General introduction
Hepatitis, an inflammation of the liver can be caused by toxic agents, viruses, drugs or occurs as a result of an autoimmune response. The term 'Viral hepatitis' is used for infections of the liver by one or more of the distinct hepatotrophic viruses. Different types of viral hepatitis are A, B, C, D, E, G and cryptogenic (caused by a virus as yet unidentified). Some viruses, such as yellow fever virus, EBV, CMV also cause hepatitis as a secondary effect. Approximately 350 million people are chronically infected with hepatitis B virus (HBV). Each year over one million people die from HBV-related chronic liver diseases, including cirrhosis and hepatocellular carcinoma (Alter, 2003). In India, 3-5% of the total population is reported to be carriers of HBV infection (Singh et al., 2000).

Hepatitis B, like any other infectious disease, is considered to be a complex disease with no clear pattern of inheritance, where the outcome of the disease is influenced by viral, environmental and host genetic factors. HBV exposure causes a broad spectrum of disease pattern ranging from no symptoms to different clinical conditions (Fig. 1). In order to dissect out the involvement of more than one gene in such a complex disease, a 'candidate gene approach' whose products are known to influence the course of the disease pathogenesis or provide protection, allows demonstration of possible susceptibility associations. Polymorphism in a variety of candidate genes involved in immune response or the genes implicated in the disease in an animal model have been subject of the study in the past. In hepatitis B related case-control studies, genetic associations have mainly focused on histocompatibility antigens (HLA), given the important role these play in immune response activation. The data, however, on genetic association of hepatitis B with: HLA genes (Giani et al., 1979; Mota et al., 1987; Thio et al., 1999; Diepolder et al. 1998; Ahn et al., 2000), mannose binding lectin gene (Hohler et al., 1998; Yuen et al., 1999; Hakozaki et al., 2002; Song et al., 2003) and vitamin D receptor (Bellamy et al., 1999), has been limited or inconsistent and further studies on other host genetic factors that influence the immune response are warranted.

In the recent past, to understand genetic susceptibility to infectious diseases, emphasis has been laid on the study of cytokines, the immunomodulatory molecules related to both innate and adaptive immune responses. Variation in cytokine release has shown to be predominantly caused by polymorphisms near or within the genes (Westendorp et al., 1999; Stuber et al., 2003; Tomasdottir et al., 2003; Galley et al., 2003; Lowe et al., 2003). Various cytokine and cytokine receptor polymorphisms have already been implicated in
Fig. 1. Outcome of exposure to HBV in a population.
infectious diseases (Van Deventer et al., 2000; Santos et al., 2002; Scola et al., 2003; Gentile et al., 2003). The background genotypes of cytokine genes, involved in influencing the outcome of the disease in HBV infection, have been suggested to be responsible for differential outcome of the disease in between population groups, since the frequency of variation for different genotypes differs between these populations. The outcome of the disease is finely influenced by the net outcome of the interaction of a high or low cytokine producing genotype of several cytokine genes, which modulates the immune response. Further, the role of genetic heterogeneity in different population groups adds to the complexity of understanding the susceptibility factors involved in complex diseases such as hepatitis. This is reflected in several observations reported in literature where tumour necrosis factor-alpha (TNF-α) genotypes were shown to be significantly associated with hepatitis B persistence in German (Hohler et al., 1998) and Korean population (Kim et al., 2003), and not associated in Japanese (Miyazoe et al., 2002) and American population (Ben-Ari et al., 2003). Similarly, IL-10 promoter polymorphisms were found to be associated with chronic progression in HBV related disease in Japanese patients (Miyazoe et al., 2002), and not associated with chronic viral infection in American population (Ben-Ari et al., 2003). In the latter study, a significant association of the susceptibility to chronic HBV infection was also reported with an IFN-gamma polymorphism and not with TGF-β1 and IL-6 polymorphisms (Ben-Ari et al., 2003).

In the present study, candidate genes such as- Fas, Transforming growth factor-betal (TGF-β1); Tumour necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) were selected since the products of these are reportedly involved in host response to HBV infection, liver damage by cell death, regeneration and disease progression. It is known that Fas is expressed on hepatocytes and its binding with Fas L, present on activated T-cells, leads to destruction of hepatocytes (Ando et al., 1994; Rouquet et al., 1995). Fas is also reportedly involved in apoptosis (Ogasawara et al., 1993), injury (Rivero et al., 2002) and anti-regenerative (Kiba et al., 2000) pathways in liver. The activities of TGF-β1, a pleiotropic cytokine, range from the arrest of liver cell regeneration and induction of apoptosis in liver diseases (Lin et al., 1992; Oberhamme et al., 1992; Takiya et al. 1995; Cain et al., 2001) to liver necroinflammation, fibrosis (Nagy et al., 1991; Murawaki et al., 1998) and immunomodulation (Chen et al., 2001; Mouri et al., 2002) in chronic hepatitis. It has also been shown that TNF-α secreted by CTLs and antigen-non-specific macrophages reduces
HBV gene expression and replication by non-cytolytic mechanisms (Guidotti et al., 1994a; Guidotti et al., 1996; Romero et al., 1996). In addition, it is involved in liver apoptosis (Bour et al., 1996; Guilhot et al., 1996) and regeneration through an IL-6 dependent mechanism (Yamada et al., 1997). IL-6, another typical pleiotropic cytokine has been implicated in the regulation of liver regeneration (Kuma et al., 1990; Cressman et al., 1996; Blindenbacher et al., 2003). An anti-injury, anti-fibrotic (Kovalovich et al., 2000 and Wuestefeld et al., 2003) and anti-apoptotic role has further been ascribed to IL-6 in liver, as it protects against TGF-β1 (Chen et al., 1999) and Fas (Kovalovich et al., 2001) mediated liver injury.

It is clearly established that HBV is a non-cytopathic virus and the liver damage during infection is the result of host-generated immune response against the virus. Cytotoxic T lymphocytes (CTLs) infiltrating the liver during viral infection are shown to cause apoptosis of viral infected hepatocytes. The mechanism of viral clearance in HBV infection is suggested to result either from apoptotic or non-cytolytic phenomena. At the same time, liver has been known for its tremendous capacity to regenerate. It has been suggested that apoptosis of hepatocytes triggers regeneration, which is an ongoing phenomenon in chronic hepatitis. This background information available in literature made it pertinent to study the status of the already mentioned candidate genes involved directly or indirectly in: viral clearance, liver apoptosis and regeneration in chronic hepatitis B.

So far, the associations of the genotypes with hepatitis B have been largely inconsistent. The genotypes of other cytokine genes involved in influencing the outcome of the disease in HBV infection could be the reason for such differential responses from one population to another. Ultimately, the outcome of the disease is influenced by the net outcome of the interaction of high or low producing genotypes of several cytokine genes, which influence modulation of immune response, survival or elimination of the pathogen and chronicity of the disease. Since the previous studies did not consider putting together the genotype backgrounds of more than one gene and complex phenotypes with the susceptibility, it was important to define this as the major objective of the present study besides monitoring the effect of single genotype variations. Gene-gene interactions have been reported in several other disorders like neural tube defects (Relton et al., 2004), Alzheimer’s disease (Robson et al., 2004), coronary heart disease (Peng et al., 2003).