus (CMV) (Tay et al., 1997), Listeria monocytogenes (Kagi et al., 1994) and Lymphohchoriomeningitis virus (LCMV) in the liver suggest that these are mediated by non-cytolytic mechanisms (Guidotti et al., 1999 a).

A similar number of virus specific CTLs observed in patients with different clinical outcomes indicates that a comparable number of intrahepatic virus-specific CD8+ cells could be associated with either protection or pathology (Maini et al., 2000). It has therefore been suggested that the bulk of intrahepatic CD8+ T cells are non-antigen specific during active liver damage (Bertoletti et al., 2000). Since the quantity of virus specific cells did not appear to be directly determining the extent of virus-induced liver pathology, it is perceived that the control of viral replication and the occurrence of liver damage might be independent events. And, the hepatic pathology could be the consequence of the large infiltrate of antigen specific mononuclear cells, since this is one variable that correlates with the extent of liver inflammation. In all probabilities the
efficiency of CTL responsiveness could likely be more important in determining viral control than the absolute numbers (Bertoletti et al., 2000).

Recently, a study on chimpanzees suggested that a dual mechanism exists for viral control in liver. A non cytolytic mechanism to clear virus through IFN-gamma dependent pathway and a cytolytic mechanism to eliminate the remaining infected cells (Thimme et al., 2003).

Role played by non-antigen specific cells

Non-antigen specific cells seem to be critical for liver damage as seen in chimpanzee with acute HBV infection where liver damage occurs concomitant with massive infiltration of T cells and the majority of such infiltration occurs after clearance of most of the virus and is therefore unlikely to be composed primarily of virus-specific CD8+ cells.

Various antigen non-specific, polymorphonuclear and mononuclear cells (i.e., NK and NKT cells, T and B lymphocytes, monocytes, macrophages, dendritic cells) are recruited into the liver when hepatitis B virus-specific CTLs are injected into HBV transgenic mice. These non-specific cells contribute to the formation of necroinflammatory foci that are scattered throughout the liver parenchyma and are typical of acute viral hepatitis in humans (Ando K et al., 1993; Kakimi et al., 2001; Sitia et al., 2002).

Liver regeneration

The liver can precisely regulate its growth and mass. While the enlarged liver mass is corrected by apoptosis, surgical resection of hepatic lobes or hepatocyte loss caused by viral or chemical injury triggers hepatocyte replication. Hepatocytes have a great replicative capacity and are capable of repopulating the liver. However, “stem-like” cells proliferate when hepatocyte replication is blocked or delayed (Fausto, 2000). The understanding of liver regeneration is based largely on studies with the partial hepatectomy model. It is important to realize that the principles derived from this model might not directly apply in acute liver injury. In partial hepatectomy, two-thirds of the rat liver is removed, leaving the remaining lobes intact and healthy. In acute liver injury, by contrast, the whole liver is involved and those cells that survive the insult and participate in the regenerative process, do so in a quite different environment. The liver has a remarkable ability to regenerate in response to liver injury due to various causes such as
partial hepatectomy, toxic exposure or virus infection (Fausto et al., 1995; Michalopoulos et al., 1997). Liver parenchymal cells, hepatocytes, are normally in the quiescent G0 phase and re-enter the cell cycle following injury to restore its mass, architecture and function quickly.

A degree of apoptosis and liver regeneration has been shown to occur during acute and chronic liver damage in woodchuck hepatitis virus infection (Guo et al., 2000).

**Liver damage, viral control and regeneration in response to HBV infection**

In the present study, candidate genes, Fas, TGF-β1, TNF-α and IL-6, whose products are reportedly involved in host response to HBV infection, liver damage by cell death, regeneration and disease progression, were selected. The functions of the candidate genes are already described in detail. This background information makes it pertinent to study the status of the candidate genes involved in viral clearance, liver apoptosis or regeneration directly or indirectly in chronic hepatitis B (Fig. 13).
Liver cell apoptosis is involved in viral, toxin, and cholestatic injury to the liver. In addition it may have a role in several pathophysiological processes of the liver, like inflammation, fibrogenesis as well as regeneration (Patel et al., 1999). Apoptosis is generally considered to be a mechanism of host defence. Induction of apoptosis interrupts viral replication and eliminates viral-infected cells. The apoptosis associated with viral infection is thought to be effected by cytotoxic T lymphocytes (CTL) through Fas ligand (FasL)/Fas antigen and perforin/granzyme B pathways (Kondo et al., 1997). Apoptosis in hepatitis B virus infection may be associated with the pathogenesis of hepatitis or may lead to impaired cell-mediated immunity that allows the virus to evade host inflammatory responses. There are evidences that inflammatory flare ups associated with viral removal during acute hepatitis are associated with apoptosis (Kondo et al., 1997), but there are no evidences to show that apoptosis is the sole way to remove viral infection. Therefore, apoptosis is an integral phenomenon associated with acute and chronic hepatitis, but the recent reports suggest that it may not be absolutely essential for viral removal.

**HBV- a non cytopathic virus**

The existence of a hepatitis B virus (HBV) carrier state for many years without evidence of parenchymal liver damage suggests that HBV is not cytopathic in man and that HBV pathogenesis is mediated by immune mechanisms. This assumption is further supported by the absence of obvious disease in HBV-infected patients with impaired immune response (e.g. Down’s syndrome, chronic renal failure, symptomatic human immunodeficiency virus infection), and by the enhanced liver damage when the immune system is stimulated by levamisole or corticosteroid withdrawal (Lau, 1993). Transgenic mice expressing the entire HBV DNA (Araki et al., 1989) or HBV surface antigen are free of liver pathology, except when the protein is expressed at a very high level (Gilles et al., 1992). Challenging of such mice with HBV-specific CTLs leads to hepatitis development (Chisari et al., 1995).

Hepatitis B virus is a non-cytopathic virus; still there is evidence that over expression of viral antigens can make the hepatocytes undergo apoptosis (Gilles et al., 1992). Hepatitis B X protein has also been shown to be involved in either inducing direct apoptosis (Terradillos et al., 1998; Kim et al., 1998; Shunting et al., 1999) or making cells susceptible to cytokine (Su et al., 1997; Janssen et al., 2003) and ultraviolet irradiation (Capovilla et al., 1997) mediated apoptosis. HBx is also shown to increase expression of
Fig. 13. Schematic representation of the role of candidate genes in liver biology after HBV infection.