Immune therapy for hepatitis B

Sheikh Mohammad Fazle Akbar[1], Mamun Al-Mahtab[2], Md. Sakilur Islam Khan[3], Ruksana Raihan[4], Ananta Shrestha[5]

[1]Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan; [2]Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh; [3]Department of Anatomy and Embryology, Ehime University Graduate School of Medicine, Ehime, Japan; [4]Department of Virology, AIMST University, Semeling, Bedong, Kedah, Malaysia; [5]Department of Hepatology, The Liver Clinic, Liver Foundation, Kathmandu, Nepal

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Correspondence to: Sheikh Mohammad Fazle Akbar, MD, PhD. Principal Investigator, Department of Medical Sciences, Toshiba General Hospital, Higashi Oi 6-3-22, Shinagawa, Tokyo 140-8522, Japan. Email: sheikh.akbar@po.toshiba.co.jp; sheikhmohammadfazle@gmail.com.

Abstract: Although several antiviral drugs are now available for treatment of patients with chronic hepatitis B (CHB), sustained off-treatment clinical responses and containment of CHB-related complications are not achieved in majority of CHB patients by antiviral therapy. In addition, use of these drugs is endowed with substantial long term risk of viral resistance and drug toxicity. The infinite treatment regimens of antiviral drugs for CHB patients are also costly and usually unbearable by most patients of developing and resource-constrained countries. Taken together, there is a pressing need to develop new and innovative therapeutic approaches for CHB patients. Immune therapy seems to be an alternate therapeutic approach for CHB patients because impaired or distorted or diminished immune responses have been detected in most of these patients. Also, investigators have shown that restoration or induction of proper types of immune responses may have therapeutic implications in CHB. Various immunomodulatory agents have been used to treat patients with CHB around the world and the outcomes of these clinical trials show that the properties of immune modulators and nature and designing of immune therapeutic regimens seem to be highly relevant in the context of treatment of CHB patients. In this review, the general properties and specific features of immune therapy for CHB have been discussed for developing the guidelines of effective regimens of immune therapy for CHB.

Keywords: Chronic hepatitis B (CHB); immune therapy; HBV antigen-specific; polyclonal immune modulators; pathogenic immunity; therapeutic immunity

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Hepatitis B virus (HBV) and prevailing therapeutic approaches

Natural course of HBV

HBV infects humans and higher primates and is the prototype of the Hepadnaviridae family. The natural course of HBV infection differs among individuals and is dependent on multiple viral and host-related factors. Epidemiological data indicate that of the 2 billion HBV-infected individuals worldwide, 80–90% (~1.7 billion at least) exhibit controlled viral replication with no or minimum liver damage (1). Conversely, 240–370 million HBV-infected individuals experience persistent HBV replication [assessed by expression of hepatitis B surface antigen (HBsAg) in the sera], and ~20% of them express ongoing HBV replication as well as progressive liver damages [patients with chronic hepatitis B (CHB) (1,2)]. CHB patients are at greater risk of developing HBV-related complications, like liver failure, liver cirrhosis (LC) and hepatocellular carcinoma (HCC), with 0.6–1.2 million

individuals dying from these complications each year (3).

With considerable information about the viral life cycle, epidemiology, immunology, pathogenesis, and complications of HBV, further progression of HBV has drastically been reduced by implementing various public health measures (4). In addition, protective vaccines against HBV are available for last three decades and millions of healthy persons have been protected from HBV infection by prophylactic vaccination (5). Even then, several million healthy and HBV-uninfected people of the world are infected with new HBV each year in different parts of the world, especially in developing countries (6). This is because of the fact that about 240–370 million chronic HBV-infected patients act as permanent and living reservoir of HBV and they transmit the virus to healthy individuals by various means. In addition about 20% chronic HBV-infected patients are prone to develop complications like LC and HCC. Taken together, proper management and treatment of CHB patients is a challenge of our time to reduce new HBV infection and also to contain HBV-related complications like LC and HCC.

Therapy of chronic HBV infection

Several antiviral drugs have been developed to treat patients with CHB during the last three decades (compiled in other sections of this issue) (7-10). Beginning in the 1980s, type-1 interferon (IFN), which has immunomodulatory and antiviral effects, has been used to treat patients with CHB. Subsequently, pegylated forms of IFN emerged as more effective and patient-friendly treatment options for treatment of CHB. In the 1990s, nucleoside analogs (NAs) that are capable of directly blocking HBV replication became available to treat CHB patients. Over time, more efficacious and safer NAs have been developed. These drugs are capable of (I) reducing or eliminating HBV DNA in sera; (II) inducing hepatitis B e antigen (HBeAg) negativity; (III) causing seroconversion to antibody to HBeAg (anti-HBe); (IV) reducing or negativity of circulating HBsAg; (V) inducing production of antibodies to HBsAg (anti-HBs); (VI) ameliorating liver damage, and delaying progression to complications (such as LC and HCC) in some, but not all, patients with CHB (11-14). However, long-term follow up revealed that treatment-induced HBeAg seroconversion with suppressed viral replication is not sustainable in considerable numbers of CHB patients. Also, loss of HBsAg with sustained negativity of HBV DNA became a rarely achieved endpoint by antiviral drug therapy in CHB patients. Most striking features indicated that HBV persists in hepatocytes even after HBsAg clearance in CHB patients, as covalently closed circular DNA (cccDNA) and these cccDNA can act as template for HBV replication when they receive an opportunity to replicate due to alteration of life style or intake of immune suppressive drugs for treatment of other morbidities (15). Thus, aspirations and hopes versus confusions and frustrations have been prevailing about IFNs and NAs for treatment of CHB in early 21st century. In fact, some well-planned, meta-analysis of these drugs revealed that IFNs and NAs are capable of improving the intermediate parameters of HBV infection; however, they may not affect the final clinical outcomes of CHB patients (16,17). Especially these drugs have some inherent limitations in developing and resource-constrained countries of the world due to poorly-developed health care delivery system of these countries (18).

Alternate therapeutic approaches for CHB patients

As the long-term therapeutic efficacy of available antiviral drugs (IFNs and NAs) could not be documented in majority of CHB patients, it is natural to ask why IFNs and NAs are not effective therapeutic approaches in CHB patients as both of these drugs are endowed with antiviral and immunomodulatory activities. Although several molecular and cellular mechanisms regarding therapeutic efficacies of IFN and NA are yet to be clarified in details, the following facts can be considered for the limited therapeutic properties of ongoing antiviral drugs in CHB:

(I) HBV replication in CHB patients produces replicating HBV DNA, cccDNA, and extra-hepatic HBV DNA. Most antiviral drugs for CHB are capable of reducing or completely blocking replication of replicating HBV. However, they are not so effective to eliminate cccDNA from HBV-infected hepatocytes (19,20). On the other hand, cccDNA may act as a template for the replication of HBV DNA. Thus, antiviral drug-induced HBV DNA negativity in sera provides little information about real antiviral potentiality of these drugs as conventional assay system only check replicating HBV DNA;

(II) HBV is a non-cytopathic virus, and its direct role on liver damage has not been elucidated in animal models or CHB patients. Although multiple HBV transgenic mice (HBV TM) lines containing different levels of HBV DNA, HBsAg, and HBeAg have been developed, none experienced liver

damage (21-23). In addition, the levels of HBV DNA or HBsAg or HBeAg or HBV genotypes do not correlate with the extent of liver damage in CHB patients (24-26). This has been shown by epidemiological data as variable levels of HBsAg, HBeAg, anti-HBe, and diverse HBV genotypes have been detected in both patients with: (i) CHB with liver damages and (ii) inactive HBV carrier with almost no liver damage. Thus, a direct role of HBV and viral products during induction and maintenance of liver damages and complications could not be substantiated in CHB. Thus it may be postulated that in addition to a direct role of HBV and its products, there are other factors that may be inherently related to induction and maintenance of liver damages in CHB patients;

(III) Since the levels of HBV DNA, or its antigens, do not correlate with the extents of liver damages in CHB patients, investigations have focused on the role of host factors in this context. Circumstantial and experimental data have suggested that the nature of host immunity may play a role in HBV control and also in HBV-induced liver damages (27-29). Host immunity is dichotomous during CHB infection, altering between pathogenic and protective/therapeutic states. Understanding such states—and the control thereof—is crucial for developing therapies against CHB, as well as for preventing HBV-related complications and deaths.

Taken together, a new field of treatment and management of CHB patients surfaced in late 1980s that was directed to manipulate host immunity of CHB patients by immune modulators; immune therapy.

Immune therapy for CHB

Immune therapy in animal models of chronic HBV infection and preclinical trials

To assess the therapeutic potentials of different types of immune modulators (both HBV antigen non-specific and HBV antigen-specific), duck hepatitis B virus (DHBV)-infected ducks, HBV-infected woodchucks, and HBV-infected chimpanzees have been used as animal models of chronic HBV infection. From mid-1980s, HBV TM were produced by microinjecting HBV genome in fertilized eggs of mice and different lines of HBV TM expressed different levels of HBV DNA, Dane particles, HBsAg, and HBeAg. The availability of HBV TM allowed dissection of different cellular and molecular events during chronic HBV infection and therapies.

Studies provided important information about immune therapy in chronic HBV infection. Induction and activation of non HBV-specific immunity in animal models of HBV infection revealed diverse therapeutic efficacy of immune therapy by these agents. Toll-like receptor (TLR) agonists, cytokines and immune modulators reduced HBV DNA and induced HBV-specific immunity in HBV TM and DHBV-infected ducks (30-32). On the other hand, liver damages were documented when cells of innate immunity were activated in these animals (33-35). Thus, confusions prevailed about the clinical implications of non-antigen-specific immune therapy in animal models of chronic HBV infection.

On the other hand, HBV antigen-specific agents mostly exhibited potent therapeutic effects in animal models of chronic HBV infections. HBsAg-based vaccines have shown HBV DNA negativity and seroconversion to anti-HBs in HBV TM (36,37). HBsAg-expressing DNA vaccines and combination of DNA vaccines with antiviral drugs have induced reduction or clearance of DHBV DNA and clearance of duck HBsAg (38,39). Immunizations of woodchucks with HBsAg-based vaccines or combination of vaccines and antiviral agents have also shown anti-HBs response and significant reductions of viral load (40). However, HBsAg-based immunization did not exhibit proper therapeutic effects in HBV-infected chimpanzees (41). Inspired by the impact of HBsAg-based vaccine in HBV TM, HBV-infected duck and HBV-infected woodchucks, cell-based vaccines were used in different animal models of HBV infection. In most cases, antigen-presenting dendritic cells (DCs) were used as an adjuvant or vehicle to stimulate immunocytes of HBV carriers. Accumulated data have shown that DC-based therapeutic vaccines represented better therapeutic options for treating HBV-infected HBV TM compared to the effects of only antigen-based therapeutic vaccines (37,42-46).

Immune therapy in patients with CHB

Non-antigen-specific immune therapeutic approaches in patients with CHB

Logic and ethical basis

Decreased levels of cytokine, impaired functions of HBV-specific immunocytes, lower levels of natural killer cells, dysfunctional antigen-presenting DC and increased activities of immune suppressor cells have been shown by various

researchers in patients with CHB (47-51). This provided an impression that the decreased immune responses of CHB patients may contribute to viral persistence and liver damages. These facts and the inspirable outcomes of immune therapeutic approaches in animal models of HBV provided rationale of immune therapy for CHB patients.

Clinical trials

A wide range of polyclonal immune modulators that include interleukin (IL)-2, IL-12, granulocyte-macrophage colony stimulating factor (GM-CSF), levamisole, thymus humoral factor-gamma 2, alpha galactosylceramide, propagermanium, liver extract, and thymosin-alpha 1 have been used in CHB patients as non-antigen-specific immune modulators. Some trials indicated that cytokines, chemokines and growth factors seems to have therapeutic efficacy in patients with CHB to down regulate HBV DNA levels and containing the extent of liver damages. However, sustained effects of these agents could not be shown. In addition, major concerns have been reported about safety and inefficacy of these agents in CHB patients. It was found that neither a biologically active but non-toxic dose of 300,000 U of IL-2, nor a toxic dose of 1.0 million U of IL-2 resulted in sustained clearance of HBeAg in CHB patients (52). Also, notable therapeutic efficacy of IL-2 was not found in patients with CHB by Artillo *et al.* (53). In fact, considerable reservation remains about the safety of IL-12 in CHB patients because 3 of 46 patients were withdrawn from therapy prematurely due to adverse events (54). Also, follow up data using GM-CSF in CHB is lacking although this agent induced altered cytokine profile in these patients (55). Ruiz-Moreno *et al.* have shown that levamisole and IFN are neither safe nor efficacious in their cohort (56). Even a combination of thymus humoral factor and IFN could induce HBV DNA negativity in only one third patients (57). Woltman *et al.* could not find any notable therapeutic effect of alpha galactosylceramide in patients with CHB (58). Hirayama *et al.* indicated that propagermanium may be an alternative therapeutic approach for CHB, however, no follow up data of their trial is available to substantiate their claim (59). Iino *et al.* showed that thymosin alpha-1 may be an effective therapeutic agent in Japanese patients with CHB (60), however, Yang *et al.* did not find any additional benefit of this drug compared to IFN monotherapy (61). Taken together, most of these agents induced upregulation of host immunity but failed to attain sustained control of HBV replication and liver damages of CHB patients. Moreover, it is really difficult to assess the real impact of these drugs as there have been no follow up study after end of treatment. Also, almost nothing has been noted about mechanism of action of polyclonal immune modulators in CHB patients. Also, there is paucity of information about phase III clinical trial that should compare the efficacy of these agents with NAs and IFNs.

However, recent studies have exposed some new and novel means of non-antigen-specific immune therapeutic approaches in patients with CHB. The antiviral function of peripheral HBV-specific T cells can be increased in patients with CHB B by blocking the interaction of programmed death (PD)-1 with its ligand PD-L1 (62).

In fact, it is extremely difficult to assess the scientific merits of these clinical trials. Most of these studies were conducted as pilot studies. Phase III clinical trial and proper follow up data about the role of non-antigen-specific immune modulators is mostly unavailable. Thus, little has been exposed regarding sustainability of antiviral and liver protection by these agents. In addition, adverse effects were documented in CHB patients during therapy with non-antigen-specific immune modulators. Also, mechanisms of action of non-antigen-specific immune modulators have not been elucidated. At present, it is elusive if non antigen-specific immune modulators can be a new and innovative therapeutic regimen for CHB patients, however, more studies and alteration of protocols may yield important information about the role of these agents in CHB patients.

HBV antigen-specific immune therapy in CHB

HBsAg-based vaccine therapy in CHB

Due to insignificant therapeutic potential and considerable reservation about the safety of most non-antigen-specific immune modulators in CHB patients, investigators have been assessing for novel and alternative immunotherapeutic approaches for these patients. Initially it has been assumed that CHB patients are tolerant to the stimulation of HBV-related antigens. However, studies in 1990s and onward have revealed that HBV-specific immune therapy may have significant therapeutic implications in CHB patients as CHB patients controlling HBV replication and containing liver damages expressed significantly higher levels of HBV antigen-specific immunocytes compared to those who could not control HBV replication and contain liver damages (32,63). Thus, a new field of immunotherapy was exposed in clinical hepatology in which HBV-related antigens were used for treating CHB patients. Although HBV expresses different antigens, HBsAg-based immune therapy was mainly accomplished in CHB patients. There are several factors underlying this initiative. The first, HBsAg is available in human consumable forms as a prophylactic vaccine from

early 1980s. The next, HBsAg-based vaccine is regarded as one of the safest vaccines around the world. It is also able to induce HBsAg-specific cellular and humoral immunity.

Based on these realities, Pol *et al.* first used a HBsAg-based vaccine for therapeutic purpose in CHB patients in 1994 (64). Their study showed that HBsAg-based vaccine therapy induced reduction of HBV DNA, HBeAg seronegativity and anti-HBe seroconversion in some CHB patients. Subsequently, different studies were done to assess the safety and efficacy of HBsAg-based vaccine therapy in CHB. Some studies showed that HBsAg-based vaccination was endowed with antiviral and liver protecting potentials in CHB patients, whereas, others could not find any notable therapeutic benefits of HBsAg-based vaccination in CHB (64-66). However, vaccine therapy with HBsAg was safe for CHB patients. In fact, most of these studies were accomplished as pilot studies and considerable heterogeneity prevailed among studies. Different types of HBsAg-based vaccines (some containing only HBsAg, while others contained HBsAg with preS1 and preS2 antigens) were used in different clinical trials. The dose of vaccines also showed considerable variation. The number of injections throughout vaccination series also varied (3–12 times) substantially among studies. Finally, the evaluation of the therapeutic efficacy of HBsAg-based vaccine therapy was done from the point of view of individual investigators. The effect of the vaccine on HBV replication was assessed by some, whereas, others checked HBeAg negativity or anti-HBe seroconversion. The effects of HBsAg-based vaccination on ALT levels were evaluated by others. As a matter of fact, none has checked the antiviral and immune modulatory potentialities of HBsAg-based vaccine during follow up time. Thus, it remained elusive if the effects of vaccine therapy were transient or sustained.

Additional tips in HBsAg-based therapeutic vaccination in CHB patients

Attempts were made to improve the therapeutic potentials of HBsAg-based vaccine therapy in CHB patients. The nature of antigen and adjuvants, dose of vaccine, and duration of therapy underwent considerable alterations during the last 18 years. Wen and her group used an antigen-antibody complex vaccine containing HBsAg and anti-HBs as a therapeutic vaccine (67), whereas others used a HBsAg-based vaccine with antiviral agents or other immune modulators to increase the therapeutic potential of these vaccines (68-72). Also, a DNA-based vaccine expressing HBsAg was used in CHB patients (73). With the advent of cell-based therapy for chronic infection and cancer in the late 1990s, some investigators loaded DC with HBsAg to produce HBsAg-pulsed DCs and used HBsAg-pulsed DCs were used as therapeutic vaccines in CHB patients (74-77). Some clinical trials inspired optimisms about the clinical utility of these therapeutic regimens; however, sustained effects of immune therapy could not be substantiated in CHB patients by HBsAg-based vaccines. Finally, a well-planned, multicenter, randomized controlled trial revealed that even a combination therapy containing both a HBsAg-based vaccine and an antiviral agent did not represent a better therapeutic approach for CHB patients (78).

HBcAg-based vaccine therapy in patients with CHB

The study of Vandepapelière *et al.* supported the concept of antigen-based immunotherapy in CHB patients, however, they suggested that only HBsAg-based vaccine may not be an ideal candidate of therapeutic vaccine; rather attention should be focused on a HBcAg-based therapeutic vaccine for treating CHB patients. The concept of using HBcAg as a therapeutic vaccine also received scientific support because patients with CHB who control HBV replication and contain progressive liver damage harbor significantly higher numbers of HBcAg-specific cytotoxic T lymphocytes (CTLs) in the liver compared to patients with CHB who are unable to control HBV replication and liver damage (32,63). In 1990s, Heathcote *et al.* used a HBcAg-based epitope vaccine to treat CHB patients (79). Livingston *et al.* showed that a vaccine based on HBcAg epitope failed to induce appropriate T helper response in CHB patients (80). However, follow up studies have not been shown and the clinical implications of HBcAg-based vaccine therapy in CHB patients remain elusive.

HBsAg/HBcAg-based immune therapy in CHB

When the immune responses of CHB patients were analyzed, it was evident that adequate levels of both HBsAg- and HBcAg-specific immune responses are essential for effective control of HBV replication and containment of liver damage in these patients (32,63). HBsAg-specific humoral immunity may be important for blocking HBV replication in the peripheral blood. On the other hand, HBcAg-specific cellular immune responses in the liver are important for controlling HBV in the liver and containment of liver damage. Thus, it seems that an effective regimen of immunotherapy may be developed for CHB patients if adequate levels of immune responses to both HBsAg and HBcAg are induced in CHB patients. Recently, a human consumable HBsAg/HBcAg vaccine has been used in HBV TM and the study showed that a combined vaccine

containing both HBsAg and HBcAg represents a potent therapeutic agent in HBV TM compared to only HBsAg-based vaccines (42,43). Inspired by the role of HBsAg/HBcAg-based vaccine in HBV TM, a phase I/II clinical trial was accomplished with this vaccine in normal volunteers and patients with CHB (81,82). Al-Mahtab *et al.* have shown that HBsAg/HBcAg-based therapeutic vaccine was safe for all CHB patients in their cohort that was administered via the nasal route and parenteral route (82). Administration of the HBsAg/HBcAg-based vaccine also induced sustained negativity of HBV DNA in 50% patients with CHB (82). Also, persistent normalization of ALT was recorded in all patients in their study (82). This study has been followed by a phase III clinical trial by the same group in which 76 patients received HBsAg/HBcAg-based therapeutic vaccine. The clinical outcome of these patients has been compared with 75 patients receiving pegylated IFN for 48 weeks (registered; ClinicalTrials.gov; NCT01374308). Preliminary data has shown that HBsAg/HBcAg-based therapeutic vaccine seems to be better than pegylated IFN in the context of antiviral action, liver protection, and progression of liver fibrosis (83).

Designing effective regimen of immune therapy for CHB patients

It has now mostly been accepted that the immune response of CHB patients is neither diminished nor increased. It seems to be distorted. Various parameters of host immunity exhibit marked heterogeneity in CHB patients. In this context, the target of immune therapy for CHB should not aim to positively or negatively regulate host immunity. Instead, the nature of immunity of CHB patients should be clarified in more details. Also, more insights should be explored about 'protective immunity' versus 'pathogenic immunity' in CHB patients. The purpose of immune therapy in CHB should be to induce 'protective immunity' and also to suppress 'pathogenic immunity'.

It seems that non antigen-specific immunity may be detrimental to the patients and concerns remain about its safety and efficacy, whereas, antigen-specific immunity may have therapeutic potentialities in CHB. However, these represent a simple explanation of a very complex issue about immune therapy of CHB patients. In fact, both innate (provided by polyclonal and non-antigen-specific immune modulators) and adaptive immunity (induced by HBV antigen-specific immune therapeutic agents) may have therapeutic implications in CHB patients. In addition, innate immunity acts as a bridge for induction of adaptive immunity. The purpose of immunotherapy for CHB patients is not only to induce an immune response. The main purpose is to evaluate whether the immune responses induced by immunotherapy translate into sustained control of HBV replication and long-term containment of liver damage. In fact, immune therapy for CHB is at their infancy and profound works are required to have proper understandings about the host immunity and their regulation in the liver and also in lymphoid tissues. It is necessary to assess the role of induced immunity by immune therapy on cccDNA. The fundamental characteristics of the immunity induced by immunotherapy versus immune induction by natural immunity during the different phases of HBV infection should be properly evaluated. Also, there is a need to assess if therapeutic approaches would be a supplementary therapeutic option or an independent novel approach for CHB therapy.

Retrieving information from transgenic mouse model of chronic HBV infection has limitations in terms of lack of liver damages in these mice. Therapeutic strategies to control HBV replication designed using the mouse model may not be effective in CHB patients because of the different pathological processes involved. Additionally, the hepatic microenvironments differ between CHB patients and HBV TM. Alternative animal models that exhibit HBV replication and liver damage may provide further insight into the scope and limitations of immunotherapy in CHB patients. These may also facilitate evaluation of the mechanisms of pathogenesis, which cannot be investigated in humans due to ethical and technical concerns.

The future of immune therapy for CHB patients depends on development of animal models with HBV replication with liver damages and also on understandings on mechanisms of immunity of CHB patients. Also, clinical trials with safer and efficacious immune therapeutic agents would aid to develop proper insights about designing of immune therapy for CHB patients.

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