Antiviral Research 96 (2012) 59–64

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Strong and multi-antigen specific immunity by hepatitis B core antigen (HBcAg)-based vaccines in a murine model of chronic hepatitis B: HBcAg is a candidate for a therapeutic vaccine against hepatitis B virus

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ARTICLE INFO

Article history:
Received 11 April 2012
Revised 24 July 2012
Accepted 24 July 2012
Available online 1 August 2012

Keywords:
Chronic hepatitis B
Immune therapy
Hepatitis B core antigen
Dendritic cells

ABSTRACT

Experimental evidence suggests that hepatitis B core antigen (HBcAg)-specific cytotoxic T lymphocytes (CTL) are essential for the control of hepatitis B virus (HBV) replication and prevention of liver damage in patients with chronic hepatitis B (CHB). However, most immune therapeutic approaches in CHB patients have been accomplished with hepatitis B surface antigen (HBsAg)-based prophylactic vaccines with unsatisfactory clinical outcomes. In this study, we prepared HBsAg-pulsed dendritic cells (DC) and HBcAg-pulsed DC by culturing spleen DC from HBV transgenic mice (HBV TM) and evaluated the immunomodulatory capabilities of these antigens, which may serve as a better therapy for CHB. The kinetics of HBsAg, antibody levels against HBsAg (anti-HBs), proliferation of HBsAg- and HBcAg-specific lymphocytes, production of antigen-specific CTL, and activation of endogenous DC were compared between HBV TM vaccinated with either HBsAg- or HBcAg-pulsed DC. Vaccination with HBsAg-pulsed DC induced HBsAg-specific immunity, but failed to induce HBcAg-specific immunity in HBV TM. However, immunization of HBV TM with HBcAg-pulsed DC resulted in: (1) HBsAg negativity, (2) production of anti-HBs, and (3) development of HBsAg- and HBcAg-specific T cells and CTL in the spleen and the liver. Additionally, significantly higher levels of activated endogenous DC were detected in HBV TM immunized with HBcAg-pulsed DC compared to HBsAg-pulsed DC ($p < 0.05$). The capacity of HBcAg to modulate both HBsAg- and HBcAg-specific immunity in HBV TM, and activation of endogenous DC in HBV TM without inducing liver damage suggests that HBcAg should be an integral component of the therapeutic vaccine against CHB.

1. Introduction

Despite the considerable information regarding the viral life cycle, epidemiology, immunology, pathogenesis and prevention of hepatitis B virus (HBV), there has been a lack of significant developments in treating patients with chronic hepatitis B (CHB). Several antiviral drugs have been developed for treating CHB patients during the last three decades. However, controversy remains about their therapeutic efficacy. A systemic review of the National Institutes of Health (NIH) Consensus Development Conference, which assessed all randomized clinical trials on antiviral drugs in CHB patients from 1989 to 2008, revealed that antiviral drug treatment did not improve the clinical outcomes and all intermediate outcomes in CHB patients in any credible randomized-controlled trial (Shamliyan et al., 2009; Wilt et al., 2008). However, others have shown that these drugs could block or delay the progression of liver disease in CHB patients (Liaw, 2009; Lin et al., 1999). Although it is difficult to determine the underlying causes of these discrepancies, as different investigators used different criteria in their therapeutic evaluations, it is generally accepted that an ongoing treatment regimen for CHB with antiviral drugs is not satisfactory, and has low efficacy and considerable adverse effects. In addition, it is now clear that antiviral drugs possess poor immunomodulatory capabilities, which may be responsible for their ineffective control of HBV replication and inadequate prevention of liver damage in CHB (Lok and McMahon, 2007; Liaw and Chu, 2009).

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http://dx.doi.org/10.1016/j.antiviral.2012.07.011

Clinical and experimental evidence suggests that the replication of HBV DNA and progression of liver damage is under control in many CHB patients, even those not receiving any antiviral drug therapy. The magnitude and nature of host immunity to HBV is important in regulating these pathological events in CHB. In support of this concept, Maini et al. (2000) demonstrated that CHB patients that are capable of controlling HBV replication and liver damage harbor higher frequencies of hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg)-specific cytotoxic T lymphocytes (CTL) compared to those that express high levels of HBV and have progressive liver damage. Taken together, it appears that the restoration of host immune responses to HBV-related antigens may have therapeutic implications in CHB patients.

Based on these observations, polyclonal immunomodulators, such as cytokines, growth factors, and other immune mediators, were used in CHB patients. However, they had limited therapeutic efficacy and considerable side effects in CHB patients (Sprengers and Janssen, 2005). Subsequently, an antigen-specific immunotherapeutic approach, or vaccine therapy, was developed for CHB patients, which used commercially available prophylactic hepatitis B (HB) vaccines for treating CHB patients. Different investigators used different types of vaccines with different immunization protocols, and thus, it is difficult to assess the real therapeutic implications of vaccine therapy in CHB patients (Wang et al., 2010; Hoa et al., 2009; Pol et al., 2001). Indeed, it appears that the HBsAg-based vaccine may not be an effective immunotherapeutic approach in CHB. A well-planned clinical trial in 80 patients with CHB used a HBsAg-based vaccine in combination with another antiviral drug also failed to exhibit substantial therapeutic effect (Vandepapelière et al., 2007). Conversely, Heathcote et al. (1999) used a HBcAg epitope-based vaccine in CHB patients and achieved moderate therapeutic effects. Recently, Luo et al. (2010) reported that antigen-pulsed dendritic cells (DC) containing epitope of HBsAg and HBcAg had therapeutic effects in hepatitis B e antigen (HBeAg)-negative patients, but not in HBeAg-positive patients.

These clinical trials with HBsAg- and HBcAg-based vaccines have raised more questions than solutions regarding immune therapy for CHB patients, as the mechanisms of action of HBsAg- or HBcAg-based vaccine in CHB have not yet been explored. However, most cellular and molecular events following vaccination with either HBsAg or HBcAg could not be evaluated in CHB patients due to ethics, safety, technical, and procedural limitations.

To develop proper insights about immunogenecity of HBsAg- or HBcAg-based therapeutic vaccines in CHB, the role of DC in adaptive immunity has been examined. DC, the most potent antigen-presenting cells, are responsible for processing and presenting antigens for induction of antigen-specific immune responses in normal conditions as well as in the immune tolerance state (Steinman and Banchereau, 2007). Studies have shown that the phenotypes and functions of DC are distorted in chronic HBV infections (van der Molen et al., 2004). One way to circumvent immune tolerance state is to produce antigen-pulsed DC and use them as a vaccine. In fact, cancer antigen-pulsed DC and HBsAg-pulsed DC have been used to induce cancer-specific immunity and HBsAg-specific immunity in cancer patients and CHB patients, respectively, when antigen-specific immune responses could not be properly induced by only cancer antigen or HBsAg (Banchereau and Palucka, 2005; Steinman and Banchereau, 2007; Akbar et al., 2010a).

The present preclinical study assessed the immunomodulatory mechanisms of HBsAg and HBcAg in a murine model of HBV, specifically HBV transgenic mice (TM). After immunizing HBV TM with antigen-pulsed DC, the immune responses of HBsAg-pulsed DC or HBcAg-pulsed DC were compared in the spleen and liver. This study may provide further insight into developing an immune therapy for CHB patients.

2. Methods

2.1. Mice

HBV TM (official designation, 1.2HB-BS10) were prepared by microinjecting the complete genome of HBV plus 619 bp of HBV DNA into the fertilized eggs of C57BL/6 mice. HBV TM are known to express HBV DNA and mRNAs of 3.5, 2.1, and 0.8 kbp of HBV in the liver (Araki et al., 1989). HBV DNA were also detected in the liver, and HBsAg was found in the sera of all HBV TM. Eight-week-old male C57BL/6 mice were purchased from Nihon Clea (Tokyo, Japan). Mice were housed in polycarbonate cages in our laboratory facilities, and maintained in a temperature- and humidity-controlled room (23 ± 1 °C) with a 12-h light/dark cycle. All mice received humane care, and the study protocol was approved by the Ethics Committee of the Graduate School of Medicine, Ehime University, Japan. Eight-week-old C3H/He mice (Nihon Clea) were used in an allogenic mixed leukocyte reaction (MLR).

2.2. Detection of HBV-related markers

HBsAg levels and antibodies against HBsAg (anti-HBs) in sera were estimated with a chemiluminescence enzyme immunoassay (Special Reference Laboratory, Tokyo, Japan) and expressed as IU/ml and mIU/ml, respectively, as previously described (Akbar et al., 2010b).

2.3. Isolation of T lymphocytes, B lymphocytes, and DC

We have previously described in detail the methodology for isolating spleen cells and liver nonparenchymal cells (NPCs) (Akbar et al., 2010b; Chen et al., 2011; Yoshida et al., 2010). To produce a single cell suspension from the spleen, spleens were cut into pieces and passed through a 40-μm-pore nylon filter (BD Falcon, Durham, NC, USA). The resulting cells were collected and suspended in culture medium containing RPMI 1640 (Iwaki, Osaka, Japan) with 10% fetal calf serum (Filtron PTY Ltd., Brooklyn, Australia).

To retrieve liver NPCs, liver tissues were cut into pieces, homogenized, passed through 70-μm-pore steel meshes (Morimoto Yakuhin Co., Matsuyama, Japan), and suspended in 35% percoll (Sigma Chemical, St. Louis, MO, USA). After centrifugation for 15 min at $450 \times g$ at room temperature, a high-density cell pellet was collected and suspended in culture medium.

T lymphocytes were isolated from the spleen single cell suspension or liver NPC by a negative selection column method using a mouse pan T isolation kit (Miltenyi Biotec, Bergish Gladbach, Germany), according to the manufacturer's directions (Chen et al., 2011; Yoshida et al., 2010).

DC were isolated from single cell suspensions of spleen and liver NPC using a density column (specific gravity 1.082), plastic adherence, re-culturing on plastic surface, and depletion of macrophages and lymphocytes or via positive selection of $CD11c^+$ cells with flow cytometry, as described (Akbar et al., 2010b, Chen et al., 2011).

2.4. Preparation of antigen-pulsed DC for immunizing HBV TM

HBsAg and HBcAg were purchased from Tokyo Institute of Immunology (Tokyo, Japan). Murine antigen-pulsed DC were prepared based on data from preliminary studies and according to our previous report (Akbar et al., 2010b; Miyake et al., 2010). Briefly, spleen DC were cultured with phosphate buffered solution (PBS) (unpulsed DC) or pyruvate dehydrogenase complex (PDC,