Immunology of hepatitis B infection

Maria-Christina Jung and Gerd R Pape

The immune response initiated by the T-cell response to viral antigens is thought to be fundamental for viral clearance and disease pathogenesis in hepatitis B virus (HBV) infection. The T-cell response during acute self-limited hepatitis B in people is characterised by a vigorous, polyclonal, and multispecific cytotoxic and helper-T-cell response. By contrast, the immune response in chronic carriers, not able to eliminate the virus, is weak or undetectable. Thus a dominant cause of viral persistence could be the existence of a weak antiviral immune response. Methodological progress in animal models allows more precise investigation of the mechanisms by which the immune system resolves viral infection or develops chronic infection. Although clearance of most virus infections is widely thought to indicate the killing of infected cells by virus-specific T cells, data suggest that non-cytolytic intracellular viral inactivation by cytokines released by virus-inactivated lymphomononuclear cells could have an important role in the clearance of this virus without killing the infected cell. Additional factors that could contribute to viral persistence, which have been partly proven in animal models, are viral inhibition of antigen processing or presentation, modulation of the response to cytotoxic mediators, immunological tolerance to viral antigens, viral mutations, and infection of immunologically privileged sites. In view of the central role of cellular immunity in disease pathogenesis, strategies have been proposed to manipulate this cellular immune response in favour of protection from disease.

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More than a third of the world's population has been infected with hepatitis B virus (HBV) and it is estimated conservatively that there are 350 million persistent carriers of HBV worldwide, 25% of whom have chronic liver disease and cirrhosis, which could progress to hepatocellular carcinoma. The annual mortality from hepatitis B infection and its sequelae is 1–2 million people worldwide.[1] HBV infection acquired in adult life is often not clinically apparent and most acutely infected adults recover completely from the disease and clear or control the virus. Roughly 5–10% of acutely infected adults become persistently infected by the virus and develop chronic hepatitis. Neonatally transmitted HBV infection, however, is rarely cleared and more than 90% of infected children develop chronic infection.

The precise pathogenetic mechanisms responsible for the various forms of associated acute and chronic liver diseases are only partly defined. Most studies indicate that HBV is not cytopathic for the infected hepatocyte.[2] Because the disease spectrum associated with these viruses is highly variable, the host response to these viruses must have a critical role in the pathogenesis of the associated diseases. Studies in human and animal models provide substantial evidence that viral hepatitis is initiated by an antigen-specific antiviral cellular immune response.

Immune response to HBV

The host response to viruses relies on a complex interaction of several cell systems; the cells of the innate immune system, the dendritic cells, which are key in priming and directing the virus-specific T-cell response; and the T cells, which are the main antiviral effectors. After infection of the hepatocyte various cellular and humoral responses have been postulated that are aimed at elimination of the virus. The earliest responses are non-specific and include the interferon system, natural killer cells, and non-specific activation of Kupffer cells. The precise role of several of these unspecific mechanisms is not well understood in HBV infection although it was recently shown that natural killer T-cell activation inhibits HBV replication in vivo.[3]

After these non-specific responses, immune responses directed specifically against viral proteins become important. The two major arms of the immune system are the humoral arm, which consists of B lymphocytes that produce antibody, and the cellular arm, which is composed of various cell types, including macrophages and T-lymphocytes (figure 1).

Dendritic cells constitute a heterogeneous group of unique antigen-presenting cells that builds the bridge between pathogens and the T-cell system. The full effect of this system in viral disease has only recently been appreciated, as well as the means for first-time identification, separation, and functional analysis of these cells—eg, the recognition of the plasmacytoid dendritic cells (pDCs) as the principal type-I-interferon-producing cells. We have limited knowledge of the dendritic cell system in viral infection and, in particular, in HBV infection. A precise definition of its function, however, is needed for

Both authors are at the Institute for Immunology and Medical Departments, University of Munich, Munich, Germany.

Correspondence: Dr Maria-Christina Jung, Medizinische Klinik 2, Klinikum Großhadern, Marchioninistrasse 15, 81377 Munich, Germany. Tel +49 89 70950; fax +49 89 7000 9540; email maria-christina.jung@med2.med.uni-muenchen.de

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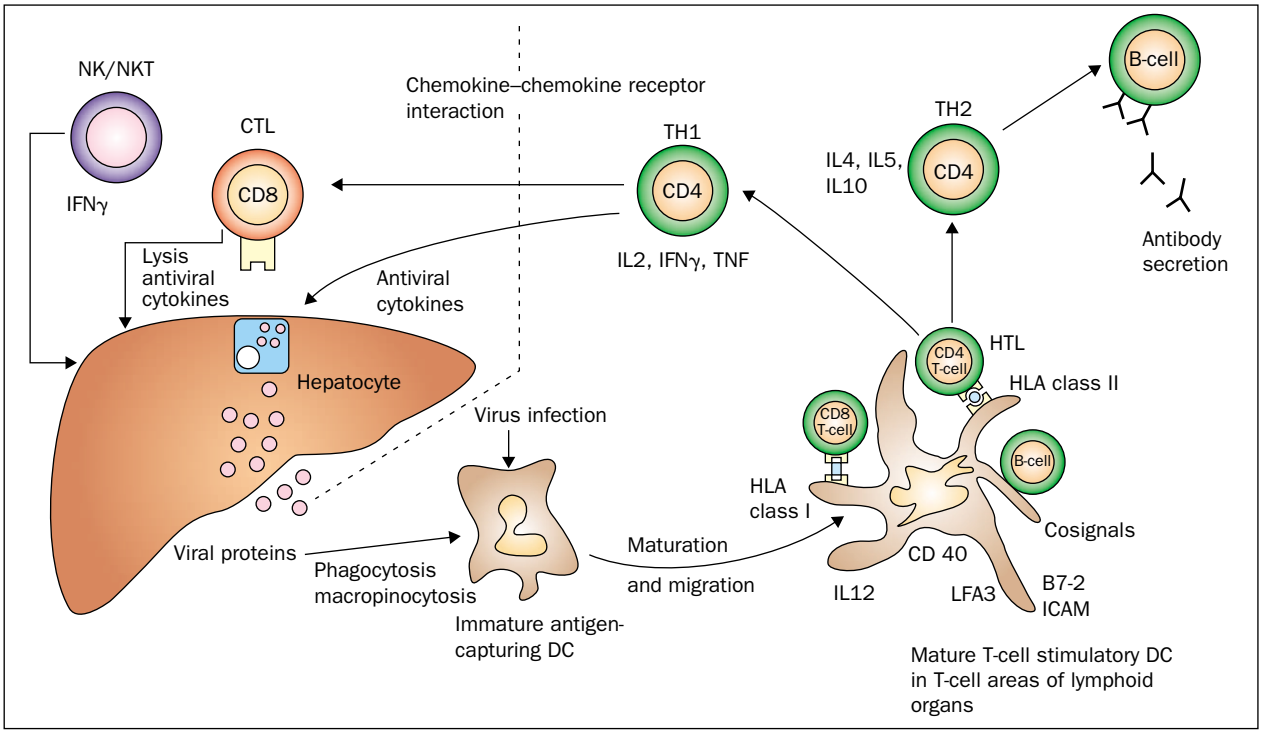

Figure 1. Interaction of different cell system in the immune response against HBV: NK/NKT=cells of the innate immune system. DC=dendritic cells. (take up viral proteins, mature and migrate to lymphoid organs where they present viral peptides on HLA class I and class II molecules to CD8+ and CD4+ T cells, polarising the T cell response in Th1 or Th2 direction. Th1 helper T cells secrete, for example, interferon-γ (IFNγ) and interleukin (IL) 2, which support macrophages and cytotoxic T cells to kill intracellular pathogens; Th2 helper T cells secrete, for example, interleukin 4, 5, and 6 supporting the B cell response. Chemokines produced in the liver specifically attract induced lymphocytes to the liver after chemokine/chemokine receptor interaction. Virus specific CTL either directly "kill" infected hepatocytes by Fas-mediated or perforin-mediated mechanisms or "cure" the hepatocytes from virus by antiviral cytokines.

understanding the host's antiviral immune response, and for design and development of therapeutic strategies in which dendritic cells are used as vectors and targets.

T cells characteristically have T-cell receptors (TCRs) that recognise processed antigen presented by MHC molecules (see figure). Most cytotoxic T cells are positive for CD8, recognise processed antigen presented by MHC-class-I molecules, and kill infected cells, to prevent viral replication. Any released virus is immediately susceptible to the effects of antibody. In addition to killing infected cells directly, CD8 T cells also produce several cytokines, including tumour necrosis factor-α (TNFα) and lymphotoxin. Interferon-γ, another product of CD8+ T cells, reinforces antiviral defences by rendering adjacent cells resistant to infection. Helper T lymphocytes are generally positive for CD4, recognise processed antigen presented by MHC-class-II molecules, and can be divided into major populations: type 1 helper T cells (Th1) secrete interferon-γ and interleukin-2 and type 2 helper T cells (Th2) secrete interleukin 4, 5, and 6.

Cytokines have a central role in influencing the type of immune response needed for optimum protection against particular types of infectious agents. For example, the release of interleukin 12 by antigen-presenting cells stimulates the production of interferon-γ by Th1 cells. This cytokine efficiently activates macrophages, enabling them to kill intracellular organisms. To generalise, the production of cytokines by Th1 cells facilitates cell-mediated immunity, including the activation of macrophages and T-cell-mediated cytotoxicity; Th2 cells help B cells produce antibodies.[4]

Chemokines are another group of proteins that has a large effect on immunologic responses. The interaction of chemokines with their respective receptors on lymphocytes is a prerequisite to attract these cells to the liver. So far, the chemokine repertoire expressed on HBV-infected liver compared with uninfected liver is not clear.

The antigens of HBV

The 3·2 kb genome of HBV consists of partly double-stranded DNA in circular construct. The precore region of the core (c) gene encodes e antigen that is truncated, secreted from the cell, and enters the circulation. The remaining core gene encodes the viral nucleocapsid protein. Soluble e antigen shares immunologic specificity with that of the nucleocapsid.

The envelope antigens that are seen in the phospholipid bilayer of the virus are dimeric and consist of small (S), medium (S plus pre-S2), and large (S plus preS2 and S1) proteins, depending on the site of initiation in transcription. The S antigen of the wildtype virus is highly conserved and bears the common a antigen determinant of HBV. The polymerase gene (pol) encodes the DNA polymerase-reverse transcriptase protein. The x gene encodes the regulatory (transactivating) proteins. Many diverse mutations happen in all four of the viral genes.[5]

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Humoral response to HBV

The antibody response to HBV-envelope antigens (HBsAg) is a T-cell-dependent process.[6]

Antibodies to HBsAg serve as neutralising antibodies. These neutralising antibodies are especially important in the prevention of viral infection, since they could prevent viral attachment and entry into the cells by absorption of the viral particles. Induction of anti-HBs alone during prophylactic vaccination is often sufficient to completely prevent viral infection, irrespective of whether this is the only operative defence mechanism against the viral infection during the course of natural infection.

The antibody is detectable in patients who have recovered from acute hepatitis B and in people immunised with HBV vaccine, but it could become undetectable in patients who have recovered fully from infection. Antibody to HBcAg is detected in virtually all patients who have ever been exposed to HBV. Unlike antibody to HBsAg this antibody is not protective; its presence alone cannot be used to distinguish acute from chronic infection.

HBcIgM antibody usually disappears within 4 to 8 months after acute infection. Since some patients with chronic hepatitis B infection become positive for IgM antibody during flares in their disease, its presence is not an absolutely reliable marker of acute illness. HBeAg is historically seen as a marker of active viral replication, and clearance of HBeAg and occurence of antibody to HBeAg are seen as marking a stop to viral replication. Studies with sensitive immunoassays indicate that antibodies to HBeAg are present even before the clearance of HBeAg and that when the HBeAg has been cleared, the virus is still replicating.[7,8] The antibody response to the viral polymerase has not been extensively studied. Reportedly, however, the carboxy-terminus of the polymerase, especially its RNAse-H domain, seems to be immunodominant at the antibody stage and these antibodies serve as early markers of infection, and possibly indicate continuing viral replication.[9]

Little is known about the antibody response to the viral transactivator protein pX, although most investigators report pX as principally associated with chronic hepatitis and hepatocellular carcinoma.[10,11]

Insights from animal systems

Woodchuck hepatitis virus (WHV)-infected woodchucks and duck hepatitis B virus (DHBV)-infected ducks are the most widely accepted and frequently used animal models for the study of mechanisms related to human HBV infection. Experimental inoculation of naive ducks with DHBV can lead to one of three outcomes—persistent viraemia, transient infection with or without viraemia, or no evidence of infection. Congenitally DHBV-infected ducks remain persistently infected for life. Studies with the WHV experimental model have also shown persistent infection after transmission of virus to neonatal animals, whereas infection of older animals is usually transient.[12,13] As in human beings, it has been proposed that variability in outcome—eg, transient or persistent infection—could depend on the balance between parameters that determine viral spread and variables of the immune system that determine the development of an immune response. It is suggested that viral parameters could include dose of inoculum, kinetics of viral replication and dissemination, and cell and tissue tropism, all of which are balanced against the specificity, kinetics, and duration of humoral and cell mediated immunity. The ability of individual ducks to resolve DHBV infection was seen to be linked to the age of the duck at the time of inoculation and the dose of inoculated virus.[14] The effect of the dose of virus on the immune response has also been studied in the woodchuck system.[15] The chronic outcome in experimental neonatal WHV was characterised by increasing initial viral load in liver and plasma, and a detectable but diminished acute hepatic inflammation.[16] Results obtained in the two animal models also indicate the importance of the cell-mediated immune response for the outcome of infection. It has been widely assumed that viral clearance is mediated chiefly by destruction of infected cells by viral antigen-specific cytotoxic T lymphocytes (CTLs) and that pathogenesis of persistent hepadnavirus infection is also mediated by these cells.[17] Studies in HBV transgenic mice provided experimental evidence for this view, taking into account the limitation that these mice are not infected by the virus.

It has been shown that CTLs have both a cytopathic and a curative potential: transgenic mice that express HBV-envelope antigens in their hepatocytes develop acute viral hepatitis after adoptive transfer of CD8+, MHC class I restricted, HBsAg-specific CTL lines and clones.[18–20] However, the direct cytopathic effect of the CTLs was limited to very few hepatocytes, possibly because the effector/target (E/T) cell ratio in the liver was low and the free-ranging CTL movement was severely limited by the architectural constraints of solid tissue. Like most cases of acute viral hepatitis in human beings, the disease is transient and mild in HBV transgenic mice, destroying no more than 5% of the hepatocytes.

However, if many HBsAg-positive ground glass hepatocytes are present in the liver, a process ensues in which the animal could die from fulminant hepatitis because ground glass cells are exquisitely sensitive to destruction by interferon-γ, and this cytokine is actively secreted by CTLs after antigen recognition. Because injury can be completely prevented by administration of neutralising antibodies to interferon-γ, it was assumed that most of the liver cell injury was mediated by non-specific inflammatory cells recruited by the CTL, probably by interferon-γ-mediated release of chemotactic and inflammatory cytokines.[21]

The transgenic mouse model has also shown that activated CTL and the cytokines they secrete can downregulate HBV gene expression and replication by non-cytotoxic intracellular inactivation mechanisms involving the degradation of viral RNA and, perhaps, the degradation of viral nucleocapsids and replicative DNA intermediates without killing the cell.[22]

This process is mediated by interferon-γ and TNFα secreted by the CTL after antigen recognition without Fas-dependent or perforin-dependent signalling pathways.

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Furthermore, it has been shown that the same events can be initiated by transfer of HBsAg-specific class-II-restricted T-cell clones into the transgenic mice when they recognise antigen presented by Kupffer cells.[23] Further support for the importance of non-cytopathic antiviral mechanisms for viral clearance came from studies in chimpanzees.[24] On the basis of the observations made in mice, acute hepatitis B was induced in two healthy, young adult chimpanzees. HBV infection was documented by virological, immunological, histopathological, and molecular analyses of serum specimens and liver biopsies that were obtained on a weekly basis throughout the course of the infection. Disappearance of HBV DNA from the liver and blood of acutely infected chimpanzees coincided with the induction of interferon-γ, which preceded the peak of T-cell infiltration and most of the liver disease. The results suggest that different populations of inflammatory cells could be responsible for early viral clearance and late viral pathogenesis in HBV infection.

Several in vivo studies of transient DHBV and WHV infection also suggested that clearance of hepadnavirus infection from the liver could happen by non-cytolytic mechanisms.[13] In experimental adult WHV infection, it has been shown that recovery from acute hepatitis in adulthood is preceded by a significantly greater hepatic expression of interferon-γ and CD3. Recovery is also preceded by increased TNFα transcription, lower hepatic virus load, and a greater degree of liver inflammation compared with acute infection associated with chronic outcome. Additionally, chronicity in experimental neonatal woodchuck seems to depend on the inability to elicit a strong acute hepatitis that is temporally deficient for the expression of interferon-γ and TNFα.[25–27] As in human beings, the T-cell proliferative response to viral antigens in DHBV and WHV is different in acute and chronic infection—ie, stronger and more frequent in acute infection and weaker or barely detectable in chronic infection.[28,29]

Virus-specific CD4+ T-cell response in people

A vigorous HLA-class-II restricted, CD4+ helper-T-cell response to multiple epitopes in the HBV nucleocapsid antigens, HBcAg and HBeAg, is detectable in the peripheral blood of almost all patients with self-limited acute hepatitis. Of the several HBcAg/HBeAg epitopes that have been defined, the epitope located between core residues 50–69 are most commonly recognised in acutely infected patients, irrespective of their HLA-backgrounds. Two additional important T-cell-recognition sites were represented by the aminoacid sequences 1–20 and 117–131, which were stimulatory for the T cells of 69% and 73% of the patients, respectively.[30] By contrast, the HLA-class-II-restricted envelope-specific response is much less vigorous in the same patients. The basis for the absence of a strong HBV envelope-specific T-cell response in acutely infected patients who respond well to the nucleocapsid antigens is not readily understood. The class-II-restricted response to the viral polymerase and X proteins has not been adequately studied.

The precise onset of specific cellular immune responses in people is not known but it is probably within weeks of infection. HBV-specific CD4+ T-cell response has been described in five patients during the incubation phase of acute hepatitis B by intracellular cytokine staining.[31] Core-specific CD4+ T cells have been shown in one patient 1 month before the onset of acute hepatitis (900 core-specific CD4+ T cells/mL). At the time of maximum liver damage core-specific CD4+ T cells were still present, but at a much lower frequency than was seen earlier in the incubation phase. The number of core-specific CD4+ T cells then decreases, reaching a frequency of 50–100 core-specific CD4+ T cells/mL after clinical resolution of infection.

The development of a vigorous CD4, MHC class-II-restricted response to core is temporally associated with the clearance of HBV from the serum, and is probably essential for efficient control of viraemia through several mechanisms.[32] These CD4 responses exert their effect by production of cytokines. The cytokine profile secreted by core-specific CD4+ T lymphocytes in self-limited acute hepatitis B showed production of Th1 cytokines dominated by the production of interferon-γ, which suggests that Th1-mediated effects could contribute to liver cell injury and recovery from disease.[33]

During chronic HBV infection, the peripheral blood HLA class-II-restricted T-cell response to all viral antigens, including HBcAg and HBeAg, is much less vigorous than in patients with acute hepatitis.[34,35] The nucleocapsid-specific T-cell response seems to be accentuated during acute exacerbations of disease, which can often be preceded by increased serum HBV DNA and HBeAg concentrations that could drop substantially as the flare in disease activity subsides.[36] T-cell clones from the liver of people with chronic HBV infection, stimulated with mitogen, produce predominantly a type 2 cytokine response.[37]

Virus-specific CD8+ T-cell response in people

Previous studies in acute symptomatic HBV infection have shown vigorous polyclonal multispecific class-I-restricted CTL responses to all HBV proteins. These studies were based on the combined use of short synthetic peptides that mimic the processed antigen fragments and eukaryotic expression vectors that direct the synthesis of HBV antigens in human cells so that they can be processed and presented in the context of HLA class-I molecules. Multiple epitopes are recognised in most HLA-A2.1 positive individuals (notably core 18–27, envelope 183–191, envelope 250–258, envelope 335–343, and polymerase 455–463).[38-43] The presence of CTL multispecificity has been repeatedly reported in patients who effectively control HBV.[40,44] Activated HBV-specific CTLs can persist long after clinical and serologic recovery from acute HBV infection, despite persistent low levels of HBV DNA, which indicates that HBV infection could remain, controlled by specific CTL activity that is maintained by low concentrations of persisting virus.[44] The association between a strong multifaceted T-cell response with acute hepatitis and viral clearance suggests a causal

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relation between these events. However, it does not prove causality, nor does it reveal the mechanisms responsible for viral clearance or disease pathogenesis during HBV infection.

By contrast with acute self-limited HBV infection, peripheral CTL responses in chronically infected patients, as measured by chromium-release assays, are difficult to detect and narrowly focused.[45,46] By the same technique it has been shown that chronically infected patients who experience a spontaneous or interferon-induced remission develop a CTL response to HBV that is similar in strength and specificity to that in patients who have recovered from acute hepatitis.[46] The results suggest that specific immunotherapeutic enhancement of the CTL response to HBV should be possible in chronically infected patients, and that it could lead to viral clearance in these people with resolution of chronic liver disease.

Similarly, HBV-specific CTLs are detected at low frequency in the livers of chronically infected patients, possibly contributing to the chronic inflammation but insufficient to mediate viral elimination.[48–50]

However, most of our understanding of the CTL response in hepatitis B has been based on the use of chromium-release assays, which measure the cytolytic effector function of CTL. We now know that the chromium-release assays are of limited sensitivity. Different effector functions of CTLs have been used to develop more sensitive assays. The most recent generation of assays measures the binding of antigen to T-cell receptors expressed on the surface of CTL. Biotinylated class I MHC molecules are loaded with peptide and linked in tetramer to streptavidin. After incubation of T cells with the tetramers, the percentage of cells binding the complexes can be measured with flow cytometry. These assays can also incorporate measurement of cell surface markers for activation and memory, and measurement of intracellular cytokine production.[51] Using these HLA-peptide tetrameric complexes the frequency and functional ability of CD8+ T cells specific for HBV has been measured during the incubation phase of acute hepatitis B, the clinically acute phase of hepatitis B, and during persistent infection with HBV.[31,41,52]

Direct analysis of the frequency of CD8+T cells in acutely infected patients, who successfully control HBV infection, shows a quantitative hierarchy of CD8+ T cells specific for core, polymerase, and envelope. Core 18-27 specific CD8+ T cells account for up to 1·3% of circulating CD8+ T cells but are always accompanied by a CD8+ T response directed against the other epitopes. Furthermore, precise, direct quantification of HBV-specific CD8+ T cells in the circulation of patients who control HBV infection reveals that the number of circulating core 18-27 specific CD8+T cells is higher in acute HBV infection compared with HBeAg-positive chronic HBV patients in whom these cells are barely detectable in the circulation. The highest frequencies of HBV-specific CTLs detected by tetramer staining coincide with the peak in serum alanine aminotransferase (ALT) concentration and fall after the ALT normalises. These cells expressed an activated phenotype and had an impaired capacity to expand in vitro and to display cytolytic activity in response to peptide stimulation. Recovery of these functions was noted when the frequency of specific CD8+ T cells decreased, as well as a progressive decrease in their expression of activation markers.

Chronic HBV-infected patients lacking evidence of liver damage but controlling HBV replication had functionally active HBV-specific CD8+T cells both in the circulation and in the liver. By contrast, patients with a high rate of HBV replication and evidence of liver inflammation showed a different pattern of virus-specific CD8+ T cells. The frequency of intrahepatic CD8+T cells specific for core 18-27, representing the immunodominant core epitope in the context of HLA A2, was much lower in these patients due to their dilution in a large infiltrate of apparently antigen-non-specific T cells. The number of intrahepatic HBV-specific CD8+ T cells was similar to that in patients without liver disease, taking into account the difference in the size of the total CD8+ T cell infiltrate. These results in chronic HBV infection show that comparable numbers of intrahepatic virus-specific CD8+ T cells could be associated with either protection or pathology, which raises the question of whether the quality rather than the quantity of HBcAg 18-27 specific T-cell response differs between both patient groups.[41]

Functional aspects of the HBV-specific immune response were assessed with HBV-specific T cells derived from the peripheral blood, because only a limited number of lymphocytes that can be isolated from a liver biopsy. HBV-specific CD8+T cells, isolated from the blood of patients with low viral load and normal ALT values, had a resting phenotype, but rapid and vigorous proliferative, interferon-γ and cytotoxic responses on re-exposure to antigen in vitro. By contrast, the number of circulating HBV-specific T cells derived from the blood of patients with high viral load and raised serum ALT levels was lower, and these cells showed a poor expansion potential in vitro.

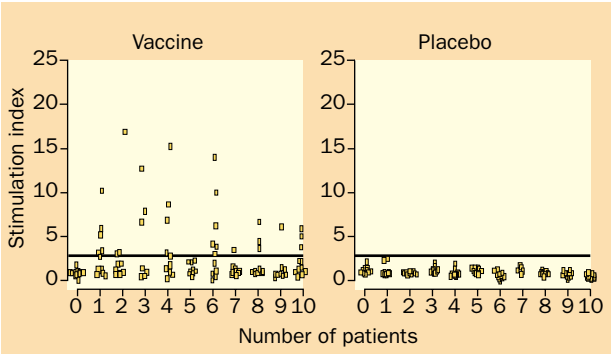

Figure 2. Induction of vaccine-specific proliferative responses in PBMC of patients with chronic hepatitis B either treated with the vaccine or with placebo. The vaccine proteins (preS1, pre S2 and S antigen components) was used in a concentration of 3μg/ml. Antigen-specific T cell proliferation is presented as the stimulation index. The index was calculated as the ratio between cpm (counts per minute in an H^3-thymidin assay) obtained in the presence of antigen to that obtained without antigen. A stimulation index >3 was considered significant. Patients received the vaccine during visits 1 to 8.

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Despite this new information, the immunological basis for viral persistence during adult-onset infection is not well understood. Perhaps the simplest explanation is quantitative —also shown in animal models[27]—and based on the kinetics of infection relative to the induction of a CTL response during the early days of an infection. As indicated in animal models, viral persistence would be predicted if the size of the inoculum or the replication rate of an incoming virus exceeds the kinetics of the immune response so that the effector-to-target cell ratio favours the virus even when the CTL response is fully in place. Other candidate mechanisms that contribute to viral persistence include infection of immunologically privileged sites, viral inhibition of antigen presentation—ie, in dendritic cells—selective immune suppression, downregulation of viral gene expression, and viral mutations that abrogate, anergise, or antagonise antigen recognition by virus-specific T cells.

New therapeutic stategies to improve antiviral immunity

In view of the central role of cellular immunity in disease pathogenesis, strategies have been proposed to manipulate this cellular immune response in favour of protection from disease.

The cellular immune response—ie, specific activity of CD8+ and CD4+ T lymphocytes—mediates clearance of HBV by CTL attack on infected hepatocytes and by the production of inflammatory cytokines. Chronic infection is due to a deficit in HBV-specific and CD8+ T-cell responses to hepatitis antigens. With this in mind, immunotherapy would seem to offer the best chance of sustained viral clearance. Vaccine therapy has produced promising effects in other diseases, including leprosy and herpes simplex.[53,54] In HBV infection it has been reported that specific vaccine therapy by standard anti-HBV vaccination reduced or circumvented HBV replication in 50% of chronic carriers.[55] We report that vaccination with preS1, pre S2, and S antigen components can overcome non-responsiveness of CD4+ T-lymphocytes to surface proteins (figure 2) in chronic HBV carriers, but did not induce the secretion of Th1 cytokines and virus-specific CD8+ T lymphocytes.[56]

In recent years, several groups have specified requirements for binding of peptides to MHC-class-I molecules, and have noted the existence of motifs that predict which peptide sequences bind to a given MHC-class-I molecule. This knowledge has led to the development and use in a dose-escalation trial of a therapeutic vaccine consisting of the HBV core antigen peptide aminoacid 18-27 as the CTL epitope, tetanus toxoid peptide 830-843 as the T-helper peptide, and two palmitic acid molecules as the lipids.[57] The vaccine was safe and able to induce a primary HBV-specific CTL response in normal patients. The magnitude of the CTL responses induced by the therapeutic vaccination was in fact found to be comparable to CTL responses associated with clearance of acute viral infection.[58] However, in persons with chronic infection, the vaccine induces only a low-level CTL activity which was not associated with viral clearance or substantial reduction of HBV-DNA.[59]

Further studies revealed an altered helper T lymphocyte function as demonstrated by altered cytokine profiles and decreased responses to tetanus toxoid. The decreased responses to tetanus toxoid correlated with hyporesponsiveness to therapeutic vaccination.[60] Based on these results the combination of therapeutic vaccines targeting CD4+ and CD8+ T lymphocytes with other nonspecific immunostimulants such as interferon-γ or interleukin-12, which might reverse helper T lymphocyte alteration should be considered. Alternatively, combination therapy with lamivudine which can restore T cell responsiveness in patients with chronic HBV infection represent an attractive clinical strategy.[61,62]

Search strategy and selection criteria

Data were identified by searches of Medline, Current Contents, and references from relevant articles; many articles were identified through searches of the extensive files of the authors. Search terms were "hepatitis B specific immune response", "immunology of hepatitis B virus", "woodchuck/duck and hepatitis B", "CD4+/CD8+ T lymphocyte in hepatitis B", "antigen presenting cells and hepatitis B", "chimpanzees and hepatitis B", "antiviral immune response", "therapeutic vaccination in hepatitis B", "treatment of hepatitis B", "immunology of virus infection", "animal models in hepatitis B", and "chemokines in hepatitis B".

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