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Review

Advances in immunomodulating therapy of HBV infection

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Abstract

Patients with chronic hepatitis B virus (HBV) infection have a higher risk of developing liver cirrhosis and hepatocellular carcinoma. Interferon- α , lamivudine and adefovir dipivoxil are the three approved treatment for chronic HBV infection and offers the only means of preventing the development of these complications. However, the efficacy of these agents, in terms of loss of Hepatitis B e antigen with or without seroconversion to Hepatitis B e antibody, normalization of serum alanine transaminase levels, loss of serum HBV DNA, and improvement in liver histology can only be achieved in 20-30% of those treated. Long-term treatment with either lamivudine or adefovir dipivoxil can result in the development of drug resistant mutants leading to an increased length of treatment with additional nucleoside analogues. These limitations of the current antiviral therapies underline the need for alternative therapies. Specific and nonspecific immunotherapeutic strategies to restore effective virus-specific T cell responses in those with chronic HBV infection offers an interesting alternative approach. These immunotherapeutic therapies include the adoptive transfer of HBV immunity, pegylated interferon and therapeutic vaccine therapies.

Key words

immunomodulating therapy, HBV infection

Author biography

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1. INTRODUCTION

Hepatitis B virus (HBV) related hepatitis is a necroinflammatory liver disease of variable severity. Most people develop acute hepatitis which is controlled by both humoral and cellular immune responses following acute infection [1]. However, around 2-20% of infected adults and 95% of infected newborns in HBV-endemic areas fail to resolve the infection and subsequently become chronic carriers [2]. Persistent infection is associated with a healthy chronic carrier state in about one-third of persons and with chronic liver disease that can lead to the development of liver cirrhosis and hepatocellular carcinoma in the remaining two-thirds.

The cellular immune response contributes to the elimination of the virus but is also responsible for liver damage caused by the lytic activity of HBV-specific cytotoxic T lymphocytes (CTL) on HBV-infected hepatocytes and by production of inflammatory cytokines [3]. Chronic HBV infection is characterized by an inefficient T helper (Th) cell response to hepatitis B surface antigen (HBsAg) and by a variable Th cell response to the HBV-related antigens such as hepatitis core antigen (HBcAg), and hepatitis e antigen (HBeAg). Increased HBcAg/HBeAg-specific Th cell responses are observed in persons with self-limited HBV-infection, whereas the HBsAg-specific Th cell response is much less vigorous [1]. However, in a proportion of vaccine recipients who have been immunized with plasma-derived or recombinant HBsAg, there is a strong response of the envelope-specific Th response suggesting that differences in antigen load or presentation may influence the strength of the HBsAg-specific T cell response [4-6].

Therapeutic intervention offers the only means of interrupting this progression. The ultimate goals of treatment are to achieve off-treatment sustained suppression of HBV replication and remission of liver disease. Agents currently approved for treatment of chronic HBV infection are divided into two main groups; the immunomodulator, interferon- α (IFN- α), and the nucleoside analogues, lamivudine and adefovir dipivoxil. The immunomodulators act by promoting cytotoxic T cell activity for lysis of infected hepatocytes and by stimulating cytokine production for control of viral replication. Nucleoside analogues on the other hand act by suppressing HBV replication at the level of DNA synthesis, and may also enhance immune clearance of infected hepatocytes [7]. As IFN- α inhibit HBV replication in only 20-40% of persons with chronic HBV [8-10], while the use of nucleoside/nucleotide analogues such as lamivudine and adefovir dipivoxil is limited by the selection of resistant mutated viruses [11,12], there is a need for alternative approaches to treatment which includes specific and non-specific immunotherapeutic strategies in order to enhance or to broaden the defective T cell responses in chronically infected patients. These immunotherapeutic strategies are the adoptive immune transfer, new forms of immunomodulatory agents and therapeutic vaccine therapies.

2. ADOPTIVE TRANSFER OF IMMUNITY

In both animal and human studies, transfer of HBV immune memory from a immune donor through bone marrow transplantation (BMT) or peripheral blood lymphocytes (PBLs) has enabled seroconversion to Hepatitis B surface antibody in HBV naïve recipients [13-16]. Furthermore, clearance of HBsAg has been observed in individual patients with chronic hepatitis B after transplantation of bone marrow from HBV immune donors [17-19]. By studying the largest series of patients, who cleared HBsAg following the engraftment of an HLA-identical bone marrow from a donor with past exposure to HBV, we found that resolution of chronic HBV infection is associated with a transfer of CD4+ T lymphocyte reactivity to HBcAg, rather than to HBV envelope proteins [20]. We have demonstrated that the CD4+ T cells are of donor origin and activation of the memory subset, CD45-RO+ T cells, occurs during the hepatitis flare, which precedes the seroconversion to anti-HBs. These results explain our earlier clinical observation that HBsAg clearance occurs only after adoptive transfer of naturally acquired immunity to HBV (anti-HBs and anti-HBc positive donors) and not in patients who received marrow with a vaccine-induced immunity (anti-HBs alone) [21]. The practical implication of the present findings of successful HBsAg clearance following adoptive transfer of immunity to HBcAg, is that therapeutic immunization of patients with chronic HBV infection should include the HBV nucleocapsid protein (or the core gene for DNA immunization) and aim to induce both HBcAg-specific CD4+ and CD8+ T cell responses.

However, although adoptive transfer is an interesting alternative for the treatment of chronic HBV infection, it is associated with certain risks. The risks of adoptive transfer are those associated with BMT and the absence of immune control that may result in infections, veno-occlusive disease and graft-versus-host disease. Fulminant hepatitis resulting in hepatic failure has also been reported following adoptive transfer of immunity to HBV [22]. Thus, although adoptive transfer of immunity to HBV is a possible approach, it is restricted to the BMT setting and is limited by its potential serious complications.

Adoptive transfer of immunity to HBV has also been reported in patients after liver transplantation [23]. Spontaneous production of anti-HBs is observed in 21 of 50 HBsAg+ patients (42%) receiving lamivudine monophylaxis after liver transplantation. Seroconversion to anti-HBs can be detected in a median of eight days. In those that developed anti-HBs seroconversion, a more rapid clearance of HBsAg is also observed and the predictor of anti-HBs production is an HBV-immune donor, suggesting the possibility of adoptive immunity transfer through a liver graft. The same could be observed in rats after kidney transplantation [24].

3. IMMUNOMODULATORY TREATMENTS OF CHRONIC HEPATITIS B INFECTION

Cytokines

Interferon-gamma, tumour necrosis factor-alpha and interleukin 1-beta has been shown to decrease the secretion of HBV DNA from HB611 cells transfected with HBV DNA in a dose dependent manner [25]. The expression of HBV mRNA is also decreased signifying that these agents can decrease the synthesis of virally encoded components of the HBV virions. The antiviral effect of these agents is postulated to be due to oxidative stress [25].

Interleukin 2 (IL-2)

Clearance of HBsAg with anti-HBs seroconversion has been demonstrated in co-infected patients with human immunodeficiency virus (HIV) [26]. However, similar results could not be reproduced when IL-2 is extended to chronic HBV patients without HIV co-infection. [27]. Further randomized trials with IL-2 versus placebo also did not demonstrate any difference between IL-2 and placebo [28,29]. A randomized trial of combination IL-2 and IFN- α 2b versus IFN- α 2b monotherapy in 37

patients also did not show any effect on serum HBV DNA clearance, HBeAg seroconversion or normalization of serum alanine transaminase (ALT) levels. However, side effects are higher in the combination group [30]. Therefore, there is no strong evidence to demonstrate the effect of IL-2 on chronic HBV infection.

Interleukin-12 (IL-12)

IL-12 is a heterodimeric cytokine produced by antigen-presenting cells that have the ability to induce interferon- γ (IFN- γ) secretion by T and natural killer cells and it can generate normal Th1 responses. As HBV-specific CTL can inhibit HBV replication in the livers of transgenic mice mediated by IFN- γ , there could be a possible therapeutic effect of IL-12 on HBV replication.

A study demonstrating this on HBV transgenic mice is conducted by Cavanaugh et al [31]. The authors are able to demonstrate a dose-dependent antiviral effect with the disappearance of HBV DNA replicative intermediates with IL-12 at doses of 100 ng once a day for three days. At further higher doses, HBV DNA replicative forms were completely abolished. Serum HBV DNA level is also decreased by IL-12 [31].

In another study with recombinant human (rHu) IL-12 on 46 patients with chronic HBV, positive serum HBV DNA level and elevated serum ALT level, a decrease in serum HBV DNA level could be observed at the end of treatment and at 12 weeks after the end of treatment. The decrease in serum HBV DNA level is also dose dependent; 25% with 0.50 $\mu\text{g}/\text{kg}$ body weight (b.w.), 13% with 0.25 $\mu\text{g}/\text{kg}$ b.w. and 7% 0.03 $\mu\text{g}/\text{kg}$ b.w. HBeAg is undetectable in two patients (13.3%) in those who received 0.25 $\mu\text{g}/\text{kg}$ b.w. and in three patients (18.8%) receiving 0.50 $\mu\text{g}/\text{kg}$ b.w [32]. Although IL-12 shows early interesting efficacy on chronic HBV infection, more large randomized controlled studies are needed before it can be recommended as a standard treatment.

Levamisole

One study showing the immunomodulatory effect of levamisole in 25 chronic HBV patients (16 HBeAg+, 9 HBeAg-) is conducted by Krastev et al. in 1999. A decrease in serum HBV DNA level is noted ($p < 0.05$) with HBeAg seroconversion to Hepatitis B e antibody in three (18.8%) HBeAg positive patients. Two patients (8.0%) in this study cleared HBsAg [33].

Oral Immune Regulation (HBV Envelope Proteins)

Oral immune regulation is the induction of immunological hypo- or hyper responsiveness toward specific antigens or, towards other antigens present at the target site [34]. Per oral administration of low doses of HBsAg+preS1+preS2 envelope proteins can induce peripheral immune tolerance and downregulation of anti-HBV immune response has been demonstrated in a murine model [35]. Safadi et al. conducted a study on the safety and efficacy of per oral HBV envelope proteins (HBsAg+preS1+preS2) in 42 chronic HBV patients [36]. A significant decrease in serum HBV DNA level can be observed in 66.6% while histological improvement in liver necroinflammatory score can be seen in 30.0%. Loss of HBeAg occurred in 26.3% and 21.1% had HBeAg to anti-HBe seroconversion. No patients had a loss of HBsAg or anti-HBs seroconversion.

Thymosin α -1

Thymosin α -1 is a 28-amino acid polypeptide isolated from thymosin fraction 5. It is an immunomodulating agent and may enhance clearance of HBV. In vitro studies have shown that thymosin α -1 can accelerate T-cell maturation and antigen recognition. It can also stimulate interferon and cytokine production and the activity of natural killer cell-mediated cytotoxicity [37,38].

Although thymosin α -1 has fewer and milder side effects compared to IFN- α , its clinical efficacy remains inconclusive. One study with 1.6 mg twice weekly subcutaneous thymosin α -1 for six months showed a response rate, defined as clearance of serum HBV DNA and HBeAg, of 40.6% vs. 9.4% in the control group ($p = 0.004$) 18 months after the completion of treatment, although the response rate between the two groups are similar at the end of treatment [39]. In a meta-analysis of 353 patients from five trials, there is no statistically significant difference in the biochemical response although odds ratio for virological response at the end of treatment, six and 12 months after treatment are 0.56 (0.20-1.52), 1.67 (0.83-3.37) and 2.67 (1.25-5.68) respectively. Interestingly, an increase in virological response over time after the discontinuation of thymosin could be observed ($p = 0.02$) [40]. The doses of thymosin α -1 in this meta-analysis are 900 $\mu\text{g}/\text{m}^2$ and 1.6 mg twice weekly respectively for at least 24 weeks.

Combination of low dose lymphoblastoid interferon and thymosin α -1 is evaluated in 15 patients. After 12 months, nine patients (60.0%) responded to treatment, which is defined as negative serum HBV DNA level and normalization of serum ALT level. Forty percent had clearance of HBsAg [41]. The efficacy of thymosin α -1 and IFN- α on HBeAg negative patients has also been shown by Saruc et al. [42]. Fifty-two HBeAg negative chronic HBV patients are nonrandomly assigned to three different groups. Group 1 ($n = 27$) received thymosin α -1 1.6 mg subcutaneously twice a week and IFN- α 2b 10 million units (MU) subcutaneously three times weekly for 26 weeks followed by IFN- α 2b for an additional 26 weeks. Group 2 ($n = 10$) received IFN- α 2b monotherapy for 52 weeks and Group 3 ($n = 15$) received IFN- α 2b and lamivudine for 52 weeks followed by continuous lamivudine. A sustained response, defined as virological and biochemical response six months after completion of therapy, is seen in 74.0% in Group 1, 40.0% in Group 2 and 26.6% in Group 3 ($p = 0.036$). At the end of the study, which is 18 months after the completion of treatment, 71.4% in Group 1, 10.0% in Group 2 and 20.0% in Group 3 had persistent sustained response ($p = 0.0003$) [42]. The results of the meta-analysis and, that of Chien et al. and Saruc et al. suggest that thymosin α -1 may be effective in suppressing viral replication with its effect being delayed until 12 months after the discontinuation of treatment [40,42].

Furthermore, combination of thymosin α -1 and famciclovir for 26 weeks versus famciclovir monotherapy or placebo showed a stronger HBV DNA reduction in the combination group with a 15.6% HBeAg seroconversion (none of the patients in the other two groups had HBeAg seroconversion) [43].

Pegylated interferon alfa

Recently, pegylated interferons have been developed with improved pharmacokinetic profiles and a more acceptable dosing regimen compared with conventional interferon (once-weekly subcutaneous injection). Clinical trials of pegylated interferons have provided encouraging results. In an early, phase II, proof-of-concept trial, peginterferon alfa-2a (40KD) proved more effective than conventional interferon alfa in all efficacy parameters assessed (loss of HBeAg, normalization of serum ALT) [44]. A later study of 266 predominantly Caucasian patients, showed that peginterferon alfa-2b (12KD) plus lamivudine combination therapy was no more effective than peginterferon alfa-2b (12KD) alone [45]. Unfortunately, this study did not have a lamivudine monotherapy

comparator arm. A new phase III study of 537 HBeAg negative, predominantly Asian patients, comparing peginterferon alfa-2a (40KD), either alone or in combination with lamivudine for 72 weeks, showed a higher normalization of serum ALT levels and a higher rate of reduction in serum HBV DNA level less than 10,000 copies/ml in the peginterferon alfa-2a (40 KD) groups (Table 1). Loss of HBsAg with or without anti-HBs seroconversion and ≥ 2 -point reduction in HAI index are also higher in patients receiving peginterferon alfa-2a (40 KD) (Table 1) [46]. New data from a Phase III study of 814 predominantly Asian patients with peginterferon alfa-2a (40KD), either alone or in combination with lamivudine, will be available soon.

Table 1. Results of phase III study with peginterferon alfa-2a (40 kDa) on HBeAg negative Chronic HBV patients.

ALT- alanine transaminase, HBV- hepatitis B virus, HBsAg- hepatitis B surface antigen, anti-HBs- antibody to hepatitis B surface antigen, HBeAg- hepatitis B e antigen.

Therapeutic Vaccine

This is another approach that can be employed to break tolerance and stimulate T-cell immune responses in chronic HBV patients. Immunization with recombinant HBsAg particles

from transgenic mice expressing either HBsAg alone or replicating the virus resulted in a reduction in serum HBsAg levels, loss of HBeAg or even the development of anti-HBs [47,48]. Pilot clinical studies on therapeutic vaccines demonstrated that specific vaccine therapy by standard vaccination could cancel or reduce HBV replication in 50.0% of chronic HBV patients [49,50]. In a controlled study involving either GenHevac B (Pre-S2/S) [Pasteur-Merieux, n=47], Recombivax (S) [Merck, Sharp and Dhome, n=34] or no vaccine (n=37), seroconversion of HBeAg to anti-HBe is seen in 13.3% of vaccinees versus 3.6% of controls after six months of follow-up. After 12 months of follow-up, the HBeAg to anti-HBe seroconversion rate is 18.9% vs. 12.5% between the two groups. None of the patients had a loss of HBsAg [51]. However, in a randomized controlled study by Yalcin et al. on 71 patients with GenHevac B (Pre-S2/S), this vaccine is not significantly associated with a higher HBsAg seroconversion. Only three out of 31 patients (9.7%) who received the vaccine cleared HBsAg with anti-HBs seroconversion. While in the control group, none of the 40 patients cleared HBsAg or developed anti-HBs seroconversion (p=0.079) [52].

Another study used adsorbed hepatitis B vaccine (MEINYU, Meiji Dairies Corporation, Tokyo) in 19 HBsAg patients with detectable serum HBV DNA (vaccinated patients, n=13 and control, n=6). This vaccine contains glycosylated (gp26) and nonglycosylated HBsAg spherical particles (23 nm in diameter) and 4.0% preS2 protein. At the end of the study there is a significant decrease in the serum HBV DNA level in the vaccinated group compared to the control group (p=0.03). However, the proportion of patients with HBsAg clearance and anti-HBs seroconversion is not available [53].

Alum-based vaccines can be used to promote the production of antibodies and a Th2 biased immune response. However, for effective therapeutic vaccination, both humoral and cytotoxic T-cell responses would be required to eradicate infected liver cells. As such, the efficacy of these vaccines can be improved by using adjuvants such as MF59 [54]. In a pilot study using MF59 as an adjuvant, anti-HBs seroconversion is achieved in 11 of 13 patients [55]. CpG DNA, a synthetic oligonucleotide that can stimulate Th1 responses, with production of IL-12 and IFN- γ is another such adjuvant [56]. Transgenic mice study with vaccines using CpG DNA as an adjuvant has been shown to lead to clearance of serum HBsAg and anti-HBs seroconversion, with a downregulation of HBV mRNA production in the liver as well [56].

Another approach with therapeutic vaccines is the use of peptide based T-cell vaccines. Heathcote et al. studied the use of a lipopeptide (CY-1899) containing a T-helper epitope from tetanus toxoid and a CTL epitope from HBV core (amino acids 18-27) in 19 chronic HBV patients [57]. This CTL activity induced by the vaccine, however, is not strong enough to clear the infection. In fact, CTL activity induced by vaccination with the highest dose are 10-fold weaker than the CTL responses induced in healthy volunteers with no HBV exposure [58].

DNA-Based Vaccines

Intramuscular injection of plasmids encoding HBV antigens is another novel approach to vaccination. It can enable the expression of encoded proteins *in vivo*. The DNA vaccines can induce immune responses against antigens synthesized *in vivo* after direct introduction of DNA encoding HBV sequences. Plasmid DNA immunization can induce both humoral immune responses and CD8+ CTL responses [59,60].

Immunization with HBsAg-encoding plasmid DNA, followed by recombinant HBsAg-expressing canarypox as booster in chimpanzees with chronic HBV resulted in a 400-fold decrease in serum HBV DNA level with stable HBsAg levels [61]. Three chimpanzees with chronic HBV immunized with a HBcAg-expressing retroviral vector showed seroconversion from HBeAg to anti-HBe in one chimpanzee. The other two chimpanzees remained HBeAg positive with stable viral load, even though one of them had detectable HBcAg-specific CTL responses [62].

A DNA vaccine against HBV using the PowderJect system has been conducted in healthy volunteers. This system delivers gold particles coated with plasmid DNA directly into the skin cells. This vaccine seems to be safe, well tolerated and is able to produce Th1 helper cell responses. But humoral anti-HBs responses, however, are weak [63].

Theoretically, the use of therapeutic vaccine may offer the greatest therapeutic potential. However, larger scale studies need to be conducted in order to determine not only its efficacy but also its safety and potential adverse effects in humans. A more important question that needs to be addressed is the potential adverse effects that may arise in the event of a hyper-responsiveness of the cytotoxic activity in the HBV infected liver cells.

	Peginterferon alfa-2a (40 KD) (n=177)	Peginterferon alfa-2a (40 KD) plus lamivudine combination (n=179)	Lamivudine (n=181)
Normalization of serum ALT level	59%	60%	44%
Serum HBV DNA <10,000 copies/ml	43%	44%	29%
Loss of HBsAg \pm anti-HBs seroconversion		3.4%	0%
Reduction in HAI by ≥ 2	47%	37%	39%

4. CONCLUSION AND RESEARCH DIRECTION

Monotherapy with nucleoside/nucleotide analogue is unlikely to cure the majority of patients with chronic hepatitis B infection. With the encouraging results being obtained from the use of pegylated interferon α for chronic HBV infection, it is highly likely that it will feature widely in future consensus guideline recommendations. However, it remains to be determined which patient should be treated, for how long and whether combination therapy with other immunological therapy or nucleoside/nucleotide analogues will further enhance its efficacy. A more rational form of therapy should entail the use of HBV-specific immune therapy either in the form of therapeutic vaccine or DNA vaccine. Hopefully, in the future, more research could be conducted to find an immunological curative solution for patients with chronic hepatitis B infection.

Conflict of interest

Dr. Lau has received research support from Roche Pharmaceutical and Gilead Sciences. Dr. Hui: none declared.

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