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EDITORIAL

T cell immunopathogenesis and immunotherapeutic strategies for chronic hepatitis B virus infection

Yukihiro Shimizu

Yukihiro Shimizu, Gastroenterology Unit, Takaoka City Hospital, 4-1 Takara-machi, Takaoka city, Toyama 933-8550, Japan
Author contributions: Shimizu Y designed and wrote the manuscript.
Correspondence to: Yukihiro Shimizu, MD, PhD, Gastoenterology Unit, Takaoka City Hospital, 4-1 Takara-machi, Takaoka city, Toyama 933-8550, Japan. rsf14240@nifty.com
Telephone: +81-766-230204 Fax: +81-766-262882
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Abstract

Hepatitis B is caused by the host immune response and T cells play a major role in the immunopathogenesis. More importantly, T cells not only destroy hepatocytes infected by hepatitis B virus (HBV), but also control HBV replication or eradicate HBV in a noncytolytic manner. Therefore, analysis of T cell immune response during acute and chronic HBV infection is important to develop a strategy for successful viral control, which could lead to immunotherapy for terminating persistent HBV infection. There have been many attempts at immunotherapy for chronic HBV infection, and some have shown promising results. High viral load has been shown to suppress antiviral immune responses and immunoinhibitory signals have been recently elucidated, therefore, viral suppression by nucleos(t)ide analogs, stimulation of antiviral immune response, and suppression of the immunoinhibitory signals must be combined to achieve desirable antiviral effects.

Key words: T cells; Immunopathogenesis; Immunotherapy; Hepatitis B virus infection

Peer reviewer: Dr. Huiping Zhou, Department of Microbiology and Immunology, Virginia Commonwealth University, 1217 East Marshall Street MB#533, Richmond, VA 23298, United States

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INTRODUCTION

Hepatitis B virus (HBV) is not cytopathic, and hepatitis B is caused by the host immune response, mainly T-cell-mediated, against virus-related peptides expressed on hepatocytes in conjunction with human leukocyte antigens (HLAs). In acute self-limiting hepatitis, a broad T-cell immune response occurs that is strong enough to eradicate the virus or suppress viral replication[1]. However, there are many mechanisms that hamper the antiviral immune response, leading to persistent infection. To develop an optimal strategy to stimulate antiviral immune response with therapeutic potential, extensive analyses of immune mechanisms for successful viral eradication and immunosuppressive mechanisms induced by viral infection during persistent infection are required. In this review, I focus on T cell immune response during HBV infection, and summarize attempted immunotherapeutic approaches against persistent HBV infection.

T CELL RESPONSE IN ACUTE HBV INFECTION

Immunological analysis has been extensively performed in transgenic and chimpanzee models of acute HBV infection. In one model, transgenic mice, in which infectious HBV virions replicate in the liver with expression of all HBV-related antigens, were injected with hepatitis B surface antigen (HBsAg)-specific cytotoxic T lymphocytes (CTLs) that had been induced in nontransgenic mice. The injected CTLs produced interferon (IFN)-γ and tumor necrosis factor (TNF)-α, which purged viral

RNA and DNA without destroying infected hepatocytes[2-4]. Importantly, this noncytolytic clearance of intracellular HBV is more efficient at controlling HBV replication than the killing of infected hepatocytes. In this sense, hepatitis is not only a harmful event but also represents an effective mechanism by which CTLs suppress HBV. Noncytolytic viral eradication can account for recovery from acute HBV infection in that most HBV is cleared from hepatocytes with only a fraction of the hepatocytes being destroyed. This was confirmed in a chimpanzee infection model; HBV DNA level was markedly decreased in the liver and blood of acutely infected chimpanzees before peak serum alanine aminotransferase (ALT) concentrations were reached[5], suggesting that this noncytopathic T cell effector mechanism results in early viral inhibition or eradication, whereas a cytopathic T cell effector mechanism is required to eliminate the remaining virus by destroying infected hepatocytes.

In humans, the HBV-specific T cell response during incubation phase of acute hepatitis B has been analyzed extensively using HLA class I tetramer and cytokine staining[6]. The data showed that maximal reduction in HBV DNA in the serum occurred before the peak of ALT elevation; again indicating that suppression of HBV replication occurs without hepatocyte injury. Moreover, infiltration of HBV-specific $CD8^+$ T cells into the liver has been observed several weeks before the peak of liver injury, suggesting that HBV-specific T cell infiltration occurs at an early stage of infection, resulting in suppression of HBV replication. Thereafter, recruitment of mostly nonspecific cells induced by cytokines or chemokines produced by HBV-specific T cells contributes to significant liver damage. Interestingly, in the HBV transgenic mouse model of acute hepatitis, administration of antibodies against the chemokines, IFN-inducible protein (IP-10) and monokine induced by interferon-(Mig), reduced the recruitment of mostly antigen-nonspecific mononuclear cells into the liver that had been induced by cytokines and chemokines produced by injected CTLs, leading to a reduction in the severity of hepatitis without affecting the antiviral activity of the CTLs[7]. These observations have important therapeutic implications, because suppression of antigen-nonspecific mononuclear cell recruitment may suppress hepatitis, while retaining the antiviral function of the CTLs.

The overall data from studies in chimpanzees and humans are essentially the same, and indicate that a sufficient T cell response to HBV at an early phase of infection is important for eradication of virus infection, and that an insufficient T cell response may lead to persistent viral infection.

The contributions of $CD4^+$ and $CD8^+$ T cells to the control of viral infection have been analyzed in a chimpanzee model of acute hepatitis B by depleting either T cell population with monoclonal antibodies. The data show that $CD8^+$ T cells are the main effector cells responsible for virus elimination[8].

Antigen specificity of T cell response in acute HBV infection

The antigen specificity of the T cell response to HBV in acute hepatitis has been analyzed, and it is clear that acute viral hepatitis involves a vigorous CTL response to multiple epitopes in the viral nucleocapsid, envelope, and polymerase proteins, whereas these are not seen in patients with chronic hepatitis[1]. Although multispecificity of the CTL response is characteristic in acute hepatitis, there is known to be a hierarchy of epitope-specific $CD8^+$ T cell responses determined by cytokine production after peptide stimulation. In acute hepatitis B, $CD8^+$ T cell response to HBc18-27 (HLA-A2 restricted epitope) is dominant followed by the response to polymerase epitope (455-463), whereas envelope epitopes are always subdominant[9]. The hierarchy is clearly distinct from that observed in chronic hepatitis, in which the $CD8^+$ T cell response to envelope epitope (183-191) is always dominant. Interestingly, chronic hepatitis patients with lower HBV DNA levels in the serum show greater responses to HBc18-27 than those with high HBV DNA. These findings imply that the T cell response to hepatitis B core antigen (HBcAg) is important for viral control, which is important for designing peptide vaccines for the treatment of chronic HBV infection.

Long-lasting T cell immune response after resolution of acute hepatitis B

In humans, most HBV is cleared after resolution of acute hepatitis. However, it has been shown that trace amounts of HBV DNA can be detected for several years after resolution of acute hepatitis, and the long-lasting memory T cell response is maintained by persistent replication of HBV[10], indicating that low levels of HBV replication could continue in most patients even in the convalescent phase of acute hepatitis in balance with immunological pressure.

T CELL RESPONSE IN CHRONIC HBV INFECTION

In peripheral blood, HBV-specific helper T lymphocytes and CTLs are barely detectable in patients with chronic hepatitis B (CHB)[11], possibly due to exhaustion by high viral load or tolerance to HBV.

In contrast, several studies have characterized intrahepatic $CD4^+$ and $CD8^+$ T lymphocytes in CHB. Intrahepatic $CD4^+$ T lymphocytes in patients with CHB have been found to contain T helper (Th)0 cells, which produce not only IFN-γ, but also interleukin (IL)-4 and IL-5, thus differing from cells in the livers of patients with chronic hepatitis C, which are mostly Th1 cells[12]. $CD4^+$ T lymphocytes that produce IL-17 infiltrate into the livers of patients with CHB and are involved in liver inflammation[13].

Livers of patients with low HBV replication contain intralobular $CD8^+$ T lymphocytes[14], and the percent-

ages of virus-specific T lymphocytes in the liver have been clarified by immunohistochemical staining with peptide-MHC tetramer. The proportion of $CD8^+$ T lymphocytes in the livers of patients with chronic HBV specific for HBc18-27, a major HBV epitope, has been found to range from 0.18% to 1.28%[15]. Maini *et al*[16] have reported that the number of HBc18-27-specific $CD8^+$ T cells, detected using tetramers, was the same in livers with low HBV DNA/ALT as in those with high HBV DNA/ALT. Hence, HBV-specific T cells recognize HBV antigens and carry out immune surveillance in the liver. Thus, they have an important role in controlling HBV replication in the liver without causing hepatic necroinflammation in low DNA/ALT anti-HBe^+ HBV carriers. It remains unknown why HBV-specific T cells fail to control effectively HBV replication in the liver with chronic hepatitis. However, recent advances in immunology have given some insight into the mechanism as described below.

IMMUNOSUPPRESSIVE MECHANISM RESPOSIBLE FOR PERSISTENT HBV INFECTION

Regulatory T cells

Regulatory T (Treg) cells expressing the forkhead family transcription factor, FoxP3, are specialized cells that exert negative control on a variety of physiological and pathological immune responses, resulting in maintenance of immunological self-tolerance[17]. They show diverse phenotypes, occurring in both $CD4^+$ and $CD8^+$ T cell subsets, and express CD25 (IL-2 receptor chain) and/or cytotoxic T-lymphocyte antigen 4 (CTLA-4) in addition to Foxp3.

In HBV infection, hepatitis B e antigen (HBeAg)-positive patients with high HBV DNA levels in the serum show elevated numbers of $CD4^+CD25^+$ Treg cells in the blood compared to patients with acute and chronic hepatitis C virus (HCV) infection[18]. Significant accumulation of $CD4^+CD25^+FoxP3^+$ Treg cells in the liver is found in patients with chronic HBV infection. Moreover, patients with high viral load have a higher proportion of Treg cells in the liver[19], suggesting that intrahepatic Treg cells suppress antiviral immune responses in the liver in chronic HBV infection.

Th cells that produce IL-17 (Th17 cells) have recently been identified as the third subset of effector T cells[20], which produce IL-17A, IL-17F, IL-22 and IL-21[21]. Recently, IL-6 has been shown to induce the generation of Th17 cells from naïve T cells together with transforming growth factor (TGF)-β and inhibits TGF-induced Treg cell differentiation[22]. Importantly, there is a reciprocal relationship between Th17 and Treg cells; not only in development, but also in their effector function, indicating that the Treg/Th17 balance may determine the quality and magnitude of immune responses in the liver[20]. Unexpectedly, the increases in circulating and intrahepatic Th17 cells are positively correlated with HBV DNA in the serum, serum ALT levels, and histological activity index of the livers with CHB, suggesting that activation of Th17 cells does not exert antiviral function in CHB[23].

Programmed death-1

Programmed death-1 (PD-1) is a surface receptor critical for the regulation of T cell function[24,25]. Binding to PD-1 by its ligands PD-L1 and PD-L2 results in the antigen-specific inhibition of T cell proliferation, cytokine production, and cytolytic function, leading to exhaustion of T cells. In the liver, PD-1 is expressed on lymphocytes; PD-L1 is expressed on lymphocytes, hepatocytes and sinusoidal endothelial cells; and PD-L2 is expressed on Kupffer cells and dendritic cells (DCs)[26]. HBeAg-positive patients with high HBV DNA levels in the serum show increased PD-1 and CTLA-4 expression on HBV-specific $CD8^+$ T cells[27]. Moreover, PD-1 expression on $CD4^+$ T cells is correlated positively with serum HBV DNA load in CHB patients[28]. Intrahepatic HBV-specific $CD8^+$ T cells express higher levels of PD-1, and upregulation of intrahepatic PD-1/PD-L1 is associated with liver inflammation and ALT elevation[29]. Although the mechanism underlying the upregulation of PD-1 on $CD8^+$ T cells in the inflamed liver is unknown, signals from PD-1 inhibit HBV-specific T cells, resulting in insufficient antiviral responses, leading to failure of viral control and persistent liver inflammation. Importantly, PD-1/PD-L1 blockade increased $CD8^+$ T cell proliferation and enhanced IFN-γ and IL-2 production by intrahepatic lymphocytes[29]. These findings suggest that inhibition of PD-1/PD-L1 may have therapeutic potential for the control of hepatitis B.

IL-10

IL-10 is an important cytokine with anti-inflammatory properties, and is produced by activated monocytes/macrophages and T cell subsets, including Treg and Th1 cells[30]. Immunosuppression by IL-10 is associated with functional exhaustion of memory T cells in chronic lymphocytic choriomeningitis virus (LCMV) infection, and blockade of IL-10 receptors could terminate chronic LCMV infection[31]. In chronic HBV infection, HBcAg stimulates the production of IL-10, which negatively regulates HBcAg-specific Th17 cell responses in CHB patients[32].

T cell immunoglobulin- and mucin-domain-containing molecule-3

It has been reported that not all exhausted T cells show upregulation of PD-1 and downregulation of CD127 (IL-7 receptor), and blockade of the PD-1/PD-L1 signaling pathway does not always restore proliferation and cytokine production[33]. Recently, another inhibitory molecule, T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3), has been reported. A high frequency of Tim3-expressing $CD4^+$ and $CD8^+$ T cells are found in chronic HBV infection, and the frequency of $Tim\text{-}3^+$ T cells is positively correlated with the severity of liver inflammation, and negatively correlated with plasma IFN-γ levels[34].

Table 1 Immunotherapeutic approaches for animal models of hepatitis B virus infection

Animal model	Immunotherapy	Results	Ref.
Peptide vaccination			
HBV transgenic mice	A synthesized fusion peptide, consisting HBcAg18-27 and HIV Tat49-57	Decrease in serum HBV DNA levels and the expression levels of HBsAg and HBcAg in the liver	[40]
Protein vaccination			
HBV transgenic mice	HBsAg vaccine	Most of the mice showed reduction of HBV DNA levels and disappearance of HBeAg and HBsAg	[41]
Woodchuck hepatitis virus infection	Combination of vaccine of HBV large surface protein and clevudine	Restoration of T-cell response to Pre-S and S region	[42]
DNA immunization			
Acute DHBV infection	DNA vaccine expressing DHBc and Pre-S/S and entecavir Boosted with fowl poxvirus vectors expressing DHBc and Pre-S/S	Clearance of DHBV infection at a rate of 100%	[43]
Chronic DHBV infection	DNA vaccine encoding the HBV large envelope and/or core protein with or without lamivudine	Reduction of viremia and liver DHBV cccDNA in 33% of ducks Seroconversion to anti-pre S in 67% of ducks showing cccDNA clearance	[44]
DC immunization			
HBV transgenic mice	Activated bone marrow-derived DCs	Break CTL tolerance to HBsAg	[45]
HBV transgenic mice	Anti-CD40 agonistic monoclonal Ab	Induction of noncytopathic inhibition of HBV replication mediated by antiviral cytokines (IL-12 and TNF-α) produced by activated intrahepatic APCs	[46]
HBV transgenic mice	HBV-specific peptide-pulsed DCs	Reductions in the serum HBsAg and HBV DNA	[47]
Cytokines and adjuvants			
HBV transgenic mice	Recombinant IL-12	Marked inhibition of HBV replication in the liver	[48]
HBV transgenic mice	α-galactosylceramide that can activate NK T cells	Complete inhibition of HBV replication	[49]
HBV transgenic mice	Recombinant IL-18	Inhibition of HBV replication noncytopathically, mediated by activation of resident intrahepatic NK cells and NK T cells	[50]
Gene therapy			
HBsAg transgenic mice	Lentivectors expressing HBsAg and IgFc fusion Ag	Induction of seroconversion to anti-HBs	[51]

HBV: Hepatitis B virus; DHBV: Duck HBV; DC: Dendritic cell; HBsAg: Hepatitis B surface antigen; HBcAg: Hepatitis B core antigen; HIV: Human immunodeficiency virus; APC: Antigen-presenting cell; IL: Interleukin; NK: Natural killer; CTL: Cytotoxic T lymphocyte; TNF: Tumor necrosis factor; cccDNA: Covalently closed circular DNA; Ab: Antibody; Ag: Antigen.

Dysfunction of DCs

DCs are specialized antigen-presenting cells that orchestrate immune responses. They stimulate innate and acquired immune responses, but also act as tolerogenic cells for immune responses in a variety of situations. In viral hepatitis, dysfunction of DCs from peripheral blood has been reported. In patients with CHB, maturation of DCs from peripheral blood of patients after incubation with cytokines is lower than that of normal subjects with lower expression of HLA-DR and co-stimulatory molecules in the former population[35], leading to low allostimulatory function of DCs from CHB patients. The mechanism of impairment of DC function in patients with CHB is unclear, but both HBV particles and purified HBsAg may have immunomodulatory capacity and may directly contribute to the dysfunction of myeloid DCs[36]. Interestingly, impaired function of monocyte-derived DCs from patients with CHB could be reversed by inhibiting viral replication with nucleos(t)ide analogs such as lamivudine[37]. Type 2 precursor plasmacytoid dendritic cells (pDCs), which are the most important cells in antiviral innate immunity, are also reported to have quantitative and qualitative impairment in patients with chronic HBV infection[38]. Recently, HBV itself was shown to inhibit the functions of pDCs[39]. These data indicate that DCs in patients with CHB have impaired function leading to insufficient T cell response to HBV, which could be the mechanism responsible for persistent viral infection.

IMMUNOTHERAPY FOR VIRAL HEPATITIS

In chronic HBV infection, strong long-term viral suppression can now be achieved with various nucleoside or nucleotide analogs. However, there are some problems that must be solved in the near future. One of the problems with treatment with nucleos(t)ide analogs is a low rate of HBe seroconversion even after long-term administration in $HBeAg^+$ patients. Moreover, reactivation rate of HBV replication is high in both $HBeAg^+$ and $HBeAg^-$ patients after cessation of treatment, although drug-free viral controls would be better than long-term administration of the drugs in terms of control of medical costs and avoidance of adverse effects of these agents. It could be possible to achieve long-term viral eradication even after cessation of nucleos(t)ide analogs, if viral suppression with nucleos(t)ide analogs could be combined with efficient immunotherapies.

Previous animal studies and human trials in HBV infection are listed in Tables 1 and 2, respectively.

IMMUNOTHERAPEUTIC APPROACHES FOR HBV INFECTION

Immunotherapeutic strategies for CHB include suppres-

Table 2 Immunotherapeutic trials for chronic hepatitis B virus infection in humans

Immunotherapy	Results	Ref.
Peptide vaccination		
A vaccine with HBc18-27 peptide comprised of a T-helper cell epitope and two palmitic acid residues	Low levels of CTL activity were induced but no significant changes in liver biochemistry or viral serology were observed	[52]
Protein vaccination		
PreS2/S (GenHevac B) or S (Recombivax)	HBe/anti-HBe seroconversion in 13% and HBV DNA negativity in 16% of the treated patients	[53]
Intradermal HBsAg vaccine and laimvudine in combination with IL-2	Induction of significant HBV DNA loss in the serum in two of five the treated patients	[54]
Oral administration of HBV envelope proteins (HBsAg + preS1 + preS2)	Induction of histological improvement in 30%, HBeAg negativity in 26.3% and HBsAg-specific T cell proliferation in 78% of the treated patients	[55]
IFN-α-2b monotherapy (9 mo) or IFN-α-2b plus pre-S2/S vaccine	Greater reduction in HBV DNA in patients with combination HBV therapy than those who received IFN-α-2b monotherapy	[56]
The combination with lamivudune and HBsAg vaccine in $HBeAg^+$ cases	No improvement of HBe seroconversion rate in comparison with lamivudine therapy alone	[57]
Combination of lamivudine and HBsAg vaccine	Induction of sustained negativity of HBV DNA in 1/4 of patients	[58]
Combination of lamivudine and HBsAg vaccine	HBV DNA became undetectable in 64% of the patients, and was decreased in the remaining patients	[59]
DNA immunization		
DNA vaccine encoding HBV envelope protein	Induction of an increase in HBV-specific IFN-γ-secreting T cells in nonresponders to conventional therapies, and HBV DNA levels were transiently decreased in 50% of vaccinated patients	[60]
DNA vaccine encoding PreS and S in patients with lamivudine breakthrough	Development of IFN-γ-producing T cells specific for preS or S antigen; Two of 10 patients showed seroconversion to anti-HBe	[61]
DC immunization		
Peripheral blood-derived DCs, activated with GM-CSF and IL-4 pulsed with HBsAg	Both patients with normal and elevated ALT responded equally to DC vaccine and 53% of the patients showed induction of HBeAg negativity	[62]
Activated DCs from PBL with GM-CSF and IL-4, pulsed with two peptides, HBc18-27 and PreS2 44-53	Undetectable HBV DNA was achieved in 46.3% and 3.1% of $HBeAg^-$ and HBeAg+ patients, respectively. ALT normalization was observed in 69% and 30.5% of HBeAg- and HBeAg+ patients, respectively	[63]
Cytokines		
GM-CSF	Safe and tolerable up to 1.0 μg/kg body weight, and induced HBV DNA negativity in 4/8 patients	[64]
Combination therapy with GM-CSF and HBsAg vaccine in HBV carrier children	Significant reduction of serum HBV DNA	[65]
High dose of IL-12 (0.5 μg/kg)	HBV DNA clearance was observed in 25% of the patients	[66]
Combination of IL-12 and lamivudine	Stimulation of T cell response to HBV with IFN-γ production. However, IL-12 was unable to suppress re-elevation of HBV DNA after cessation of lamivudine	[67]
Combination of IL-12 and IL-18	Stimulation of IFN-γ production by CD4+ T cells isolated from peripheral blood in response to HBcAg, and the effect was greater than those observed with either cytokine alone	[68]
α-galactosylceramide	Poorly tolerated and showed no clear suppressive effect on serum HBV DNA or ALT levels	[69]
Tα1		
Combination of Tα1 and IFN-α	No statistically significant differences as compared with IFN-α monotherapy with respect to HBeAg seroconversion, changes in histology, normalization of ALT or loss of HBV DNA	[70]
Tα1 alone	At 12 mo after cessation of therapy, 36.4% of patients treated with 1.6 mg of Tα1 achieved ALT normalization, 15% achieved HBV DNA clearance by transcription-mediated amplification, and 22.8% achieved clearance of HBeAg	[71]
Comparative effect of Tα1 and IFN-α	Tα1 treatment was more effective in achieving ALT normalization and HBV DNA negativity at the end of the follow-up period than IFN-α	[72]
Combination of Tα1 and lamivudine	No any additional antiviral effect compared with lamivudine monotherapy as determined by HBe seroconversion and the emergence of viral breakthrough	[73]
Combination therapy with lamivudine and Tα1	Induction of significantly higher rates of ALT normalization, virological response, and HBeAg seroconversion than lamivudine monotherapy	[74]

HBV: Hepatitis B virus; Tα1: Thymosin α1; IFN: Interferon; ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: Interleukin; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; HBsAg: Hepatitis B surface antigen; HBcAg: Hepatitis B core antigen; PBL: Peripheral blood lymphocytes.

sion of viral replication, stimulation of T cell immune response to hepatitis virus, activation of nonspecific cells, and administration of cytokines with antiviral activity (Tables 1 and 2).

Suppression of viral replication

High viral load has been shown to suppress $CD4^+$ and $CD8^+$ T cells in addition to induction of Treg cells, which could be reversed by antiviral therapy in CHB[75].

Therefore, immunotherapy followed by restoration of virus-specific T cell response with antiviral therapy could be more efficient in CHB.

Stimulation of immune response to HBV

Peptide immunization: A peptide vaccine containing highly immunogenic HBc18-27 has been developed and administered to CHB patients[52], but the results were disappointing because there was no induction of a significant antiviral T cell response.

Protein immunization: In a model of HBV in transgenic mice, vaccine on the base of surface antigen in complete Freund's adjuvant once monthly for 1 year induced reduction in HBV DNA, and the disappearance of HBeAg and HBsAg in most mice treated[41]. Moreover, it is important to note that some mice developed anti-HBs in the sera. However, several human trials with HBsAg vaccine showed limited efficacy if used as monotherapy.

Recently, hepatitis B vaccine containing not only S protein but also preS has been used with increased immunogenicity[53,55], or has been combined with lamivudine or IFN-α[56], leading to potential improvement of clinical efficacy. However, analysis of the T cell epitope hierarchy has indicated that the most important epitope for viral control is HBc18-27, and not the HBsAg epitope in HLA-A2 patients[9], suggesting the necessity to reconsider antigen selection for vaccination that could lead to better viral control.

DNA immunization: Injection of plasmid DNA has been shown to elicit strongly both cellular and humoral immune responses, and is now known to be safe and well-tolerated both in mice and humans. In a model of duck HBV infection, DNA vaccine encoding HBV large envelope and/or core protein was shown to induce reduction in not only viremia but also covalently closed circular DNA (cccDNA) in the liver in one thirds of ducks receiving DNA monotherapy or combination treatment with lamivudine[44]. This finding is encouraging because clearance of cccDNA from the liver is the goal of treatment for HBV infection, but is difficult to achieve using IFN-α or nucleos(t)ide analogs. Clinical trials have also been performed in HBV infection with some encouraging results, which remain to be confirmed by future randomized large-scale trials.

DC immunization: DCs are specialized antigen-presenting cells that can induce strong immune responses in T and B cells. We have previously shown that activated bone-marrow-derived DCs can break CTL tolerance to HBsAg in HBV transgenic mice[45]. Thereafter, several immunotherapies with activated DCs have been applied in both animals and humans. In a recent study performed in HBV transgenic mice, peptide-pulsed DCs were shown to reduce significantly the concentrations of serum HBsAg and HBV DNA[47], indicating therapeutic potential in chronic HBV infection. Recently, DCs treated with peptide inhibitors of IL-10 have been shown to induce strong anti-HCV T cell responses in HCV transgenic mice[76], suggesting a strategy to augment the immunogenic function of DCs. Moreover, when intrahepatic antigen-presenting cells, including DCs, are activated by injection of an anti-CD40 agonistic antibody, HBV replication is inhibited by a noncytopathic mechanism, possibly through production of antiviral cytokines such as TNF-α and IL-12[46]. Although no CTL response against HBV antigens was reported in this study, the *in vivo* activation of DCs could be an alternative way for inducing antiviral immune responses, including possible activation of CTLs against HBV. In humans, injection of activated DCs loaded with HBV peptide or protein has achieved a reduction in HBV DNA level in some patients[62,63]. HBeAg negativity was achieved in more than half of the treated patients in one study[62]. Although preparation of activated and mature DCs incurs financial costs and requires experienced researchers, immunotherapy with DCs is a promising method.

Natural killer T cells: A single injection of α-galactosylceramide abolished HBV replication by activating natural killer (NK) T cells in the liver in HBV transgenic mice[49]. However, α-galactosylceramide was poorly tolerated in humans and showed no clear antiviral effect[69], possibly due to smaller numbers of NKT cells in the human liver than in the mouse liver.

Cytokines and thymosin-1: Cytokines such as IL-12[48] and IL-18[50] have been shown to inhibit HBV replication noncytopathically in HBV transgenic mice. In humans, granulocyte–macrophage colony-stimulating factor[64,65] and IL-12[66,67] have been used for treatment with some antiviral effects. They have been used as monotherapy or in combination with hepatitis B vaccine or lamivudine.

Thymosin (T)α1, a synthetic 28-amino acid peptide, is able to enhance the Thl immune response and also exerts a direct antiviral mechanism of action. It has been used for the treatment of chronic HBV infection in humans[70-73], and has shown some antiviral efficacy. Although antiviral effect by the addition of Tα1 to lamivudine or IFN-α therapy was controversial, a meta-analysis has demonstrated that combination therapy with lamivudine and Tα1 shows significantly higher rates of ALT normalization, virological response, and HBeAg seroconversion as compared with lamivudine monotherapy[74]. It is of note that HBeAg seroconversion rate was 45% in the combination group, which was significantly higher than that with lamivudine monotherapy (15%).

Blockade of immunoinhibitory signals

Recently, there have been several basic attempts to improve the efficacy of immunotherapy. Among these reports, augmentation or restoration of T cell response by blocking the inhibitory signals has been extensively analyzed *in vitro*. It has been demonstrated that exhausted T

cells express not only PD-1, but also CTLA-4[77], CD244[78] or Tim-3[33], and blocking of these molecules in combination could be better than blocking any single molecule to achieve full activation of the exhausted T cells.

CONCLUSION

There have been several attempts to apply immunotherapy for the control of chronic HBV infection, and some of the data are promising. Viral suppression, stimulation of antiviral immune response with cytokines or immunization with peptide, protein, DNA or DCs, and suppression of the immunoinhibitory signals must be combined to achieve desirable antiviral effects, although further studies are required to explore the best protocols and their most efficient combinations.

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