Immune therapy including dendritic cell based therapy in chronic hepatitis B virus infection

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Abstract

Hepatitis B virus (HBV) infection is a global public health problem. Of the approximately 2 billion people who have been infected worldwide, more than 400 million are chronic carriers of HBV. Considerable numbers of chronic HBV carriers suffer from progressive liver diseases. In addition, all HBV carriers are permanent source of this virus. There is no curative therapy for chronic HBV carrier. Antiviral drugs are recommended for about 10% patients, however, these drugs are costly, have limited efficacy, and possess considerable side effects.

Recent studies have shown that immune responses of the host to the HBV are critically involved at every stage of chronic HBV infection: (1) These influence acquisition of chronic HBV carrier state, (2) They are important in the context of liver damages, (3) Recovery from chronic HBV-related liver diseases is dependent on nature and extent of HBV-specific immune responses. However, induction of adequate levels of HBV-specific immune responses in chronic HBV carriers is difficult. During the last one decade, hepatitis B vaccine has been administrated to chronic HBV carriers as a therapeutic approach (vaccine therapy). The present regimen of vaccine therapy is safe and cheap, but not so effective. A dendritic cell-based therapeutic vaccine has recently been developed for treating chronic HBV infection. In this review, we will discuss about the concept, scientific logics, strategies and techniques of development of HBV-specific immune therapies including vaccine therapy and dendritic cell-based vaccine therapy for treating chronic HBV infection.

Key words: Hepatitis B virus; Dendritic cells; Immune therapy; Vaccine therapy
are costly and a significant proportion of patients with CHB are not eligible for treatment due to various reasons. On the other hand, lamivudine may need life-long usage. Treatment with lamivudine gives rise to emergence of mutant HBV and breakthrough hepatitis in considerable numbers of patients with CHB. These problems related to currently available anti-viral therapies necessitate the development of alternative therapeutic approaches for chronic HBV carriers.

HBV is a non cytopathic virus and the immune responses of the hosts to HBV are important in different aspects of HBV infection. Although the cellular and molecular mechanisms underlying host/HBV interactions are not completely understood, various evidences suggest that host immune responses may have dual role in chronic HBV carriers. In one hand, liver damages in chronic HBV carriers are initiated and maintained by host immune responses. On the other hand, recovery from chronic HBV carrier state is dependent on the intensity and nature of host immune responses. Accordingly, induction or suppression of generalized or polyclonal immune responses in HBV carriers is not likely to have therapeutic potentials. In line of this, it has been shown that although chronic HBV carriers exhibit impaired T helper 1 (Th1) responses, Th1-inducing cytokines (interleukin (IL)-12 and IFN-gamma) possess no notable therapeutic effects in these patients.

Only recently, some investigators have shown functional differences of HBV-specific immune responses and non HBV-specific immune responses in chronic HBV infection. It appears that products of non HBV-specific immune responses are responsible for liver damages. On the other hand, HBV-specific immune responses possess antiviral potentials without inducing liver damage in chronic HBV carriers.

Although this indicates that induction of HBV-specific immune responses in chronic HBV carriers may have therapeutic effect, it is extremely difficult to achieve this in vivo. Indeed, this is a confusing task. All chronic HBV carriers harbor HBV DNA and all HBV-related antigens. However, they do not exhibit adequate levels of HBV-specific immune responses. Initially, it has been assumed that chronic HBV carriers may be tolerant to some HBV-related antigens such as hepatitis B surface antigen (HBsAg). However, recent studies have revealed that HBsAg-specific immune responses are present in chronic HBV carriers, although the magnitude of immune responses may not adequate to clear or suppress the virus in chronic HBV carriers.

### ROLE OF IMMUNE RESPONSES DURING ACQUISITION AND PROGRESSION OF CHRONIC HBV INFECTION AND ALSO DURING RECOVERY

Two billion people of the world exhibit either present or past infection with HBV and about 400 million of them are chronically infected with HBV. This indicates that less than 20% HBV-infected persons become chronic HBV carriers. It is natural to ask why some persons become chronic HBV carriers, whereas, others control the virus after an acute infection. Again, it is not clear why some chronic HBV carriers do not show any evidence of liver diseases, whereas, others suffer from progressive liver diseases like CH, LC and HCC. Finally, it is elusive why some chronic HBV carriers recover spontaneously. Although the exact cellular and molecular mechanisms underlying these are not completely clear, experimental evidences indicate that viral factor alone do not influence the courses of HBV diseases. Acquisition of HBV carrier state, degrees of liver damages in chronic HBV carriers and recovery from HBV carrier state are not dependent on any particular viral factor such as the amounts of the HBV, genotypes of the HBV, mutations at certain region of the HBV or expression of certain proteins of the HBV. Recent investigations have revealed that immune responses of the hosts to HBV may be important in this context (Table 1).

| HBV carriers | Possible causes |
|--------------|-----------------|
| A: Acquisition of chronic State | A: Neonates of HBV-carrier mothers | Inability to induce HBV-specific immunity |
| B: Immune suppressed Individuals | Chronic hepatitis B | Accumulation of non-specific lymphocytes |
| C: Recovery from chronic HBV infection | A: Natural recovery | A: Strong and multispecific |
| B: Acute hepatitis | B: Antiviral therapy effective in immune modulatory phase |
| C: By antiviral drugs |

Vertical transmission of HBV from HBV-infected mothers (especially hepatitis B e antigen-positive) to neonates usually results in chronic HBV carrier state. This may be related to the concept of ‘Neonatal Tolerance’ as described by Burnett et al. On the other hand, this may be due to impaired functional capacities of neonatal antigen-presenting cells.

Some chronic HBV carriers never show any feature of liver diseases during their life time, although they harbor the HBV from their birth day. On the other hand, considerable numbers of chronic HBV carriers exhibit features of necroinflammatory liver diseases. Many of them also suffer from progressive liver diseases such as LC and HCC. HBV is a non-cytopathic virus and it is assumed that HBV-specific HLA-class restricted cytotoxic T lymphocytes (CTLs) act as the principle event determining the intensity of liver damages. However, recent studies indicate that direct destruction of hepatocytes by the CTLs may not the only or principle mechanism of liver damages in patients with CHB. A series of investigations have shown that most of the liver-infiltrating lymphocytes are not HBV-specific. Liver injury could occur as a consequence of large infiltrate of antigen non-specific mononuclear cells. Different types of proinflammatory
cytokines such as IFN-gamma and tumor necrosis factor alpha may be responsible for induction of liver damages because neutralization of these cytokines prevent liver damages in various experimental models of hepatitis\textsuperscript{[14]}

Host immune responses are also important during recovery from chronic HBV carrier state. The HBV-specific immune responses are strong and multispecific in patients with acute hepatitis B who control the virus replication after an acute infection. On the other hand, these are narrowly focused, weak and mono-specific in chronic HBV carriers who are unable to control the virus\textsuperscript{[13]}. This indicates that if proper immune responses against the HBV are initiated after HBV infection, the patients are likely to recover. This is also manifested by the fact that antiviral drugs are mostly effective in immunodulatory phase, but not in immunotolerant phase, of chronic HBV carriers\textsuperscript{[13]}. 

### DEVELOPMENT OF EVIDENCE-BASED IMMUNE THERAPY FOR CHRONIC HBV INFECTION

As described above, immune responses of the hosts to HBV play important roles during acquisition of chronic HBV infection, during progression of liver damages in chronic HBV carriers and also for recovery from chronic HBV carrier state. In this context, it is natural ask what is meant by ‘immune response of the host’ in chronic HBV infection. This is a very complex issue and this review is not primarily intended to give a detail account of cellular and molecular events related to this. Here, we will focus on HBV-related ‘immune responses’ those are relevant for developing immune therapy for chronic HBV carriers.

All chronic HBV carriers studied to date, even the immune competent adults who acquire HBV infection, display infrequent, narrowly-focused, and weak HBV-specific immune responses\textsuperscript{[13]}. The purpose of immune therapy in patients with chronic HBV infection is aimed at induction of strong HBV-specific immune responses \textit{in vivo}.

It has been assumed that HBV-specific CTLs are primarily responsible for clearance of HBV-infected hepatocytes in chronic HBV carriers. CTLs are thought to destroy HBV-infected hepatocytes by cytolytic mechanisms. However, the overall concept regarding CTL and their role in destroying virus-infected cells has been changed not only in the context of HBV infection but also in other viral infections. First, CTL is not the only effector cells capable of destroying HBV-infected hepatocytes. Rather, other cells of both innate and adaptive immune system such as HBV-specific CD4 T cells, natural killer cells, macrophages, and neutrophils may also regulate replication of HBV and destruction of hepatocytes\textsuperscript{[14]}. The next, CTLs are not only committed to destroy HBV-infected cells by directly interacting with hepatocytes. Rather, one of the major functions of CTL is to produce various types of cytokines. Series of experiments by different investigators have shown that cytokines are able to induce destruction of HBV or reduced replication of HBV by a non cytopathic mechanism\textsuperscript{[13,14,16]}

Moreover, both HBV-specific and non HBV-specific immune responses are seen in patients with CHB. Several investigators have shown that HBV-specific immune responses are needed for control of HBV replication. On the other hand, non HBV-specific immune responses may induce destruction of hepatocytes. Patients with CHB express high levels of proinflammatory cytokines and accumulation of mononuclear cells in the liver. Recently, it has been shown that most of these mononuclear cells are non HBV-specific\textsuperscript{[17,18]}

Taken together, these features indicate that induction of immune responses is not the main target of immune therapy for chronic HBV carriers. Rather, induction and maintenance of HBV-specific immune responses may have therapeutic implications. The challenging issue is to accomplish this in patients with chronic HBV infection.

### VACCINE THERAPY: INDUCTION OF HBV-SPECIFIC IMMUNE RESPONSES

As an approach to induce HBV-specific immune responses in chronic HBV carriers, vaccines containing HBsAg and/or other HBV-related antigens have been administered in patients with CHB. Dienstag \textit{et al} have used hepatitis B vaccine in patients with CHB in early 1980s, however, that study has not been regarded as vaccine therapy because the purpose of that investigation was to evaluate induction of anti-HBs in chronic HBV carriers\textsuperscript{[19]}. The first clinical trial of vaccine therapy was done by Pol \textit{et al} in 1994\textsuperscript{[20]}

Subsequently, clinical trials of vaccine therapy have been performed during last one decade (Table 2)\textsuperscript{[21-27]}. Most of these studies were pilot studies. The present regimen of vaccine therapy has been well tolerated in patients with CHB. It has caused reduced HBV replication, seroconversion to anti-HBe and normalization of serum transaminases in some patients with CHB. However, there is no standard protocol of vaccine therapy. Vaccine therapy has mainly been conducted as pilot studies. All investigators have used their own protocol to perform vaccine therapy. Variable doses of HBsAg (10-20 micrograms) have been used. In some clinical trials, only HBsAg-containing vaccines were used, whereas, in others vaccines containing HBsAg plus preS1 and preS2 proteins were used. The numbers of immunization also varied among studies (3-12 times). Most importantly, there are no standard criteria to assess the response of vaccine therapy.

### CAN VACCINE THERAPY STAND THE TASTE OF TIME?

The present regimen of vaccine therapy is unlikely to be an independent therapeutic approach for patients with CHB. A lot of works must be done regarding vaccine therapy. There should be randomized controlled trials of vaccine therapy in different countries. More studies are needed regarding optimum doses of vaccine, compositions of vaccine and numbers of immunization. This therapy is safe for human usage. No side effects of this therapy have yet been published. Vaccines are commercially available and comparatively cheaper. At present, therapeutic efficacy of vaccine therapy is not so satisfactory. But, that is not so important if we consider its future potentials. The concept
of vaccine therapy is unique. Treatment of chronic infection by inducing antigen-specific immune responses deserves attention. There are ample opportunities to improve the present regimen of vaccine therapy.

At present, it appears that the present regimen of vaccine therapy can be used in some specific patients with CHB such as in patients with anti-HBe positive CHB and patients with low levels of HBV replication. The next, vaccine therapy can be used in combination with other antiviral agents. Vaccine therapy can be started after using antiviral drugs for some times in patients with CHB. This may allow better action of this therapy due to decrease of HBV load by antiviral drugs. Again, vaccine therapy can be used first and then antiviral agents can be applied after induction of host immune responses by vaccine therapy. This may be manifested in better therapeutic potential of antiviral agents in CHB patients. We have recently reported a combination therapy of lamivudine and vaccine therapy in patients with CHB. All patients receiving combination therapy became negative for HBV DNA in the sera, whereas, only 56% patients with lamivudine monotherapy lost HBV DNA in the sera within 12 mo. Also, patients with combination therapy did not develop HBV DNA mutation or breakthrough hepatitis.[29].

 DEVELOPMENT OF NEXT GENERATION VACCINE FOR TREATMENT OF CHRONIC HBV INFECTION

Vaccine therapy in patients with CHB was started without adequate discussions regarding its scientific validity and utility. Vaccine therapy was done without paying full attention to common consensuses of immunology and virology. It was surprising to most clinicians why administration of vaccine containing HBsAg in CHB patients will have therapeutic effect because all CHB patients have abundant amounts of HBsAg in the sera and the liver. Moreover, it has been assumed that HBsAg is a tolerogenic antigen in CHB patients. Also the mechanism of action of vaccine therapy in patients with CHB has not been properly analyzed. In general, new therapeutic approaches are usually tested in animal models of human diseases and then these are applied in patients. Vaccine therapy is an exceptional type of therapy. It was first done in patients with CHB without performing preclinical trials in animal models of chronic HBV infection.

In fact, there has been no good animal model of chronic HBV infection. Natural infection of the HBV is limited to some animals only. Lack of an animal model was a major limitation to study immune pathogenesis of chronic HBV infection. During 1980s, HBV transgenic mice (HBV-TM) were produced by microinjecting HBV genome into fertilized eggs of mice[29]. Naturally, there are many differences between HBV-TM and patients with CHB, but HBV-TM represent a suitable immunological animal model of HBV infection. We and others used HBV-TM to develop insights about HBV immunopathogenesis to study various cellular events that can not be investigated directly in human.[29,30].

We used a line of HBV-TM that expressed HBsAg and HBV DNA in the sera. This HBV-TM also expressed HBV-related mRNAs in the liver[31]. HBV-TM did not express antibody to HBsAg (anti-HBs) and HBsAg-specific lymphocytes were not detected in HBV-TM. We showed that HBV-TM were unresponsiveness to HBsAg due to impaired function of antigen-presenting dendritic cells (DCs)[32]. Moreover, the immune response defects of HBV-TM were overcome by activating endogenous DCs of HBV-TM[33,34]. Next, four controlled trials of vaccine therapy were conducted in HBV-TM. HBV-TM were immunized with vaccine containing HBsAg once in a month for 12 times. It was possible to induce both HBsAg-specific immune responses in HBV-TM by administration of vaccine containing HBsAg[35-38]. Study on the mechanism of action of vaccine therapy revealed that vaccine therapy induced HBsAg-specific immune responses by activating autologous DCs[39]. Also, the functions of DCs prior to start of therapy had prognostic value during vaccine therapy[40].

 DEVELOPMENT OF HBsAg-PULSED DC

DCs are essential for induction and maintenance of antigen-specific specific immune responses. DCs recognize antigens at the site of their localization. They internalize antigens and process them at their endosomal compartments. This leads to expression of antigenic peptides at the surface of DCs. These DCs migrate to lymphoid tissues and activate lymphocytes to become either CTL or CD4+ T cells or antibody-producing B cells[39,40].

During vaccine therapy in HBV-TM, vaccine containing HBsAg was administered to HBV carriers to induce HBsAg-specific immune responses. This was done with the assumption that DCs of HBV-TM will recognize injected HBsAg. Subsequently, HBsAg would be captured, internalized, and processed by DCs of HBV-TM. It was assumed that DCs would activate lymphocytes to induce HBsAg-specific immune responses (Figure 1). But, DCs of HBV-TM had impaired functional capacities and thus endogenous DCs of HBV-TM are not potent inducer of HBsAg-specific immune responses.
We postulated that a powerful regimen of vaccine therapy can be developed by loading DCs with HBsAg in vitro (HBsAg-pulsed DCs). If HBsAg-pulsed DCs exhibit markers of activation and maturation, HBsAg-pulsed DCs will be able to directly induce HBsAg-specific immune responses without any influence of endogenous DCs of chronic HBV carriers. Accordingly, we cultured murine spleen DCs and HBsAg together to produce HBsAg-pulsed DCs. HBsAg-pulsed DCs were more immunogenic than unpulsed DCs. Only two injections of HBsAg-pulsed DCs caused negativity of HBsAg from HBV-TM (Figure 2). HBV-TM injected with HBsAg-pulsed DCs also developed anti-HBs in the sera[41]. Administration of HBsAg-pulsed DCs also induced anti-HBs in immunosuppressed HBV-TM[42,43].

**TRANSLATION OF IMMUNOLOGICAL KNOWLEDGE FROM THE BENCHES TO PATIENT’S BEDSIDES**

Wang et al. have first reported about functional impairment of DCs in patients with CHB[44]. Subsequently, we localized HBV DNA and HBV RNA in human blood DCs and also showed low IL-12 production capacity of DCs from CHB patients[45]. Similar data regarding functional defects of DCs of chronic HBV carriers have been reported by other investigators during the last 3 years[46,47]. These studies provided the scientific basis for developing DC-based therapy for patients with CHB.

We followed a very careful protocol to produce HBsAg-pulsed blood DCs for human usage[48]. DCs were enriched from human peripheral blood and cultured with HBsAg to prepare HBsAg-pulsed DCs. We used commercial vaccine as a source of HBsAg. The functional capacities of HBsAg-pulsed DCs were assessed in vitro. Next, HBsAg-pulsed DCs were administered to human volunteers. There was no features of inflammation, abnormal liver and kidney functions in any volunteers. No volunteer showed any features of autoantibody or autoimmune diseases. Administration of HBsAg-pulsed DCs induced anti-HBs antibody in all volunteers, although the levels of antibody varied among volunteers.

Then, a study was conducted to assess the safety of HBsAg-pulsed DCs in patients with CHB. We prepared DCs from peripheral blood of patients with CHB. Human blood DCs were cultured with HBsAg in commercial vaccine to prepare HBsAg-pulsed DCs. HBsAg-pulsed DCs were administered to patients with CHB. No adverse effects were detected in these patients. Recently, a group of researcher from China has used HBsAg-pulsed DCs for treating patients with CHB[49]. The data of their study is highly encouraging because HBsAg-pulsed DCs exhibited both antiviral and immune modulatory capacities in some patients with CHB. Moreover, they have reported that patients of CHB with normal transaminase levels may also be benefited from this therapy. Although the data of this study is highly stimulating, more information is necessary about the source of HBsAg and characterization of HBsAg-pulsed DCs. Also, detailed data about study protocol will allow undertaking more clinical trials of these therapeutic approaches.

**PRESENT AND FUTURE OF IMMUNE THERAPY AGAINST CHRONIC HBV INFECTION WITH AN EMPHASIS OF IT’S UTILITY IN DEVELOPING NATIONS**

The life cycle of HBV, presence of cccDNA of HBV at hepatocytes, and existence of HBV at different extrahepatic sites are major obstacles for eliminating this virus from chronic HBV carriers. It is now apparent that complete eradication of the HBV from chronic HBV carriers may be an unachievable goal[5].

It is also evident that there should be different strategies of HBV control program in developing and developing nations of the world. Less than 10% of total HBV carriers live in developed countries. Moreover, both vertical and horizontal transmissions of HBV have effectively been controlled in most of these countries.

On the other hand, the health care delivery system and economic conditions of developing nations are completely different from those of developed nations, where more than 90% chronic HBV carriers reside. The public health measures are not well developed in most developing nations. Contaminated needles and syringes are used in many facilities, especially in rural areas. There are many chronic HBV carriers among professional blood donors. The numbers of drug abusers are increasing in many of these courtiers. Thus, the numbers of HBV carriers will remain unchanged or increase in most developing nations. China alone harbors about 130 million HBV carriers. Also, there are several million HBV carriers in India.

Regarding treatment, the medical infrastructures of most developed nations allow usage of costly antiviral drugs, regular follow up of treated patients, proper handling of complications and adequate approaches to fight against the mutant HBV. Newer nucleoside analogues are coming to the market. In future, combination therapy with different nucleoside analogues will mainly be used.
in patients with CHB in most developed countries. In developed countries, national liver and gastroenterology associations update therapeutic regimens for chronic HBV infection from time to time.

However, usage of antiviral drugs in patients with chronic HBV infection and proper follow up of these patients are difficult in developing nations. IFNs can be afforded by a very small percentage of patients with CHB in these countries. Most of them are not aware of limitation of antiviral drugs in chronic HBV infection. It is generally told that antiviral drugs are effective in about 30% patients with CHB. However, antiviral drugs are recommended for only 10% patients with chronic HBV infection. These drugs are not recommended for majority of chronic HBV carriers. In most developing nations, there is no appropriate system to follow emergence of mutant HBV following lamivudine therapy.

Vaccine therapy in patients with CHB was first conducted in 1994. However, few randomized controlled trials of vaccine therapy have been conducted till now. This situation is different regarding clinical trials of antiviral drugs in developed countries. Antiviral drugs are developed by multinational companies. In contrary, vaccine therapy for chronic HBV carriers has been developed by scientists. This is a cheap therapeutic option for patients with CHB. It is also safe. Vaccine therapy may be popularized if data of controlled trials show their real potentialities. There should be randomized controlled trials of vaccine therapy in patients with CHB in developing countries.

One of the main purposes of immune therapy is to achieve what antiviral drugs could not accomplish in most chronic HBV carriers. The potentiality of vaccine therapy for treating chronic HBV carriers lies in its concept, not in its present ways of application. Truly speaking, this therapy is still in its infancy. Several clinical and experimental studies should be done to optimize protocols of vaccine therapy. At present, commercial vaccines contain HBsAg and/or other components of surface antigens such as preS and preS2 antigens are used in vaccine therapy against chronic HBV carriers. Vaccines containing hepatitis B core antigen or polymerase antigen can also be used in future for treating patients with CHB. However, the main problem is to get safe and human consumable forms of these antigens.

Till now, antigen-pulsed DCs have been used almost exclusively in patients with cancers. Although there are ample opportunities of using antigen-pulsed DCs in chronic infection, allergy and autoimmunity, very few investigators have tried to materialize this in nonmalignant patients. However, we have learnt many important lesions from DC-based therapy in patients with cancers. In the context of DC-based therapy, there is a need to get proper antigens. The next is about the method of preparation of immunogenic antigen-pulsed DCs. Culture of DCs and antigens may lead to production of immunogenic as well as tolerogenic DCs. Accordingly, it is needed to check the nature of antigen-pulsed DCs before administration those to patients. Again, safety is a major concern when antigen-pulsed DCs are administered to patients with chronic HBV infection. In contrast to patients with cancers, patients with chronic HBV infection are immune competent. Administration of antigen-pulsed DCs may induce autoimmunity in these patients.

At present, clinical trials have been conducting with HBsAg-pulsed DCs. In future, hepatitis B core antigen-pulsed DCs may be used in patients with CHB. The major limitation is to get human grade HBV-related antigens. We used commercial vaccine containing HBsAg to pulse DCs with HBsAg. Chen et al.[49] have used HBsAg-pulsed DCs in patients with CHB, but they have not mention about the source of HBsAg. The availability of GMP standard HBsAg and other HBV-related antigens will contribute significantly for conducting more clinical trials with antigen-pulsed DCs in patients with CHB. A major challenge will be to achieve GMP standard in developing nations for conducting cell-based therapies including DC-based therapy. This issue can be solved by establishing scientific collaboration among developing countries, developed countries and international agency like World Health Organization.

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