Review

The Immune Response Induced by Hepatitis B Virus Principal Antigens

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Hepatitis B virus (HBV) infection occurs primarily in hepatocytes in the liver with release of infectious virions and non-infectious empty surface antigen particles into the bloodstream. HBV replication is non-cytopathic. Transient infections run a course of several months, and chronic infections are often life-long. Chronic infections can lead to liver failure with cirrhosis and hepatocellular carcinoma. It is generally accepted that neutralizing anti-HBs antibodies plays a key role in recovery from HBV infection by containing the spread of infection in the infected host and facilitating the removal and destruction of viral particles. However, the immune response initiated by the T-cell response to viral antigens is also important for viral clearance and disease pathogenesis in HBV infection. The three structural forms of the viral proteins, the HBsAg, the particulate HBcAg, and the nonparticulate HBeAg, may preferentially elicit different Th cell subsets. The different IgG subclass profiles of anti-HBs, anti-HBc, and anti-HBe in different HBV infection status were revealed. Moreover, the different IgG subclass profiles in chronic carriers did not change with different ALT and AST levels and may reflect the difference between stimulating antigens, immune response, and the stages of viral disease and provide the basis for the use of vaccines and prophylactic treatments for individuals at high risk of human HBV infection. This review elucidates the detailed understanding of the immune responses induced during transient and persistent infection, and the development of immunotherapy and immunodiagnosis in patients with HBV infection, and possible means of reducing the liver damage. *Cellular & Molecular Immunology*. 2006;3(2):97-106.

Key Words: Hepatitis B virus, IgG subclass, HBsAg, HBcAg, HBeAg

Introduction

Hepatitis B virus (HBV) currently infects more than 400 million people worldwide. Despite the availability of hepatitis B vaccine, the overall prevalence of hepatitis B virus infection has declined little in recent years. HBV is not directly hepatotoxic but its interaction with the host immune system creates opportunity for HBV DNA integration into the host genome. HBV causes liver injury by an immune response against the virus-infected liver cells, although immunosuppression appears to enhance replication and lead to induce cytotoxicity. The interplay of the host immune response and the viral ability to replicate is a prime determinant of the likelihood of liver injury, its intensity, and progression to cirrhosis. A series of stages evolve in the life cycle of each patient's infection, with associated decreases in viral load at each successive stage. Viral mutations in the polymerase or the core gene affect replication and may enhance liver injury. Recently, genotypes have been identified that are linked to clinical outcomes, drug responses, and mutations. This review summarizes the immune response in acute, self-limited and chronic hepatitis B, and its differential effects on viral replication and liver injury.

Classification

Human hepatitis B virus (HBV), as well as a number of

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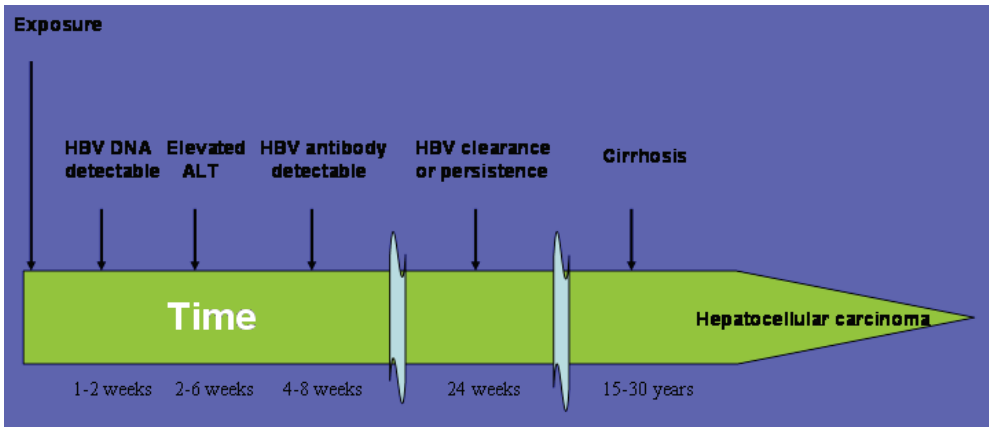

Figure 1. Natural history of HBV infection: chronology and complications.

viruses that infect other mammalian hosts (woodchucks, ground squirrels, arctic squirrels, woolly monkeys), is a member of the orthohepadnavirus genus in the *Hepadnaviridae* family (1). All members of this family are small, hepatotropic DNA viruses which display a similar virion morphology and genome organization, and replicate *via* reverse transcription of an RNA intermediate. Eight major serological subtypes (ayw, ayw2, ayw3, ayw4, ayr, adw2, adw4, and adr) are found and have distinct geographical distributions with some overlap (2). DNA sequencing has now allowed replacement of the initial serotypic classification of HBV strains by a more systematic genotype system that currently consists of 7 members (genotypes A-G) (2).

HBV particle structure and replication

The HBV virion has a diameter of 40-45 nm and possesses an envelope composed of viral surface antigens embedded in host derived lipid, and an icosahedral nucleocapsid composed of viral core antigen (3). The viral nucleocapsid contains the DNA genome and the viral polymerase protein (4-7). Humans with persistent HBV infection have high levels of viral replication in > 95% of the hepatocytes in the liver, and up to 1×10^{10}/ml viral particles and 5×10^{12}/ml non-infectious surface antigen particles in serum (6).

The HBV genome is a relaxed circular double-stranded DNA molecule that is 3,200 base pairs (bp) in length. The DNA molecule has a defined nick in the negative strand and an almost complete positive strand. Adjacent to the area of the nick and the gap are two direct repeat sequences (DR1 and DR2) that facilitate strand transfer during replication of the viral genome (4). The HBV DNA genomes have a viral polymerase protein molecule covalently attached to the 5' end of the negative strand, positioned within DR1. Attachment of this protein occurs during initiation of DNA synthesis (6, 7). The HBV DNA genome contains three partially overlapping open reading frames (ORFs). The C-ORF codes for the core antigen (HBcAg) and for a pre-core protein which is co-translationally processed and secreted as HBeAg. The S-ORF codes for the surface antigens (HBsAg), termed PreS/S and S proteins, and the P-ORF codes for the viral polymerase (pol) that has RNA- and DNA-dependent DNA polymerase and RNase H activities (5, 6). A fourth ORF, the so-called X-ORF, codes for a protein with trans-activating activity (1, 6). During HBV infection, filamentous forms of surface antigen containing particles are produced as well as 22 nm spherical particles. Also circulating in the blood of infected humans is a secreted form of the viral core antigen, the so-called e antigen (HBeAg) (8), which is thought to play an important role in the maintenance of persistent infection.

HBV transmission and infection outcomes

HBV infection of adult is usually transient, with the development of neutralizing antibodies and immunity to reinfection (8). However, other cases resulted in prolonged HBV infection that was accompanied by moderately severe chronic hepatitis and interface hepatitis (8). In humans, HBV infection of neonates usually leads to persistent infection, while HBV infection of adults results in persistent infection in 5-10% of individuals and transient infection with immunity to reinfection in the remainder (9). In humans with persistent HBV infection, liver damage is often observed ranging from mild chronic persistent hepatitis to chronic active hepatitis, cirrhosis and hepatocellular carcinoma. Figure 1 shows the natural history of HBV infection.

The extent of liver damage has been shown to correlate with the presence or absence of HBeAg in serum (8). The basis for impairment of the ability of HBV-infected patients to clear viral infection has not been adequately addressed, although it is generally accepted that a failure to mount an adequate immune response is likely to be responsible. A recent model for the outcome of infection with non-cytopathic viruses (9) proposed that variability in outcome (e.g., transient or persistent infection) may depend on the balance between parameters which determine viral spread and variables of the immune system that determine the

development of an immune response.

HBV mutants--clinical significance

HBV mutants have recently been identified in patients with acute, fulminant, or chronic infections. Sequence analysis of virus isolates from many individual patients has revealed the occurrence of certain mutational hot spots in the genome, some of which appear to correlate with the patient's immunological and/or disease status; however, cause and effect are not always easily discernible (10). This holds particularly for the issue of whether virus variants exist that have an increased pathogenic potential; due to the scarcity of appropriate *in vivo* experiment models, such hypotheses are difficult to prove. Similarly, because of the compact organization of the HBV genome, almost every single mutation may have pleiotropic phenotypic effects.

Naturally occurring mutations have been identified in all viral genes and regulatory elements, most notably in the genes coding for the structural envelope and nucleocapsid proteins (11). Mutations in the gene coding for the HBsAg may result in infection or viral persistence despite the presence of antibodies against HBsAg (anti-HBs). Mutations in the gene encoding the pre-core/core protein (pre-core stop codon mutant) result in a loss of HBeAg (HBeAg minus mutant) and seroconversion to antibodies to HBeAg (anti-HBe) with persistence of HBV replication (10). Mutations in the core gene may lead among others to an "immune escape" due to a T cell receptor antagonism (10). Mutations in the gene coding for the polymerase/reverse transcriptase can be associated with viral persistence or resistance to nucleoside analogues (10). Thus, HBV mutations may affect the natural course of infection, viral clearance and response to antiviral therapy. The exact contribution of specific mutations to diagnosis and therapy of HBV infection as well as patient management in clinical practice remain to be established. In addition, despite the availability of an effective prophylactic vaccine, further extensive efforts are required to monitor the emergence of vaccination- and therapy-resistant HBV variants and to prevent their spread in the general population.

Humoral immune responses to HBV proteins

Antibody responses to each of the HBV proteins have been detected in the sera of humans following transient HBV infection. Anti-HBs antibodies are a marker of resolution of transient HBV infection. It is generally accepted that neutralizing anti-HBs antibodies plays a key role in recovery from infection with HBV by containing the spread of infection in the infected host and facilitating the removal and destruction of viral particles (12). These antibodies have also been shown to prevent reinfection by blocking the ability of virus particles binding to receptors on target cells. In chronic HBV infection, antibodies to the viral surface proteins are generally not detected in serum although it is possible their presence is masked by the formation of immune complexes with surface antigen particles present in the bloodstream (13). However, our previous studies have successfully detected anti-HBs in different populations and suggested that anti-HBs IgG1 is the only dominant subclass in chronic carriers as well as recovered individuals and vaccinees by using a self-prepared ELISA plate (14-16). Antibodies to the HBV core protein (anti-HBc) can be readily detected in the serum of patients with chronic HBV infection as antibodies to e antigen that developed following seroconversion from e antigenaemia. These antibodies are thought to be unable to neutralize viral infectivity and are presented in the sera of chronic HBV-infected patients tested for many years (17). While infected with persistent HBV infection, anti-core antibodies are detected and persist throughout the course of infection (18). They do not resolve their HBV infection and do not develop anti-HBs antibodies (9). A number of studies have defined neutralizing and non-neutralizing epitopes within the HBV preS/S and S proteins. These have been mapped predominantly within the pre-S domain (17), and only one within the S domain (19). In addition, there is an observed correlation between presence or absence of HBeAg in serum and liver damage in humans with persistent HBV infection (8). It has been postulated that in persistently infected individuals the HBV-specific immune response is too weak to eliminate HBV from all infected hepatocytes, but sufficiently strong to continuously destroy HBV-infected hepatocytes and to induce chronic inflammatory liver disease. Furthermore, a clear correlation was demonstrated between increasing level of serum HBV DNA and acute exacerbation of patients with chronic hepatitis B. It suggests that host's immune response which causes acute exacerbation of liver injury in patients with chronic hepatitis B is triggered by the change of viremia and HBV replication (20). Moreover, patients with severe chronic active hepatitis and persistent hepatitis B virus replication are at very high risk of rapid progression to cirrhosis (18).

The different protein compositions of the hepatitis B virus can evoke the generation of different IgG subclass patterns. The different specific IgG subclasses against these antigens reflect the difference between stimulating antigens, immune response, and the stages of viral disease (17). It was reported that the anti-HBs immunoglobulin for vaccinees immunized with cDNA HBsAg consisted mainly of IgG1 and IgG2 (18), while that of individuals vaccinated with plasma-purified HBsAg consisted of IgG1 (19). In individuals naturally infected, the anti-HBs IgG consisted mainly of IgG3 and IgG1 (20). Recently, our studies also suggested that anti-HBs subclass IgG1 was predominant in cured patients, chronic carriers and vaccinees (14). The IgG subclass pattern of the relative anti-HBs IgG subclass titers was IgG1 > IgG3 ≈ IgG4 in both chronic carriers and recovered individuals (16). The samples from both chronic carriers and vaccinees exhibited a significantly higher concentration of total IgG and IgG1 than samples in recovered individuals (14, 16). Evaluation of the differential effects of vaccines demonstrated that the resulting anti-HBs immunoglobulin consisted predominantly of IgG1 (14, 16). For anti-HBc, our studies suggested that the anti-HBc IgG subclass pattern was IgG1 > IgG3 > IgG4 in chronic carriers, and IgG3 > IgG1 > IgG4 in

Table 1. The anti-HBs, anti-HBc, and anti-HBe IgG subclass profiles in populations with different HBV infection status

Population	Anti-HBs	Anti-HBc	Anti-HBe
Chronic carriers	IgG1 > IgG3 ≈ IgG4	IgG1 > IgG3 > IgG4	IgG1 > IgG4 > IgG3
Recovered individuals	IgG1 > IgG3 ≈ IgG4	IgG3 > IgG1 > IgG4	IgG1 > IgG3 > IgG4
Vaccinees	IgG1 dominant	N.A.	N.A.

N.A.: Not available.

recovered individual (15, 21). This was in accordance with the data reported by other groups (22-25). We further suggested the anti-HBc IgG1/IgG3 ratio may be related with HBV status. However, in spite of the different anti-HBc IgG1/IgG3 patterns demonstrated in different populations, both anti-HBc IgG1 and IgG3 concentrations were significantly higher in chronic carriers (21). For anti-HBe, our results suggested that the anti-HBe IgG subclass pattern was IgG1 > IgG4 > IgG3 in chronic carriers, and IgG1 > IgG3 > IgG4 in recovered individuals (unpublished data). The anti-HBe IgG subclass pattern in chronic carriers result is consistent with that of Sällberg et al. (25). Moreover, the IgG2 level of anti-HBe, which is the same as our anti-HBc (15, 21) and anti-HBs (14, 16) studies, is the lowest IgG isotype both in male and female of chronic carriers and recovered individuals (unpublished data). The IgG subclass profiles of anti-HBs, anti-HBc, and anti-HBe in chronic carriers did not change with liver inflammation (ALT and AST abnormal), and were independent of sex and age (16, 21, unpublished data). Furthermore, in inverse to that of our anti-HBs results (14, 16), the mean O.D. values of anti-HBc (15, 21) and anti-HBe (unpublished data) total IgG and all IgG subclasses except for IgG2, in both male and female, were significantly higher in chronic carriers than in recovered individuals. Table 1 summarizes the anti-HBs, anti-HBc, and anti-HBe IgG subclass profiles in populations with different HBV infection status. These studies promote a deeper understanding of the relationship between these patterns and immunity in virus clearance.

Differences in secreted immunoglobulins, manifested in classes, subclasses, and subclass patterns, may be the result of the conformational binding of different antigenic structures to MHC class I or class II molecules (17). However, the specific role that each Ig plays in the clearance of HBV remains unclear. Hence, the different specific IgG subclasses against these antigens may reflect the difference between stimulating antigens, immune response, and the stages of viral disease.

Cell mediated immune responses to HBV infection

One of the challenges in understanding viral pathogenesis is the elucidation of the full repertoire of immune responses that control the replication of the invading pathogen. Generally speaking, such control mechanisms can be either noncytocidal or cytocidal. Noncytocidal responses can result from the release of cytokines or other substances with antiviral potential, without necessarily injuring the infected cell. By contrast, cytocidal responses aid in suppressing the pathogen but can also contribute to tissue injury and disease *via* killing infected cells.

The T-cell response during acute self-limited hepatitis B in people is characterized by a vigorous, polyclonal, and multispecific cytotoxic and helper-T-cell response. Although clearance of most virus infections is widely thought to indicate the killing of infected cells by virus-specific T cells, it was suggested that non-cytolytic intracellular viral inactivation by cytokines released by virus-inactivated lymphomononuclear cells have an important role in the clearance of this virus without killing the infected cells (26). Recent studies using a transgenic mouse model of hepatitis B virus infection have also shown that adoptive transferred, virus-specific cytotoxic T cells can abolish hepatitis B virus gene expression and replication in the liver without killing the hepatocytes (27). Additional factors that may contribute to viral persistence are immunological tolerance to viral antigens, viral inhibition of antigen processing or presentation, infection of immunologically privileged sites, modulation of the response to cytotoxic mediators, and viral mutations (28).

The three structural forms of the viral proteins, the HBsAg, the particulate HBcAg, and the nonparticulate HBeAg, may preferentially elicit different Th cell subsets. In previous studies, HBeAg has been shown to induce a Th2 immune response in mice, whereas HBcAg induced a Th1 response (29, 30). The Th2 response to the HBeAg was dominant over the Th1 response to the HBcAg, resulting in the depletion of HBcAg-specific Th1 cells *in vivo* (29). It is also accepted that cell mediated immune responses are important in the elimination of viruses that do not have a lytic cycle in the host and for any tissue damage seen during either transient or persistent infection, or both (26). In the direct elimination performed by killer T cells, the cell-mediated immune response also needs assistance from Th cells, which work in two ways (31). First, the Th1 cells stimulate macrophages, which then clear virus particles. Second, the Th2 cells stimulate B cells to generate immuno-globulins, which adhere to the surface of virus particles and induce opsonization (32). It has been shown that low doses of virus were able to induce a protective cytotoxic T lymphocyte (CTL) response (Th1-mediated), whereas high doses of virus failed to do so and induced a non-protective humoral (Th2-mediated) response instead (33). This suggests

that the initial viral dose may be critical in determining whether hosts develop protective or non-protective immunity. Therefore the same may be true for adults whose ability to mount a vigorous immune response to certain viral antigens is particularly sensitive to antigen load. Further studies have shown that the mode of antigen presentation and the dose may determine whether immunological tolerance or a vigorous immune response is the final outcome (33). This may serve to explain the outcomes of HBV infection.

Natural killer (NK) cells are abundant in the normal liver, accounting for around one-third of intrahepatic lymphocytes and are important in the defense against HBV infection as innate immune responses. NKT cells bear both T cell and NK cell markers and are important in anti-tumor immunity. HBV-associated hepatocellular carcinoma expresses HBsAg on its cell surface and may serve as a tumor-associated antigen (34). It is believed that whether directly activated by HBV infection or indirectly activated by other lymphocytes, NK cells exert their antiviral functions by natural cytotoxicity and production of high levels of cytokines (35). Moreover, activated NK cells play an important role in regulating adaptive immune responses by interaction with other lymphocytes and may contribute to the lymphocyte-mediated liver injury during HBV infection (35). Recently, it was suggested that therapeutic activation of NKT cells may represent the innate immune response, like the adaptive immune response, and has the potential to control viral replication during natural HBV infection (36).

Dendritic cells are antigen presenting cells that play a role in T-cell activation. There was a correlated simultaneous decrease numbers of circulating $CD8^+$ T cells and NK cells in HBV-infected cirrhotic patients (37). Furthermore, cirrhotic patients with opportunistic infections have lower numbers of $CD8^+$ T cells and NK cells compared to those without opportunistic infections. The decline of host immune response was suggested to partially contribute to the disease progression of HBV infection and opportunistic infections (37).

Based on available data derived from studies in HBV, it was assumed that a human leucocyte antigen (HLA) class I-restricted CTL response to one or more HBV-encoded antigens displayed at the hepatocyte membrane is a major effector mechanism of hepatocellular injury and clearance of infected cells (38). Elucidation of the immunological and virological basis for HBV persistence may yield immunotherapeutic strategies to terminate chronic HBV infection. Accordingly, it has been assumed that viral clearance is mediated mainly by destruction of infected cells by viral antigen-specific CTL (39) and that pathogenesis of persistent HBV infection is also mediated by these cells (40). Multiple CTL epitopes within both HBsAg and HBcAg can be detected by sensitizing target cells with synthetic peptides. However, the direct cytopathic effect of the CTL was limited to very few hepatocytes, possibly because the effector : target cell ratio in the liver was low and the free ranging CTL movement was severely limited by the architectural constraints of solid tissue (41). Several studies also suggested that clearance of HBV infection from the liver may occur by non-cytolytic mechanisms (8). In these studies > 95% of hepatocytes were shown to support viral replication at the peak of infection (9).

Based on virological and biochemical parameters, patients with chronic HBV infection can be divided into distinct clinical phases: the immune-tolerance phase, the immune-clearance phase, and the inactive carrier state (42). It was demonstrated that clear differences in the intrahepatic cellular infiltrate were found between the various clinical phases of chronic HBV infection (43). Compared with normal controls, the numbers of leucocytes, lymphocytes, T-lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+CD28^+$ T cells and $CD8^+CD28^+$ T cells were decreased in both the patients with chronic hepatitis B and asymptomatic HBV carriers, the number of NK cells of the patients with chronic hepatitis B decreased as well (44). Moreover, it was also revealed that the proportions of $CD8^+CD38^+$ T cells and $CD4^+CD45RA^-$ memory T cells were markedly increased and the proportion and number of $CD4^+CD45RA^+CD62L^+$ T cells were decreased in patients with chronic hepatitis B when compared with normal controls and asymptomatic HBV carriers (45). These data suggested that there is an imbalance in peripheral blood T-lymphocyte subsets and turbulence in cellular immunity in the patients with chronic hepatitis B and asymptomatic HBV carriers, which may be associated with HBV persistent infection.

Cytokines in viral hepatitis

Cytokines play a key role in the defense against viral infections, both directly, through inhibition of viral replication, and indirectly, through determination of the predominant Th1/Th2 pattern of host response. However, in the context of an inflammatory response against a virus, cytokines may also lead to liver damage (46). The importance of this is best demonstrated in HBV. In acute HBV infection, a vigorous polyclonal cellular immune response is critical; thus Th1 cytokine release is essential to initiating an effective immune response. Among the cytokines, IFN-γ is one of the most important mediators in the immune system. It is also known to exert inhibitory effects on viral replication (47, 48) and has been generally considered to show more strict species specificity than IFN-α (47). The cytokines released by $CD4^+$ and $CD8^+$ cells also play an important role in down-regulation of HBV replication, demonstrating that it is possible to control a viral infection without the death of infected cells (49). However, if there is a defect in the acute response, HBV becomes chronic and consequently the presence of an ongoing suboptimal inflammatory response can activate the process of hepatic fibrosis.

HBV may have specific mechanisms to inhibit cytokine production, highlighting the critical role of these molecules in recovery from infection (50). On the other hand, there are some of the immune evasion strategies adopted by HBV. These include the antagonism of immune function through the use of homologues of cytokine receptors, expression of

viral proteins which interact with cytokine signal transduction and expression of cytokine mimics and host proteins that influence the Th1 and/or Th2 cytokine responses. These immunomodulatory strategies can protect the host from the lethal inflammatory effects as well as inhibit the local inflammatory response elicited to kill the HBV. Other strategies include the alterations in cytokine expression such as demonstrated with the HBcAg and terminal protein which can inhibit IFN-β gene expression (51).

The circulating cytokine profile in chronic hepatitis B was related to the replication level of the virus and the activity of liver disease (52). Recently, it was reported (53) that IL-18 inhibits hepatitis B virus replication in the livers of transgenic mice. Interferon was used as part of the treatment for chronic hepatitis B (54). Several of the IFN-γ-induced genes have antiviral activity in other systems, raising the possibility that they may also inhibit HBV replication. Combined therapy with ribavirin and IFN-α for chronic hepatitis B not only significantly reduces viremia levels but also induces lasting $CD4^+$ T-cell proliferation and Th1 cytokine release at the site of infection (55, 56). It was also found that chronic hepatitis patients with low HBeAg levels were more likely to respond to IFN-α therapy (57). Moreover, the analysis conducted by Milich et al. suggested that the presence of serum HBeAg ablated the expected Th1-mediated anti-HBc antibody response, shifting it toward a Th2 phenotype (30). The treatment with IFN-α followed by a HBeAg shot, could change the antibody pattern elicited for the mice (29), that was the way the immune response switched from Th2 pathway to Th1 pathway (58). The alteration of IgG subclass patterns which were induced by Th1 cytokine treatment (e.g., IL-2 and IFN-γ) (59, 60), ribavirin (55), or both (55) to modulate the immune response from Th2 towards Th1 pathway, may promote the treatment for HBV infection.

The role of immune system during liver fibrosis and cancer

The association of HBV infection and liver cancer is well documented in many studies. Patients with chronic hepatitis B have increased risk of hepatocellular carcinoma (HCC), in particular those with active liver disease and cirrhosis. For those individuals with high levels of viral replication, chronic active hepatitis with progression to cirrhosis, liver failure and HCC is common. Chronic inflammation and cirrhosis, accompanied by regenerative process, function as a tumor promoter, providing a common pathway from chronic HBV infection to HCC (61). In the Caucasian, people acquired chronic HBV infection usually during adulthood. There is biochemical and histologic regression after HBeAg seroconversion and the risk of death from hepatitis B-related causes is low in these patients. In the Asian, people acquired chronic HBV infection usually during birth or early childhood. There is a prolonged phase of immunotolerance in these patients. Moreover, the progression to cirrhosis can be relatively silent and can occur even in children. After HBeAg seroconversion, precore and core promotor mutations occur frequently in the Asian population. However, there is little correlation between the occurrence of these mutations and alanine aminotransferase elevation in patients who are positive for anti-HBe (61). Similarly, our previous studies also suggested that the IgG subclass profiles of anti-HBs, anti-HBc, and anti-HBe in chronic carriers did not change with different ALT and AST levels (16, 21, unpublished data). It is therefore reasonable to suggest that there is no correlation between the occurrence of the precore and core promotor mutations and the IgG subclass profiles of anti-HBs, anti-HBc, and anti-HBe. Although cirrhosis develops during the process of HBeAg seroconversion, most of the complications of cirrhosis and of hepatocellular carcinoma occur after HBeAg seroconversion and these complications may still occur even after HBsAg seroclearance.

For liver cancer, the integration and protein expression of HBX-encoded X protein was shown to impose alteration in cell proliferation cycle and apoptosis process. Other factors including viral-induced alterations in p53 and telemerase, HBV genotypes, co-infection with HCV or delta agents, patient's lifestyle such as smoking, alcohol excesses, and genetic factors of the host patient were also suggested to involve. For example, HCC tumor progression may be brought about by mutation of the p53 tumor suppressor gene. Another mechanisms of host defense are the production of transforming growth factor β1 (TGF-β1), and the induction of cytotoxic T lymphocytes; the failure of there mechanisms permits the process of hepatocarcinogenesis (61). The processes of necroinflammation, cell proliferation and fibrosis facilitate the initial carcinogenic development (62). In addition, cirrhotic cases with evidence of HBV infection with or without HBs antigenemia tend to have lower NK activity than normal controls (63). One other possible factor is dendritic cells. The depressed function of dendritic cells is associated with pathogenesis of HCC with HBV (64).

Treatment with IFN-α of chronic hepatitis is able to delay or prevent the progression to liver cirrhosis and development of HCC. Newer antiviral agents for the treatment of HBV and their long-term effect on the natural history of HBV are yet to be proven (65). Recent advances in immunotherapy may become a modality for patients with HCC. For example, clinical application of vaccine immunotherapy with NY-ESO-1 derived peptides in HLA-A2 positive HCC patients was shown to be possible (61).

Host genetic factors influencing the outcome of hepatitis

The mechanisms that determine viral clearance or viral persistence in chronic viral hepatitis have yet to be identified. Recent advances in molecular genetics have permitted the detection of variations in immune response, often associated with polymorphism in the human genome. Differences in host susceptibility to infectious disease and disease severity cannot be attributed solely to the virulence of viral agents. Several recent advances concerning the influence of human

genes in chronic viral hepatitis B are discussed below:

HLA molecules and the major histocompatibility complex (MHC)
The HLA complex consists of class I (HLA-A, -B, and -C) and class II (HLA-DRB1, -DQA1, -DQB1, -DPA1, and -DPB1) alleles, which are glycoproteins situated on the cellular surface and encoded by MHC genes. Class I HLA molecules consist of a heavy 45-kD chain and a 15-kD β2 microglobulin chain. Class II HLA molecules are heterodimeric and consist of α and β chains of approximately 30-kD each. As primary step of immune response, HLAs present antigens to both $CD4^+$ helper T cells and $CD8^+$ CTLs.

Class I HLA molecules generally present antigens that are generated endogenously, including epitopes from viruses and other intracellular pathogens. In order to kill the infected cells, CTLs must recognize the combination of viral epitope and class I antigen coexpressed on the surface of hepatocytes. Class II HLA molecules occur on antigen presenting cells or dendritic cells that present extracellularly derived antigens (including viral peptides) to $CD4^+$ T cells in order to stimulate cytokine release, thereby generating humoral and cell-mediated immune responses. Most genetic studies involving HBV susceptibility have focused on its correlations with HLA I and II (66, 67). If specific alleles of the class II loci are efficient for viral clearance or for slowing disease progression, then it follows that a $CD4^+$ helper T cell response would be implicated as a critical factor in viral clearance. On the other hand, HLA class I correlations would suggest that a $CD8^+$ CTL response is also a critical factor in eliminating HBV.

The DRB1*1302, A*0301, DR2, DR6 and DR13 alleles are shown to correlate with better HBV outcomes. In Gambia, Thursz et al. studied both children and adults and found that the MHC class II allele DRB1*1302 was more frequent in individuals that had cleared the infection than in those presenting persistent infection (68). A German study examined the effect of DRB1*1302 in adults and also suggested that this allele is protective against viral persistence (69). In a study of Caucasian subjects, a single class I allele (HLA-A*0301) was associated with HBV clearance with an odds ratio (OR) of 0.4770. A study of Qatari adults showed different correlations between HLA class II alleles and HBV infection, and the results indicated that HLADR2 was a protective factor (70). Another Korean study showed that HLA-DR6 and HLA-DR13 were correlated with HBV clearance (71).

In a study involving 91 African-American adults who were regular intravenous drug users, 31 presenting self-limited HBV infections and 60 with persistent HBV infections, HBV persistence was shown to correlate with the DQA1*0501 and DQB1*0301 alleles found in a common DQA1-DQB1 haplotype (72). It was found that the three loci of the DQA1*0501-DQB1*0301-DRB1*1102 haplotype were significantly correlated with viral persistence. In another study, HLA-DRB1*0301, HLADQA1*0501 and HLA-DQB1*0301 were found to be closely correlated with susceptibility to chronic hepatitis B, whereas HLA-DRB1 *1101/1104 and HLA-DQA1*0301 were correlated with resistance to chronic hepatitis B37. The HLA-B*08 allele has been associated with B virus persistence both independently (OR, 1.59) and as part of the conserved Caucasian haplotype A*01-B*08-DRB1*03. The B*44-Cw*1601 (OR, 2.23) and B*44-Cw*0501 (OR, 1.99) haplotypes have also been associated with viral persistence (73). The HLA-DR7 allele was also found to be a risk factor for hepatitis B in another study (70). Further investigations have demonstrated that HLA-DR9 was significantly more frequent in chronic HBV carriers (71).

Other candidate genes associated with HBV
Genes that encode TNF-α, as well as mannose binding protein (MBP), and vitamin D receptor have been studied as potential candidate genes, due to the role they may play in HBV pathogenesis. Patients infected with HBV have increased levels of TNF-α and upregulation of TNF-α receptors (74). In addition, CTL secretion of TNF-α was able to stop HBV gene expression in transgenic mice infected with HBV (75). Furthermore, it is suggested that polymorphisms in the TNF-α gene may influence HBV persistence (75-77).

Mutations in the MBP gene (codons 52, 54, and 57), as well as in its promoter lead to low serum concentrations of MBP, preventing both its ability to activate complement and to act as an opsonin (78). The HBV envelope has a mannose-rich oligosaccharide to which MBP could bind. Therefore, these mutations may be important in HBV pathogenesis (79). The mutation of codon 52 in the MBP gene has been shown to correlate with persistent HBV infection in British Caucasians but not in Chinese Asians (80).

The active form of vitamin D, in addition to its role in calcium regulation, is an immunomodulatory hormone that inhibits Th1 response and activates Th2 response. The codon 352 (genotype tt) in one polymorphism of vitamin D receptor gene was suggested to associate with HBV clearance in another study (80).

Conclusion

The current results suggest that HBV acts like a stealth virus early in infection, remaining undetected and spreading until the onset of the adaptive immune response several weeks later. Figure 2 summarizes the role of adaptive immune responses to HBV infection. This figure makes the point that antibody is the only component of the adaptive immune response that provides a defense against a cell-free virion, and that T cells are the primary mechanisms in the adaptive immune response to clear virus-infected cells. When MHC restricted T cells enter the liver and recognize antigen, they kill some of the infected cells and secrete IFN-γ, which induces the expression of a large number of genes that enhance antigen processing and presentation; recruit macrophages, NK or dendritic cells, and T cells that also produce IFN-γ; and amplify the process. These highly coordinated

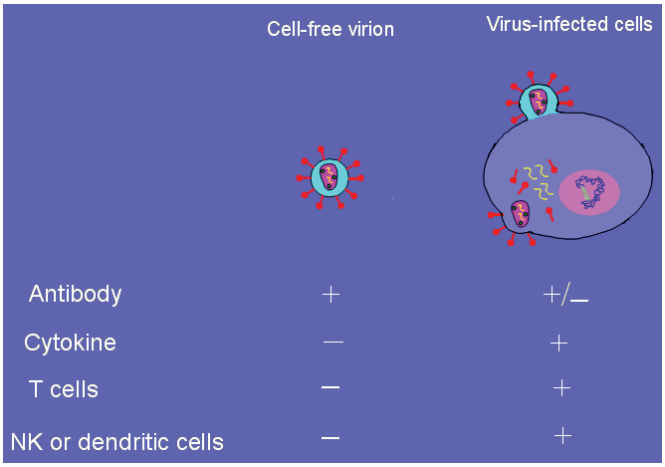

Figure 2. The role of adaptive immune responses to HBV infection.

cellular and molecular events continue until the infection is terminated, at which point they rapidly subside.

A better understanding of the pathogenetic mechanisms and natural course of hepatitis flares, wiser selection of patients and the timing of drug therapy are crucial to achieve better treatment result. Furthermore, although CTLs are considered to play a critical role, other effector mechanisms (e.g., cytokines or neutralizing antibodies) are likely to contribute to viral dynamics and the eventual outcome of infection. Furthermore, genetic studies performed in patients with chronic viral hepatitis have contributed to the understanding of its pathogenesis. Various MHC alleles that are correlated with more favorable outcomes in cases of viral hepatitis have been identified in diverse populations. Identifying correlation between specific genes and the evolution of viral hepatitis infection is a good approach toward better understanding of the pathogenesis of hepatitis. The understanding of the immune response upon HBV infection is useful to develop appropriate therapeutic strategies for controlling viral hepatitis, as well as to improve current knowledge regarding hepatitis prognosis.

References

1. Schoedel F, Sprengel R, Weimer T, Fernholz D, Schneider R, Will H. Animal hepatitis B viruses. In: Klein G, editor. Adv Viral Oncol. 1989;8:73-100.
2. El Khouri M, dos Santos VA. Hepatitis B: epidemiological, immunological, and serological considerations emphasizing mutation. Rev Hosp Clin Fac Med Sao Paulo. 2004;59:216-224.
3. Bruss V. Envelopment of the hepatitis B virus nucleocapsid. Virus Res. 2004;106:199-209.
4. Wang GH, Seeger C. Novel mechanism for reverse transcription in hepatitis B viruses. J Virol. 1993;67:6507-6512.
5. Ganem D. Hepadnaviridae and their replication. In: Fields BN, Knipe DM, Howley PM, editors. Fundamental virology, 3rd ed. New York: Lippincott Raven; 1996:1199-1233.
6. Nassal M, Schaller H. Hepatitis B virus replication - an update. J Viral Hepatol. 1996;3:217-226.
7. Summers JW, Mason WS. Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. Cell. 1982;29:403-415.
8. Niederau C, Heintges T, Lange S, et al. Long-term follow-up of HBeAg-positive patients treated with interferon α for chronic hepatitis B. New Eng J Med. 1996;334:1422-1427.
9. Zinkernagel RM. Immunology taught by viruses. Science. 1996; 271:173-178.
10. Baumert TF, Barth H, Blum HE. Genetic variants of hepatitis B virus and their clinical relevance. Minerva Gastroenterol Dietol. 2005;51:95-108.
11. Tong S. Mechanism of HBV genome variability and replication of HBV mutants. J Clin Virol. 2005;34:134-138.
12. Brown JL, Carman WF, Thomas HC. The hepatitis B virus. Baillieres Clin Gastroenterol. 1990;4:721-747.
13. Hu KQ. Occult hepatitis B virus infection and its clinical implications. J Viral Hepat. 2002;9:243-257.
14. Wang L, Lin SJ, Tsai JH, Tsai CH, Tsai CC, Yang CC. Anti-HBs IgG1 subclass is predominant in HBV recovered individuals, chronic carriers and vaccinees by evaluating self-prepared ELISA plates. Med Microbiol Immunol. 2005; 194:33-38.
15. Yang CC, Lin CC, Wang L, Ku CS, Chen CK. Anti-HBc IgG subclasses in different populations by comparing a variety of ELISA plates. J Immunoassay Immunochem. 2001;22:33-45.
16. Tsai TH, Huang CF, Wei JCC, et al. The study of IgG subclass profiles of anti-HBs in populations with different status of HBV infection. Viral Immunol. 2006; in press.
17. Wilson IA, Stanfield RL, Jewell DA, Ghiara JB, Fremont DH, Stura EA. Immune recognition of viral antigen. Infect Agents Dis. 1994;3:155-161.
18. Borzi RM, Dal Mote P, Honorati MC, Facchini A. IgG subclass distribution of anti-HBs antibodies following vaccination with cDNA HBsAg. J Immunol Meth. 1992;146:17-23.
19. Carotenuto P, Quinti I, Pontesilli O, et al. Response to hepatitis B vaccine in a cohort of Gambian children. Ped Inf Dis. 1995; 14:215-220.
20. Morell A, Roth-Wicky B, Skvaril F. Immunoglobulin G subclass restriction of antibodies against hepatitis B surface Antigen. Infect Immun. 1983;39:565-568.
21. Yang YY, Huang CF, Wei JCC, et al. The study of IgG subclass profiles of anti-HBc in populations with different status of HBV infection. Cell Mol Immunol. 2005;2:393-398.
22. Skavril F, Joller-Jemelka H. IgG subclasses of anti-HBs antibodies in vaccinated and nonvaccinated individuals and in anti-HBs immunoglobulin preparations. Int Archs Allergy Appl Immunol. 1984;73:330-337.
23. Akbar SM, Horiike FN, Onji M, Ohta Y. Immunoglobulin G subclasses of antibodies to hepatitis B core antigen in HBs antigen positive liver diseases. J Gast Hepat. 1991;6:71-75.
24. Torgano G, Vecchi M, Podda M, et al. Primary biliary cirrhosis is associated with specific changes in liver IgG bearing cell subpopulations. J Hepat. 1995;22:545-550.
25. Sällberg M, Norder H, Magnius LO. Comparison of class and subclass distribution of antibodies to the hepatitis B core and B e antigens in chronic hepatitis B. J Med Virol. 1990;30:1-6.
26. Sarzotti M, Robbins EJ, Hoffman PM. Induction of protective CTL responses in newborn mice by murine retrovirus. Science. 1996;271:1726-1728.
27. Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity. 1996;4:25-36.

28. Guidotti LG, Chisari FV. To kill or to cure: options in host defense against viral infection. Curr Opin Immunol. 1996;8: 478-483.
29. Milich DR, Schodel F, Hughes JL, Jones JE, Peterson DL. The hepatitis B virus core and e antigens elicit different Th cell subsets: antigen structure can affect Th cell phenotype. J Virol. 1997;71:2192-2201.
30. Milich DR, Chen MK, Hughes JL, Jones JE. The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: a mechanism for persistence. J Immunol. 1998;160:2013-2021.
31. Forsthuber T, Yip HC, Lehmann PV. Induction of TH1 and TH2 immunity in neonatal mice. Science. 1996;271:1728-1730.
32. Barrios C, Brawand P, Berney M, Brandt C, Lambert PH, Siegrist CA. Neonatal and early life immune responses to various forms of vaccine antigens qualitatively differ from adult responses: predominance of a Th-2 biased pattern that persists after adult boosting. Eur J Immunol. 1996;26:1489-1496.
33. Ridge JP, Fuchs EJ, Matzinger P. Neonatal tolerance revisited: turning on newborn cells with dendritic cells. Science. 1996; 271:1723-1726.
34. Kakimi K, Guidotti LG, Koezuka Y, Chisari FV. Natural killer T cell activation inhibits hepatitis B virus replication *in vivo*. J Exp Med. 2000;192:921-930.
35. Chen Y, Wei H, Gao B, Hu Z, Zheng S, Tian Z. Activation and function of hepatic NK cells in hepatitis B infection: an under investigated innate immune response. J Viral Hepat. 2005;12: 38-45.
36. Baron JL, Gardiner L, Nishimura S, Shinkai K, Locksley R, Ganem D. Activation of a nonclassical NKT cell subset in a transgenic mouse model of hepatitis B virus infection. Immunity. 2002;16:583-594.
37. Duan XZ, Wang FS, Wang M, et al. Identification of phenotype and interferon-α-producing capability of circulating type II dendritic cells and its clinical implication in HBV-infected patients. Zhonghua Yi Xue Za Zhi. 2003;83:548-552.
38. Ando K, Guidotti LG, Wirth S, et al. Class I restricted cytotoxic T lymphocytes are directly cytopathic for their target cells *in vivo*. J Immunol. 1994;152:3245-3253.
39. Chisari FV, Ferrari C, Mondelli MU. Hepatitis B virus structure and biology. Microb Pathogen. 1989;6:311-325.
40. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol. 1995;13:29-60.
41. Ando K, Guidotti LG, Wirth S, et al. Mechanisms of class I restricted immunopathology. A transgenic mouse model of fulminant hepatitis. J Exp Med. 1993;178:1541-1554.
42. Thomas HC, Pignatelli M, Scully LJ. Viruses and immune reactions in the liver. Scand J Gastroenterol. 1985 Suppl;114: 105-117.
43. Sprengers D, van der Molen RG, Kusters JG, et al. Different composition of intrahepatic lymphocytes in the immune-tolerance and immune-clearance phase of chronic hepatitis B. J Med Virol. 2006;78:561-568.
44. Tian Y, Qiu ZF, Li TS. Difference and significance of peripheral blood T-lymphocyte subsets in patients with chronic hepatitis B and asymptomatic HBV carriers. Zhonghua Yi Xue Za Zhi. 2005;85:3354-3358.
45. Tang TJ, Kwekkeboom J, Mancham S, et al. Intrahepatic $CD8^+$ T-lymphocyte response is important for therapy-induced viral clearance in chronic hepatitis B infection. J Hepatol. 2005;43: 45-52.
46. de Lalla C, Galli G, Aldrighetti L, et al. Production of profibrotic cytokines by invariant NKT cells characterizes cirrhosis progression in chronic viral hepatitis. J Immunol. 2004; 173:1417-1425.
47. Farrar MF, Schreiber RD. The molecular cell biology of interferon-γ and its receptor. Annu Rev Immunol. 1993;11:571-611.
48. Boehm U, Klamp T, Groot M, Howard JC. Cellular responses to interferon-γ. Annu Rev Immunol. 1997;15:749-795.
49. Jung MC, Pape GR. Immunology of hepatitis B infection. Lancet Infect Dis. 2002;2:43-50.
50. Rapicetta M, Ferrari C, Levrero M. Viral determinants and host immune responses in the pathogenesis of HBV infection. J Med Virol. 2002;67:454-457.
51. Ilan Y. Immune downregulation leads to upregulation of an antiviral response: a lesson from the hepatitis B virus. Microb Infect. 2002;4:1317-1326.
52. Bozkaya H, Bozdayi M, Turkyilmaz R, et al. Circulating IL-2, IL-10 and TNF-α in chronic hepatitis B: their relations to HBeAg status and the activity of liver disease. Hepatogastro-enterology. 2000;47:1675-1679.
53. Lynch DH, Ramsdell F, Alderson MR. Fas and FasL in the homeostatic regulation of immune responses. Immunol Today. 1995;16:569-574.
54. Chen X, Li M, Le X, Ma W, Zhou B. Recombinant hepatitis B core antigen carrying preS1 epitopes induce immune response against chronic HBV infection. Vaccine. 2004;22:439-446.
55. Hultgren C, Milich DR, Weiland O, Sallberg M. The antiviral compound ribavirin modulates the T helper (Th) 1/Th2 subset balance in hepatitis B and C virus-specific immune responses. J Gen Virol. 1998;79:2381-2391.
56. Rico MA, Quiroga JA, Subira D, et al. Hepatitis B virus-specific T-cell proliferation and cytokine secretion in chronic hepatitis B e antibody-positive patients treated with ribavirin and interferon alpha. Hepatology. 2001;339:295-300.
57. Heijtink RA, Janssen HL, Hip WC, Osterhaus AD, Schalm SW. Interferon-α therapy for chronic hepatitis B: early response related to pre-treatment changes in viral replication. J Med Virol. 2001;63:217-219.
58. Finkelman FD, Sevtic A, Gresser I, et al. Regulation by interferon α of immunoglobulin isotype selection and lymphokine production in mice. J Exp Med. 1991;174:1179-1188.
59. Milich DR, Jones J, Hughes J, Maruyama T. Role of T cell tolerance in the persistence of hepatitis B virus infection. J Immunother. 1993;14:226-233.
60. Gregorek H, Madalinski K, Woynarowski M, Mikolajewicz J, Syczewska M, Socha J. IgG subclass distribution of hepatitis B surface antigen antibodies induced in children with chronic hepatitis B infection after interferon-α therapy. J Infect Dis. 2000;181:2059-2062.
61. Marotta F, Vangieri B, Cecere A, Gattoni A. The pathogenesis of hepatocellular carcinoma is multifactorial event. Novel immunological treatment in prospect. Clin Ter. 2004;155:187-199.
62. Kakumu S, Ito S, Ishikawa T, et al. Decreased function of peripheral blood dendritic cells in patients with hepatocellular carcinoma with hepatitis B and C virus infection. J Gastroenterol Hepatol. 2000;15:431-436.
63. Yoffe B, Pollack S, Ben-Porath E, Zinder O, Barzilai D, Gershon H. Natural killer cell activity in post-necrotic cirrhotic patients as related to hepatitis-B virus infection and plasma zinc levels. Immunol Lett. 1986;14:15-19.
64. Yuen MF, Lai CL. Natural history of chronic hepatitis B virus infection. J Gastroenterol Hepatol. 2000;15 Suppl:E20-24.
65. Imperial JC. Natural history of chronic hepatitis B and C. J Gastroenterol Hepatol. 1999;14 Suppl:S1-5.
66. Mota AH, Fainboim H, Terg R, Fainboim L. Association of

chronic active hepatitis and HLA B35 in patients with hepatitis B virus. Tissue Antigens. 1987;30:238-240.
67. Verdon R, Pol S, Landais P, et al. Absence of association between HLA antigens and chronicity of viral hepatitis in haemodialyzed patients. J Hepat. 1994;21:388-393.
68. Thursz MR, Kwiatkowski D, Allsopp CEM, et al. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. New Engl J Med. 1995;332:1065-1069.
69. Hohler T, Gerken G, Notghi A, et al. HLA-DRB1*1301 and *1302 protect against chronic hepatitis B. J Hepat. 1997;26: 503-507.
70. Aikawa T, Kojima M, Onishi H, et al. HLA DRB1 and DQB1 alleles and haplotypes influencing the progression of hepatitis C. J Med Virol. 1996;49:274-278.
71. Ahn SH, Han KH, Park JY, et al. Association between hepatitis B virus infection and HLA-DR type in Korea. Hepatology. 2000;31:1371-1373.
72. Thio CL, Carrington M, Marti D, et al. Class II HLA alleles and hepatitis B virus persistence in African Americans. J Infect Dis. 1999;179:1004-1006.
73. Thio CL, Thomas DL, Karacki P, et al. Comprehensive analysis of class I and class II HLA antigens and chronic hepatitis B virus infection. J Virol. 2003;77:12083-12087.
74. Sheron N, Lau J, Daniels H, et al. Increased production of tumour necrosis factor α in chronic hepatitis B virus infection. J Hepat. 1991;12:241-245.
75. Guidotti LG, IshikaWA T, Hobbs MV, et al. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity. 1996;4:25-36.
76. Hohler T, Kruger A, Gerken G, et al. A tumor necrosis factor-α (TNF-α) promoter polymorphism is associated with chronic hepatitis B infection. Clin Exp Immunol. 1998; 111:579-582.
77. Wilson AG, de Vries N, Paciot F, et al. An allelic polymorphism within the human tumor necrosis factor α promoter region is strongly associated with HLAA1, B8 and DR3 alleles. J Exp Med. 1993;177:557-560.
78. Summerfield JA, Ryder S, Sumiya M, et al. Mannose binding protein gene mutations associated with unusual and severe infection in adults. Lancet. 1995;345:886-889.
79. Thomas HC, Foster GR, Sumiya M, et al. Mutation of gene of mannosebinding protein associated with chronic hepatitis B viral infection. Lancet. 1996;348:1417-1419.
80. Bellamy R, Ruwende C, Corrah T, et al. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. J Infect Dis. 1999;179:721-724.