PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 May 14; 12(18): 2876-2883
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

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

Sk Md Fazle Akbar, Norio Horiike, Morikazu Onji

Sk Md Fazle Akbar, Norio Horiike, Morikazu Onji, Third Department of Internal Medicine, Ehime University School of Medicine, Ehime 791-0295, Japan
Supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan to Sk. Md. Fazle Akbar, No. C17590651 and Morikazu Onji, No. C17590652
Correspondence to: Dr. Sk. Md. Fazle Akbar, PhD, Third Department of Internal Medicine, Ehime University School of Medicine, To on City, Ehime 791-0295, Japan. akbar@m.ehime-u.ac.jp
Telephone: +81-89-9605308 Fax: +81-89-9605310
Received: 2005-09-28 Accepted: 2005-10-26

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 carriers. 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 administered 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

Akbar SMF, Horiike N, Onji M. Immune therapy including dendritic cell based therapy in chronic hepatitis B virus infection. *World J Gastroenterol* 2006; 12(18): 2876-2883

http://www.wjgnet.com/1007-9327/12/2876.asp

INTRODUCTION

Hepatitis B Virus (HBV) is a hepatotropic, noncytopathic, DNA virus which can cause acute as well as chronic infection. Chronic HBV infection remains a significant public health problem world-wide. It is estimated that there are at least 400 million HBV carriers in the world and that up to one million die annually due to HBV-associated liver diseases. Although the majority of the chronic HBV carriers may remain in an inactive phase associated with low viral replication and histological remission, a significant proportion will develop chronic hepatitis B (CHB), which is characterized by the presence of high viral replication, and chronic necroinflammation of the liver. Patients with CHB develop serious complications like liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Indeed, all chronic HBV carriers are at higher risk for HCC when compared with general population; the risk becomes much higher when LC develops. In addition, all chronic HBV carriers (inactive HBV carrier, patients with CHB [both treated and untreated], patients with LC and HCC) are permanent and living sources of HBV. Presence of million of chronic HBV carrier is practically responsible for the incidence of acute and fulminant hepatitis due to HBV[1].

No specific treatment is provided to 90% of chronic HBV carrier ('do nothing strategy'). Antiviral drugs are recommended only for patients with high HBV replication and necroinflammatory liver diseases. These drugs include type-1 interferons (IFN) and nucleosides analogues such as lamivudine and adefovir. It is difficult to assess the real therapeutic impact of antiviral drugs in chronic HBV carriers. On one hand, these drugs are recommended in some selected patients with CHB. On the other hand, the response varies considerably depending on virological and immunological statuses of the patients. Finally, many terms such as complete responder, biological responder, virological responder, transient responder, and sustained responder are used to assess responses to antiviral drugs. In general, antiviral drugs exhibit some beneficial effects in about 20%-30% of treated patients. However, IFNs

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[2,3]. 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[4,5]. 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[6].

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[7,8].

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[9,10]

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[1,2]. Recent investigations have revealed that immune responses of the hosts to HBV may be important in this context (Table 1). 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*[11]. On the other hand, this may be due to impaired functional capacities of neonatal antigen-presenting cells[12].

Table 1 Role of immune responses in chronic HBV carrier

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

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[13]. 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 factoralpha may be responsible for induction of liver damages because neutralization of these cytokines prevent liver damages in various experimental models of hepatitis[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[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 immunetolerant phase, of chronic HBV carriers[15].

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[13]. The purpose of immune therapy in patients with chronic HBV infection is aimed at induction of strong HBV-specific immune responses *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[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 sown that cytokines are able to induce destruction of HBV or reduced replication of HBV by a non cytopathic mechanism[5,14,16].

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[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 *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[19]. The first clinical trial of vaccine therapy was done by Pol *et al* in 1994[20]. Subsequently, clinical trials of vaccine therapy have been performed during last one decade (Table 2)[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[28].

Table 2 Vaccine therapy in patients with chronic hepatitis B. The criteria of response to this therapy have been determined by individual investigators

Protocol	Response	Reference
HBsAg/pre S2, 3 times	Responders: 12 of 32 (38%)	Pol *et al*[20]
HBsAg/anti-HBs vaccine; 3 times	Responders: 9 of 15 (60%)	Wen *et al*[21]
PreS2/S, 3 times (controlled trial)	Responders: 18.8%	Pol *et al*[22]
HBsAg; 12 times	Responders (1 yr: 55%, 2 yr: 77%)	Horiike *et al*[23]
PreS2 vaccine; 3 times	Responders: 60%	Senturk *et al*[24]
S/pre S2; 6 times	Decreased HBV DNA	Ren *et al*[25]
HBsAg/preS2; 3 times	Immune tolerant phage: no significant change	Dikici *et al*[26]
PreS2; 3 times	anti-HBs; 3 of 31 cases	Yalcin *et al*[27]
Combination of lamivudine and HB vaccine	HBV DNA negativation in all 15 cases at 1 yr	Horiike *et al*[28]

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 those 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 over come 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 humoral and cellular 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[37]. Also, the functions of DCs prior to start of therapy had prognostic value during vaccine therapy[38].

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 year[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.

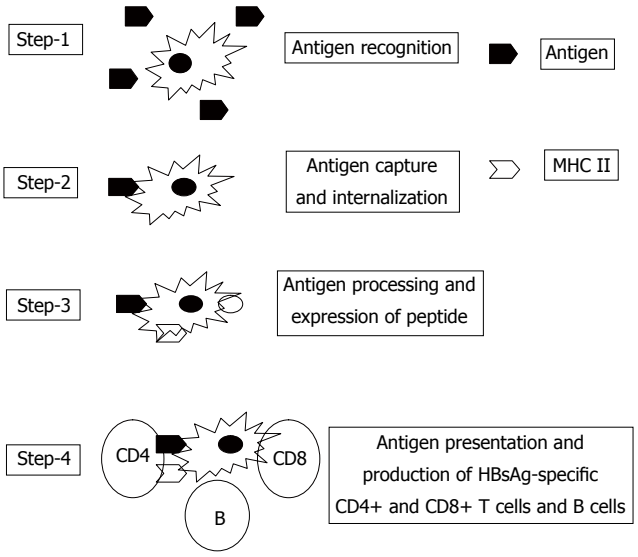

Figure 1 DC/antigen interactions have been shown in 4 steps. Three of these steps are accomplished *in vitro* (step 1-3) when HBsAg-pulsed DCs are prepared.

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[2].

It is also evident that there should be different strategies of HBV control program in developed 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

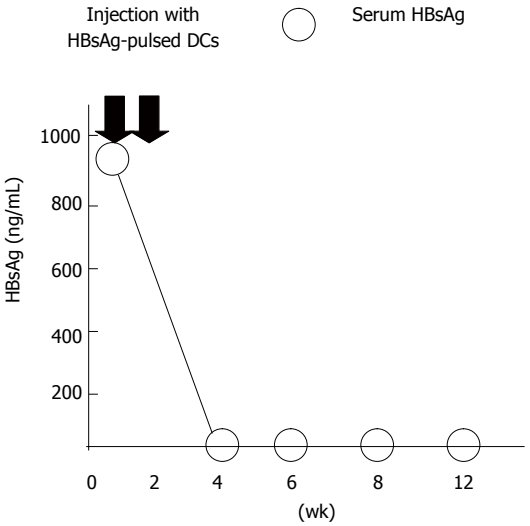

Figure 2 Potent immune modulatory capacity of HBsAg-pulsed DCs in HBV transgenic mice. HBV transgenic mice expressed HBsAg in the sera but not anti-HBs. After two injections of HBsAg-pulsed DCs, these mice became negative for HBsAg in the sera.

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.

REFERENCES

1 **Fattovich G**. Natural history and prognosis of hepatitis B. *Semin Liver Dis* 2003; **23:** 47-58
2 **Fung SK**, Lok AS. Update on viral hepatitis in 2004. *Curr Opin Gastroenterol* 2005; **21:** 300-307
3 **Esteban R**. Management of chronic hepatitis B: an overview. *Semin Liver Dis* 2002; **22** Suppl 1: 1-6
4 **Bertoletti A**, Maini MK. Protection or damage: a dual role for the virus-specific cytotoxic T lymphocyte response in hepatitis B and C infection? *Curr Opin Microbiol* 2000; **3:** 387-392
5 **Rehermann B**. Immune responses in hepatitis B virus infection. *Semin Liver Dis* 2003; **23:** 21-38
6 **Sprengers D,** Janssen HL. Immunomodulatory therapy for chronic hepatitis B virus infection. *Fundam Clin Pharmacol* 2005; **19:** 17-26
7 **Maini MK**, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, Williams R, Vergani D,

Naoumov NV, Ferrari C, Bertoletti A. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000; **191:** 1269-1280

8 **Reignat S**, Webster GJ, Brown D, Ogg GS, King A, Seneviratne SL, Dusheiko G, Williams R, Maini MK, Bertoletti A. Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. *J Exp Med* 2002; **195:** 1089-1101

9 **Maruyama T**, McLachlan A, Iino S, Koike K, Kurokawa K, Milich DR. The serology of chronic hepatitis B infection revisited. *J Clin Invest* 1993; **91**: 2586-2595

10 **Milich DR**, Sallberg M, Maruyama T. The humoral immune response in acute and chronic hepatitis B virus infection. *Springer Semin Immunopathol* 1995; **17**:149-166

11 **Burnet FM**, Stone JD, Edey M. The failure of antibody production in the chick embryo. *Aust J Exp Biol Med Sci* 1950; **28:** 291-297

12 **Matzinger P.** Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994; **12:** 991-1045

13 **Chisari FV**, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; **13**: 29-60

14 **Webster G**, Bertoletti A. Quantity and quality of virus-specific CD8 cell response: relevance to the design of a therapeutic vaccine for chronic HBV infection. *Mol Immunol* 2001; **38**: 467-473

15 **Boni C**, Bertoletti A, Penna A, Cavalli A, Pilli M, Urbani S, Scognamiglio P, Boehme R, Panebianco R, Fiaccadori F, Ferrari C. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. *J Clin Invest* 1998; **102:** 968-975

16 **Guidotti LG**, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001;**19:** 65-91

17 **Bertoletti A**, Sette A, Chisari FV, Penna A, Levrero M, De Carli M, Fiaccadori F, Ferrari C: Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. *Nature* 1994; **369:** 407-410

18 **Maini MK**, Boni C, Ogg GS, King AS, Reignat S, Lee CK, Larrubia JR, Webster GJ, McMichael AJ, Ferrari C, Williams R, Vergani D, Bertoletti A. Direct ex vivo analysis of hepatitis B virus-specific CD8(+) T cells associated with the control of infection. *Gastroenterology* 1999; **117:** 1386-1396

19 **Dienstag JL**, Stevens CE, Bhan AK, Szmuness W. Hepatitis B vaccine administered to chronic carriers of hepatitis b surface antigen. *Ann Intern Med* 1982; **96:** 575-579

20 **Pol S**, Driss F, Michel ML, Nalpas B, Berthelot P, Brechot C. Specific vaccine therapy in chronic hepatitis B infection. *Lancet* 1994; **344:** 342

21 **Wen YM,** Wu XH, Hu DC, Zhang QP, Guo SQ. Hepatitis B vaccine and anti-HBs complex as approach for vaccine therapy. *Lancet* 1995; **345:** 1575-1576

22 **Pol S,** Nalpas B, Driss F, Michel ML, Tiollais P, Denis J, Brécho C. Efficacy and limitations of a specific immunotherapy in chronic hepatitis B. *J Hepatol* 2001; **34:** 917-921

23 **Horiike N**, Md Fazle Akbar S, Ninomiya T, Abe M, Michitaka K, Onji M. Activation and maturation of antigen-presenting dendritic cells during vaccine therapy in patients with chronic hepatitis due to hepatitis B virus. *Hepatol Res* 2002; **23:** 38-47

24 **Senturk H**, Tabak F, Akdogan M, Erdem L, Mert A, Ozaras R, Sander E, Ozbay G, Badur S. Therapeutic vaccination in chronic hepatitis B. *J Gastroenterol Hepatol* 2002; **17:** 72-76

25 **Ren F**, Hino K, Yamaguchi Y, Funatsuki K, Hayashi A, Ishiko H, Furutani M, Yamasaki T, Korenaga K, Yamashita S, Konishi T, Okita K. Cytokine-dependent anti-viral role of CD4-positive T cells in therapeutic vaccination against chronic hepatitis B viral infection. *J Med Virol* 2003; **71:** 376-384

26 **Dikici B**, Kalayci AG, Ozgenc F, Bosnak M, Davutoglu M, Ece A, Ozkan T, Ozeke T, Yagci RV, Haspolat K. Therapeutic vaccination in the immunotolerant phase of children with chronic hepatitis B infection. *Pediatr Infect Dis J* 2003; **22:** 345-349

27 **Yalcin K**, Acar M, Degertekin H. Specific hepatitis B vaccine therapy in inactive HBsAg carriers: a randomized controlled trial. *Infection* 2003; **31:** 221-225

28 **Kumagi T,** Horiike N, Michitaka K, Hasebe A, Kawai K, Tokumoto Y, Nakanishi S, Furukawa S, Hiasa Y, Matsui H, Kurose K, Matsuura B, Onji M. Recent clinical features of Wilson' s disease with hepatic presentation. *J Gastroenterol* 2004; **39:** 1165-1169

29 **Chisari FV**, Pinkert CA, Milich DR, Filippi P, McLachlan A, Palmiter RD, Brinster RL. A transgenic mouse model of the chronic hepatitis B surface antigen carrier state. *Science* 1985; **230:** 1157-1160

30 **Akbar SK**, Onji M. Hepatitis B virus (HBV)-transgenic mice as an investigative tool to study immunopathology during HBV infection. *Int J Exp Pathol* 1998; **79:** 279-291

31 **Araki K**, Miyazaki J, Hino O, Tomita N, Chisaka O, Matsubara K, Yamamura K. Expression and replication of hepatitis B virus genome in transgenic mice. *Proc Natl Acad Sci U S A* 1989; **86:** 207-211

32 **Akbar SM,** Onji M, Inaba K, Yamamura K, Ohta Y. Low responsiveness of hepatitis B virus-transgenic mice in antibody response to T-cell-dependent antigen: defect in antigen-presenting activity of dendritic cells. *Immunology* 1993; **78:** 468-475

33 **Akbar SM**, Inaba K, Onji M. Upregulation of MHC class II antigen on dendritic cells from hepatitis B virus transgenic mice by interferon-gamma: abrogation of immune response defect to a T-cell-dependent antigen. *Immunology* 1996; **87:** 519-527

34 **Kurose K,** Akbar SM, Yamamoto K, Onji M. Production of antibody to hepatitis B surface antigen (anti-HBs) by murine hepatitis B virus carriers: neonatal tolerance versus antigen presentation by dendritic cells. *Immunology* 1997; **92:** 494-500

35 **Akbar SM**, Kajino K, Tanimoto K, Kurose K, Masumoto T, Michitaka K, Horiike N, Onji M. Placebo-controlled trial of vaccination with hepatitis B virus surface antigen in hepatitis B virus transgenic mice. *J Hepatol* 1997; **26:** 131-137

36 **Akbar SM**, Yamamoto K, Abe M, Ninomiya T, Tanimoto K, Masumoto T, Michitaka K, Horiike N, Onji M. Potent synergistic effect of sho-saiko-to, a herbal medicine, during vaccine therapy in a murine model of hepatitis B virus carrier. *Eur J Clin Invest* 1999; **29:** 786-792

37 **Akbar SM**, Abe M, Masumoto T, Horiike N, Onji M. Mechanism of action of vaccine therapy in murine hepatitis B virus carriers: vaccine-induced activation of antigen presenting dendritic cells. *J Hepatol* 1999; **30:** 755-764

38 **Akbar SK**, Horiike N, Onji M. Prognostic importance of antigen-presenting dendritic cells during vaccine therapy in a murine hepatitis B virus carrier. *Immunology* 1999; **96:** 98-108

39 **Banchereau J**, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; **392:** 245-252

40 **Morikazu Onji**. Dendritic cells in clinics. Springer, 2004: 9-40

41 **Akbar SM,** Furukawa S, Hasebe A, Horiike N, Michitaka K, Onji M. Production and efficacy of a dendritic cell-based therapeutic vaccine for murine chronic hepatitis B virus carrierer. *Int J Mol Med* 2004; **14:** 295-299

42 **Furukawa S**, Akbar SM, Hasebe A, Horiike N, Onji M. Induction and maintenance of anti-HBs in immunosuppressed murine hepatitis B virus carriers by a novel vaccination approach: implications for use in hepatitis B virus-infected subjects with liver transplantation. *J Gastroenterol* 2004; **39:** 851-858

43 **Furukawa S,** Akbar SM, Hasebe A, Horiike N, Onji M. Production of hepatitis B surface antigen-pulsed dendritic cells from immunosuppressed murine hepatitis B virus carrier: evaluation of immunogenicity of antigen-pulsed dendritic cells in vivo. *Immunobiology* 2004; **209:** 551-557

44 **Wang FS**, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY. Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2001; **7:** 537-541

45 **Arima S**, Akbar SM, Michitaka K, Horiike N, Nuriya H, Kohara M, Onji M. Impaired function of antigen-presenting dendritic cells in patients with chronic hepatitis B: localization of HBV DNA and HBV RNA in blood DC by in situ hybridization. *Int J Mol Med* 2003; **11:** 169-174

46 **Beckebaum S**, Cicinnati VR, Zhang X, Ferencik S, Frilling A, Grosse-Wilde H, Broelsch CE, Gerken G. Hepatitis B virus-induced defect of monocyte-derived dendritic cells leads to impaired T helper type 1 response in vitro: mechanisms for viral immune escape. *Immunology* 2003; **109:** 487-495

47 **van der Molen RG,** Sprengers D, Binda RS, de Jong EC, Niesters HG, Kusters JG, Kwekkeboom J, Janssen HL. Functional impairment of myeloid and plasmacytoid dendritic cells of patients with chronic hepatitis B. *Hepatology* 2004; **40**: 738-746
48 **Fazle Akbar SM**, Furukawa S, Onji M, Murata Y, Niya T, Kanno S, Murakami H, Horiike N. Safety and efficacy of hepatitis B surface antigen-pulsed dendritic cells in human volunteers. *Hepatol Res* 2004; **29:** 136-141
49 **Chen M,** Li YG, Zhang DZ, Wang ZY, Zeng WQ, Shi XF, Guo Y, Guo SH, Ren H. Therapeutic effect of autologous dendritic cell vaccine on patients with chronic hepatitis B: a clinical study. *World J Gastroenterol* 2005; **11:** 1806-1808

S- Editor Wang J **L- Editor** Kumar M **E- Editor** Cao L