Biochemical and Biophysical Research Communications 450 (2014) 61–66

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Soluble CD40 ligand-activated B cells from patients with chronic hepatitis B virus infection as antigen presenting cells to induce hepatitis B virus specific cytotoxic T lymphocytes

Yong Liu [a,1], Rui Huang [b,1], Yali Xiong [c], Qi Zhao [c], Guangmei Chen [d], Juan Xia [c], Chao Wu [c,*]

[a] Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing 210008, China
[b] Department of Infectious Diseases, Nanjing Drum Tower Hospital Clinical College of Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210008, China
[c] Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing 210008, China
[d] Department of Infectious Diseases, The Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210008, China

ARTICLE INFO

Article history:
Received 25 April 2014
Available online 24 May 2014

Keywords:
Hepatitis B
B cells
Soluble CD40 ligand
Cytotoxic T lymphocytes
Antigen presenting cells

ABSTRACT

Chronic hepatitis B virus (HBV) infection is the result of an inadequate antiviral immune response to the virus. In this study, we aimed to investigate whether the soluble CD40 ligand-activated B (CD40-B) cells could present antigen and induce specific cytotoxic T lymphocytes (CTLs) in patients with chronic HBV infection. We observed that after activated by sCD40L, the expression of CD80, CD86, major histocompatibility complex (MHC) I and II molecules on the CD40-B cells was significantly increased. Cytometry and fluorescence microscopy showed that more than 41.34% CD40-B cells were loaded by the HBcAg peptide. Furthermore, after been activated and HBcAg18–27 antigen peptide pulsed, B cells obtained from patients with chronic HBV infection could induce HBcAg18–27 specific CTLs in vitro. Taken together, our results show that B cells from patients with chronic HBV infection can be activated by sCD40L and may function as antigen presenting cells and induce HBV-specific CTLs.

1. Introduction

Hepatitis B virus (HBV) infection is a global public health challenge. Approximately 350–400 million people are chronically infected by this virus [1,2]. Chronic hepatitis B (CHB) is potential to progress to severe clinical outcomes such as cirrhosis, hepatocellular carcinoma (HCC) and even death [1].

The treatment of CHB has improved considerably over the past two decades. Potent antiviral drugs such as nucleoside/nucleotide analogs (e.g., entecavir or tenofovir) and pegylated interferon alpha are now available in the treatment of chronic HBV infection. However, existing treatment is rarely curative, with ongoing problems including viral resistance, intolerance and toxicity [3]. In addition, these drugs rarely result in the long-term immunological control of HBV infection. Therefore, novel therapeutic approaches are urgently needed. In recent years, attention has been focused on the development of an immunotherapy for viral control [4,5].

It is well known the main cause of viral persistence during HBV infection is an inadequate antiviral immune response to the viral antigens [6–8]. Therefore, it represents a promising strategy to protect against HBV infection that induced specific cytotoxic T lymphocytes (CTLs) responses to HBV [9,10]. Specific CTLs were introduced by efficient antigen presentation, thus antigen presenting cells (APCs) is critical for the process. Dendritic cells (DCs) are the most prominent APCs. Many studies have demonstrated that activated DCs could evoke a higher CTLs response to hepatitis B core antigen (HBcAg) in vivo [11–14]. However, several significant disadvantages have limited the clinical applications of DC vaccine. DCs are rare in the peripheral blood and cannot be expanded without laborious and expensive efforts ex vivo [15]. Therefore, investigating the ability of other more abundant APC types to induce a CD8+ T cell response might help to design better vaccination strategy to HBV.

Abbreviations: APCs, antigen presenting cells; CD40L, CD40 ligand; CD40-B, CD40L-activated B; CHB, chronic hepatitis B; CTLs, cytotoxic T lymphocytes; DCs, dendritic cells; HBcAg, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; MHC, major histocompatibility complex; PBMCs, peripheral blood mononuclear cells; sCD40L, soluble CD40 ligand.

* Corresponding author. Address: Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, 321 Zhongshan Road, Nanjing 210008, China. Fax: +86 25 83307115.
E-mail address: dr.wu@nju.edu.cn (C. Wu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.05.080
0006-291X/

B cells have been shown to act as APCs and they represent a promising alternative source of potent APCs for cellular immunotherapy [16]. It has been reported that CD40 ligand (CD40L) stimulated B cells could serve as effective tumor APC in the clinical setting [17]. CD40L-activated B cells (CD40-B cells) are a promising alternative to DCs as professional APCs for immunotherapy [16].

CD40L belongs to the tumor necrosis factor superfamily [18] and plays a key role in B–T-cell interactions. CD40L is mainly expressed on activated CD4 T cells and it interacts with the receptor CD40 expressed on B cells and DCs [19]. CD40–CD40L interactions play an important role in regulating both cellular immune and humoral responses. CD40L stimulates CD40+ B cells to express a number of accessory molecules such as CD80, CD86 which are important in the process of T-cell activation [20]. Furthermore, CD40L can enhance the ability of normal B cells to act as APCs [21,22]. In this study, we investigated whether B cells could be activated by recombinant human soluble CD40L (sCD40L) and present HBcAg peptide of HBV to T cells and induce HBcAg-specific CTLs.

2. Materials and methods

2.1. Blood samples

Nine patients with chronic HBV infection and three healthy donors were enrolled in this study, and all of them were HLA*0201 positive. Patients with chronic HBV infection were defined as a positive hepatitis B surface antigen (HBsAg) for at least 6 months and without evidence of cirrhosis, HCC and co-infection with hepatitis C virus, hepatitis D virus, hepatitis G virus or human immunodeficiency virus. All the HBV DNA levels of the nine enrolled patients were higher than 1×10^5 IU/ml. Three healthy individuals with positive anti-HBs were enrolled as control group. The patients and healthy donors gave the consent and all the experiments were approved by the Ethics Committee of Nanjing Drum Tower Hospital, Nanjing University Medical School, in accordance with guidelines of the Nation Health and Medical Research Council of China.

At least 30 ml peripheral blood was obtained by venipuncture from 9 enrolled patients and 3 healthy individuals. The peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-density centrifugation, and then B lymphocytes were selected by anti-CD20 monoclonal antibody conjugated with magnetic beads (Miltenyi Canada). Approximately 3×10^7 PBMCs were acquired, and at least 1×10^6 B cells were purified from the PBMCs. The rest PBMCs were cultured in RPMI 1640 with 10% human AB serum and recombinant human (rh) IL-2 (10 ng/ml, R&D Systems, Minneapolis, USA).

2.2. Activation of B cells by sCD40L

B cells were cultured in wells of 6-well plates (1×10^5 cells in 4 ml/well) in iscoves modified Dulbecco medium (IMDM) (Gibco BRL, Carlsbad, USA) culture medium with 10% human AB serum, rh IL-4 (2 ng/ml, R&D Systems, Minneapolis, USA), and rh sCD40L (2 μg/ml, R&D Systems, Minneapolis, USA), and all cells were cultured at 37 °C in 5% CO_2 cell incubator for 4 days. B cells without sCD40L were cultured in the same medium served as controls. The culture was maintained by replacing half medium with the same medium every 2 days, in which rh IL-4 and sCD40L were freshly added.

2.3. Assay for cell surface molecules

Activated B cells with sCD40L were analyzed by flow cytometry to detect the cell surface molecules, including CD86, CD80, major histocompatibility complex (MHC) classes I and II. In brief, B cells were incubated with anti-CD19-APC, anti-CD86-FITC, anti-CD86-PE, anti-MHC-II-FITC and anti-MHC-I-PE for 20 min, negative isotype control staining reactions were in parallel performed with a saturating concentration of irrelevant mouse IgG1-FITC and IgG1-PE, all the antibodies were purchased from BD Biosciences (BD PharMingen, San Diego, CA). FACS analysis was performed on the BD FACSCanto flow cytometer. B cells were gate on CD19-positive cells for analyzing the cell surface molecules.

2.4. HBcAg18–27 antigen peptide loaded

HBV derived peptides were used for peptide pulsing study as previously described [23,24] with modifications. The HLA-A*0201-binding peptide of HBV core 18–27 (HBcAg18–27, Phe-Leu-Pro-Ser-Asp-Phe-Phe-Pro-Ser-Val, FLPSDFFPSV), a confirmed HLA-A 2.1-restricted CTL epitope and derived from the HBV core protein [25], was obtained from Sangon (Shanghai, China). After 4 days culture, B cells were co-cultured with HBcAg18–27 peptide (50 μg/ml) for 16 h, then cells were collected, washed 2 times by RPMI1640 culture medium. B cells were also incubated with FITC-conjugated peptide under the same condition for detecting the results of peptide loaded. The cells were performed immediately on the BD FACSCanto flow cytometer for detecting the ratio of B cells specific binding of HBV core peptide and observed by fluorescence microscope (Zeiss, Goettingen, Germany) for determining the position of HBV core peptide in B cells.

2.5. HBcAg18–27 specific cytotoxic T lymphocytes

After loading HBcAg18–27 peptide, the activated B cells were harvested from culture, washed 2 times, and resuspended in IMDM culture medium contained 10% bovine fetal serum at 2×10^5 cells/mL and seeded into 6-well plates (1 ml/well). Then 1 ml CD20 negative lymphocyte cells (1×10^6 cells/ml) were added to the wells. Each sample had control group which did not contain CD40-B cells. The cells were cultured with rh IL-2 (10 ng/ml) for 4 days, then the cells were harvested, washed, and mixed with anti-CD8-FITC and PE-labeled HLA-A2 pentamer complexes against the HBV core 18–27 peptide (ProImmune, Oxford, UK) to detect HBcAg18–27-specific CD8+ T cells. The cells were washed by PBS and analyzed by BD FACSCanto flow cytometer.

2.6. Statistical analysis

The results were analyzed by SPSS 13.0 soft ware (SPSS, Inc., Chicago, IL, USA). Results of experimental data are expressed as the mean ± standard error of the mean (SEM). Student t test and or Mann–Whitney U test were used as appropriate to determine the statistical significance. $P < 0.05$ was considered to be statistically significant difference.

3. Results

3.1. Purified B lymphocytes

A positive-selection procedure was chosen for purification of B cells from PBMCs. After magnetic enrichment of CD20-positive B cells from PBMCs, B cells were stained with anti-CD19-FITC and analyzed by flow cytometry. FACS analysis showed B cells were enriched to more than 90% (Fig. 1).

3.2. Expression of costimulatory molecules of CD40-B cells

To investigate whether CD40-B cells may serve as APCs, we detected the expression of costimulatory molecules, including

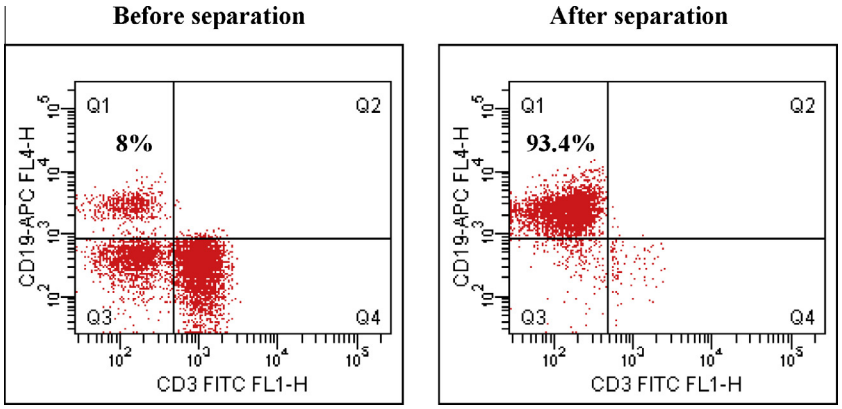

Fig. 1. Representative example of B cells purified by magnetic beads separation from the PBMCs of a patient with chronic HBV infection. B cells were enriched to more than 90%.

CD80, CD86, MHC classes I and II on the cell surface by flow cytometry. After activated by sCD40L, more than 25% and 60% of B cells expressed CD80 and CD86 respectively, and the expression of MHC classes I and II on B cells was also significantly higher than that on control (Fig. 2). The results indicated that compared with control, the levels of these molecules on the CD40-B cells were significantly increased. Thus, the B cells activated by sCD40L may have the function of APCs.

3.3. HBcAg18–27 antigen peptide loading on CD40-B cells

To clarify whether CD40-B cells have the function of antigen presentation, we cultured CD40-B cells in the presence of green fluorescent protein (GFP)-binding HBcAg18–27 peptide. Cytometry analysis showed there were 41.34% of CD40-B cells loaded by HBcAg18–27 while only 0.01% on B cells without sCD40L (Fig. 3A and B), which indicated that there was hepatitis B core peptide in the cells or on the surface of the cells. Thus, the B cells might be loaded by the hepatitis B core peptide. We further observed the cells under fluorescence microscope and found that the red fluorescence located at B cell surface was CD19-PE and the green fluorescence (the green FITC fluorescence and the red CD19-PE superimposition demonstration is a yellow) located at B cell cytoblastema was hepatitis B core peptide. The CD40-B cells showed strong fluorescence after peptide pulsing at concentrations even lower than 25 μg/ml (Fig. 3C). All of the above results indicated that the CD40-B cells could load the hepatitis B core peptide.

3.4. HBcAg18–27 specific cytotoxic T lymphocytes

To investigate whether the CD40-B cells could present the hepatitis B core peptide to T cells and induce specific CTL responses, we co-cultured the autologous T cells and CD40-B cells loaded by hepatitis B core peptide, and then detected the CTL responses against peptide of HBcAg18–27 by pentamer analysis. In patients with chronic HBV infection, the percentage of HBcAg18–27 specific CTL was 0.226 ± 0.083% after T cells co-cultured with CD40-B cells, while the percentage was only 0.034 ± 0.012% in the absence of CD40-B cells ($P = 0.009$, Fig. 4). In healthy controls, the HBcAg18–27 specific CTL population was slightly higher after T cells co-cultured with CD40-B cells (0.434 ± 0.194%) compared with the absence of CD40-B cells (0.111 ± 0.076%). The difference did not achieved to be significant ($P = 0.197$) because of the small sample size in the group. No significant differences were found between the groups of healthy individuals and patients with chronic HBV infection in the

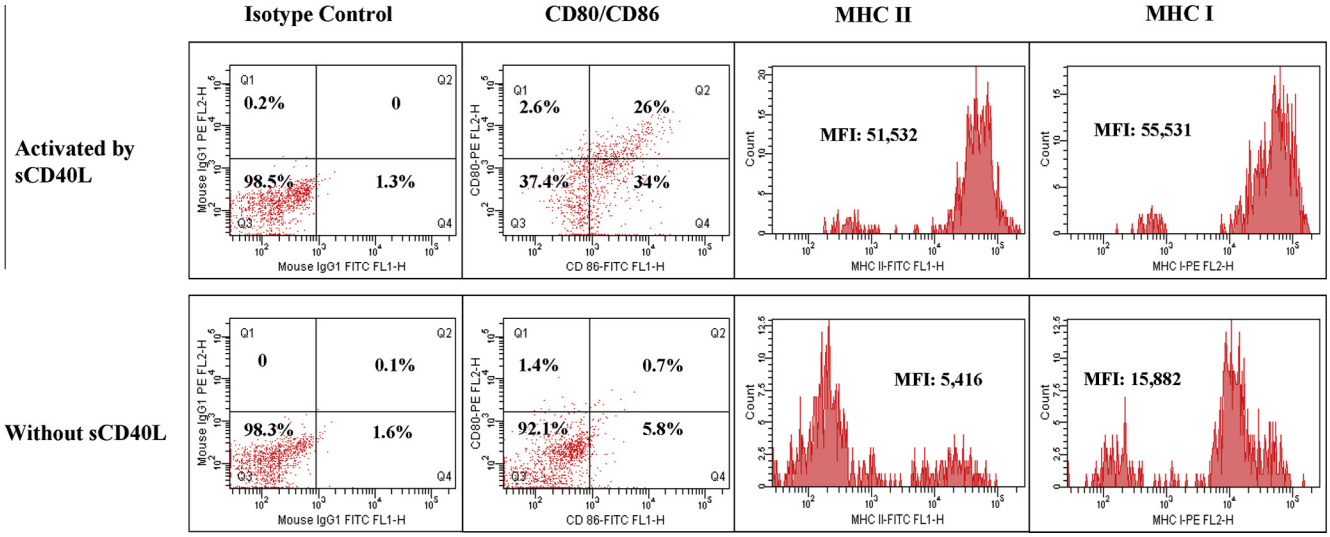

Fig. 2. Expression of CD80, CD86, MHC I and II on sCD40L activated human B cells. The expression of surface molecules MHC I, MHC II, CD80, and CD86 on B cells were examined by gating CD19 positive cells by FACS analysis. The expression of CD80 and CD86 on B cells was expressed as the percent in all B cells. The expression of MHC I and MHC II on B cells was expressed as the mean fluorescence intensity (MFI). The data are representative experimental results from a patient with chronic HBV infection.

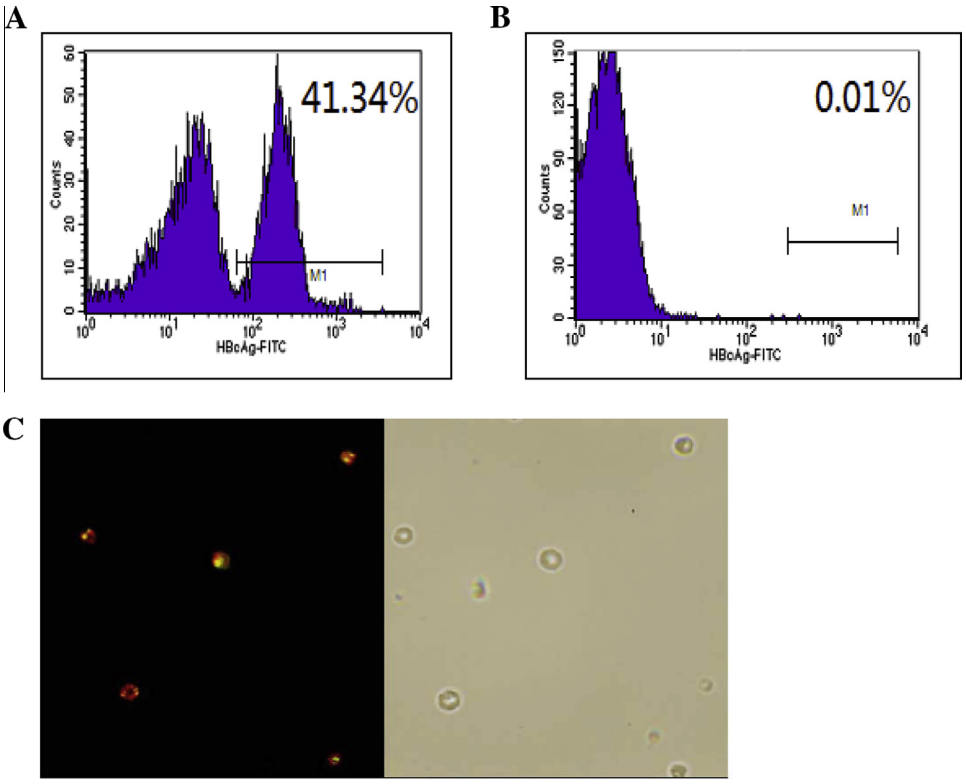

Fig. 3. B cells loaded hepatitis B core peptide was detected by FACS analysis. The data are representative experimental results from a patient with chronic HBV infection. (A) The ratio of antigen loading on B cells stimulated by sCD40L was 41.34%; (B) The ratio on B cells without sCD40L was 0.01%. (C) To observe the B cells specific binding of hepatitis B core peptide by fluorescence microscope, the red fluorescence located at B cell surface was CD19-PE and the yellow fluorescence (the green FITC fluorescence and the red CD19-PE superimposition demonstration is a yellow) located in B cell cytoplasm was hepatitis B core protein (FITC-FLPSDFFPSV). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

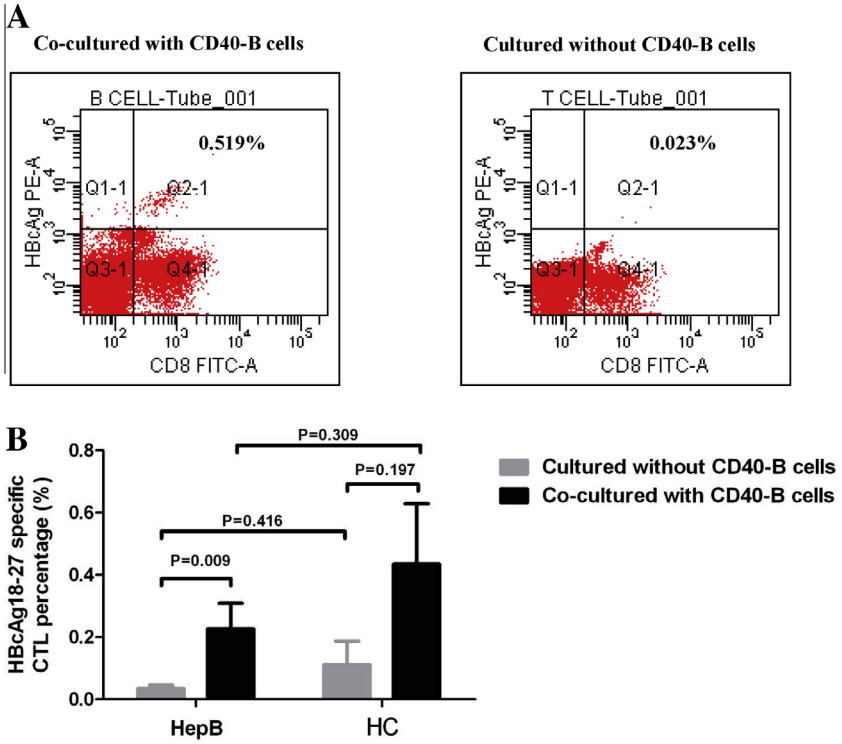

Fig. 4. Pentamer analysis of the induction of CTL response against HBcAg18–27 peptide. (A) Representative dot plots showing of the induction of CTL response by CD40-B cells from a patient with chronic HBV infection. (B) Percentage of HBcAg18–27 specific CTLs after been co-cultured with CD40-B cells or cultured without CD40-B cells. Results are expressed as mean; error bars denote standard error of the mean. HepB: patients with chronic HBV infection, HC: healthy controls.

HBcAg18–27 specific CTLs percentage after co-cultured with CD40-B cells ($P = 0.309$) or absence of CD40-B cells ($P = 0.416$).

4. Discussion

In this study, we used human sCD40L to activate B cells and the CD40-B cells could load the HBcAg peptide. Furthermore, after been activated and HBcAg18–27 antigen peptide pulsing, the B cells from patients with chronic HBV infection could induce HBcAg18–27 specific CTLs in vitro.

Unlike some studies, in which B cells were co-cultured with NIH3T3 cells or other tumor cells which steadily express CD40L [26,27], our results demonstrated that B cells could be activated by rh sCD40L. While previous culture system has introduced the extraneous source germ cells and has limited the further clinical application researches, the accomplishment of present work may be taken as an alternative approach to activate primary human B cells in vitro. In addition, compared with DCs which constitute only 0.1–0.5% of human PBMCs, the rate of B cells in human PBMCs is significantly higher which constitute 7–23% [28].

Previous reports have shown that B cells are tolerogenic APCs but they used small resting B cells lacking CD80 and CD86 expression [29]. It has been demonstrated that in order to fully extend their function as APCs and the capacity to induce T-cell activation, B cells need to be activated properly [16]. Our experiments indicated that the rh sCD40L could activate the B cells from PBMCs, and also induce a strong up-regulation of CD80 and CD86 on human B lymphocytes. The main function of MHC II molecules is to present processed antigens to CD4 T-lymphocytes. Constitutive expression of MHC II molecules is confined to APCs of the immune system [30]. In our study, the expression of MHC II molecules on B cells was significantly increased after the B cells activated by sCD40L. All of these results indicate that the CD40-B cells have the characteristics of APCs.

Many studies have suggested that a strong intrahepatic CTL response to HBV can noncytopathically suppress viral replication [14,31,32]. HBcAg is thought to be a key target for the host immune response in controlling the infection, because the presence of HBcAg-specific T cells has been associated with clearance of acute and chronic HBV infections, meanwhile prophylactic and therapeutic vaccines that induce HBcAg-specific T cells have shown some efficiency in infectious models [33,34]. In this study, CD40-B cells could be loaded by the HBcAg peptide. Furthermore, the peptide-pulsed CD40-B cells could induce HBcAg18–27 specific CTLs. It suggested that CD40-B cells could be alternatively used as a potential source of APCs for adoptive immunotherapy.

The present study primarily demonstrated that the B cells from patients with chronic HBV infection could be activated by sCD40L and loaded with HBcAg peptide and the CD40-B cells loaded with HBcAg peptide could induce the production of HBV-specific T cells. It suggested that they might be the optimal APCs with which to generate antigen-specific T cells ex vivo for HBV adoptive immunotherapy. It may provide a new cell-based vaccine and a potential novel therapeutic strategy for patients with chronic HBV infection.

5. Competing interests

The authors declared that they have no competing interests.

Acknowledgments

This work was supported by grants from National Natural Science Foundation of China (No. 30872234), Jiangsu Province's Outstanding Medical Academic Leader Program (No. LJ201154) and Jiangsu Province's Clinical Medicine and Technology Special Program (No. BL2012034).

References

[1] D. Lavanchy, Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures, J. Viral Hepat. 11 (2004) 97–107.
[2] Y.F. Liaw, C.M. Chu, Hepatitis B virus infection, Lancet 373 (2009) 582–592.
[3] M.L. Michel, Q. Deng, M. Mancini-Bourgine, Therapeutic vaccines and immune-based therapies for the treatment of chronic hepatitis B: perspectives and challenges, J. Hepatol. 54 (2011) 1286–1296.
[4] D. Grimm, M. Heeg, R. Thimme, Hepatitis B virus: from immunobiology to immunotherapy, Clin. Sci. (Lond) 124 (2013) 77–85.
[5] A. Bertoletti, A.J. Gehring, Immune therapeutic strategies in chronic hepatitis B virus infection: virus or inflammation control?, PLoS Pathog 9 (2013) e1003784.
[6] B. Rehermann, Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells, Nat. Med. 19 (2013) 859–868.
[7] A.M. Woltman, M.L. Op den Brouw, P.J. Biesta, C.C. Shi, H.J. Janssen, Hepatitis B virus lacks immune activating capacity, but actively inhibits plasmacytoid dendritic cell function, PLoS One 6 (2011) e15324.
[8] A. Bertoletti, C. Ferrari, Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection, Gut 61 (2012) 1754–1764.
[9] W.O. Böcher, B. Dekel, W. Schwerin, M. Geissler, S. Hoffmann, A. Rohwer, F. Arditti, A. Cooper, H. Bernhard, A. Berrebi, S. Rose-John, Y. Shaul, P.R. Galle, H.F. Löhr, Y. Reisner, Induction of strong hepatitis B virus (HBV) specific T helper cell and cytotoxic T lymphocyte responses by therapeutic vaccination in the trimera mouse model of chronic HBV infection, Eur. J. Immunol. 31 (2001) 2071–2079.
[10] C. Zhou, G. Peng, X. Jin, J. Tang, Z. Chen, Vaccination with a fusion DNA vaccine encoding hepatitis B surface antigen fused to the extracellular domain of CTLA4 enhances HBV-specific immune responses in mice: implication of its potential use as a therapeutic vaccine, Clin. Immunol. 137 (2010) 190–198.
[11] J.H. Chen, Y.S. Yu, X.H. Chen, H.H. Liu, G.Q. Zang, Z.H. Tang, Enhancement of CTLs induced by DCs loaded with ubiquitinated hepatitis B virus core antigen, World J. Gastroenterol. 18 (2012) 1319–1327.
[12] W. Chen, M. Shi, F. Shi, Y. Mao, Z. Tang, B. Zhang, H. Zhang, L. Chen, L. Chen, S. Xin, F.S. Wang, HBcAg-pulsed dendritic cell vaccine induces Th1 polarization and production of hepatitis B virus-specific cytotoxic T lymphocytes, Hepatol. Res. 39 (2009) 355–365.
[13] X. Chen, Y. Yu, Q. Pan, Z. Tang, J. Han, G. Zang, Enhancement of cytotoxic T lymphocyte activity by dendritic cells loaded with Tat-protein transduction domain-fused hepatitis B virus core antigen, Acta Biochim. Biophys. Sin. (Shanghai) 40 (2008) 996–1004.
[14] S.M. Akbar, S. Chen, M. Al-Mahtab, M. Abe, Y. Hiasa, M. Onji, 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, Antiviral Res. 96 (2012) 59–64.
[15] K.M. Ardeshna, A.R. Pizzey, N.S. Thomas, S. Orr, D.C. Linch, S. Devereux, Monocyte-derived dendritic cells do not proliferate and are not susceptible to retroviral transduction, Br. J. Haematol. 108 (2000) 817–824.
[16] K. Wennhold, A. Shimabukuro-Vornhagen, S. Theurich, M. von Bergwelt-Baildon, CD40-activated B cells as antigen-presenting cells: the final sprint toward clinical application, Expert Rev. Vaccines 12 (2013) 631–637.
[17] J.L. Schultze, S. Michalak, M.J. Seamon, G. Dranoff, K. Jung, J. Daley, J.C. Delgado, J.G. Gribben, L.M. Nadler, CD40-activated human B cells: an alternative source of highly efficient antigen presenting cells to generate autologous antigen-specific T cells for adoptive immunotherapy, J. Clin. Invest. 100 (1997) 2757–2765.
[18] B. Del Bello, M.A. Valentini, F. Zunino, M. Comporti, E. Maellaro, Cleavage of Bcl-2 in oxidant- and cisplatin-induced apoptosis of human melanoma cells, Oncogene 20 (2001) 4591–4595.
[19] G. Tan, L. Heqing, C. Jiangbo, J. Ming, M. Yanhong, L. Xianghe, S. Hong, G. Li, Apoptosis induced by low-dose paclitaxel is associated with p53 upregulation in nasopharyngeal carcinoma cells, Int. J. Cancer 97 (2002) 168–172.
[20] C. van Kooten, J. Banchereau, Functions of CD40 on B cells, dendritic cells and other cells, Curr. Opin. Immunol. 9 (1997) 330–337.
[21] Y.S. Kim, Y.J. Kim, J.M. Lee, S.H. Han, H.J. Ko, H.J. Park, A. Pereboev, H.H. Nguyen, C.Y. Kang, CD40-targeted recombinant adenovirus significantly enhances the efficacy of antitumor vaccines based on dendritic cells and B cells, Hum. Gene Ther. 21 (2010) 1697–1706.
[22] N. Voorzanger-Rousselot, M. Favrot, J.Y. Blay, Resistance to cytotoxic chemotherapy induced by CD40 ligand in lymphoma cells, Blood 92 (1998) 3381–3387.
[23] M. Bocchia, T. Korontsvit, Q. Xu, S. Mackinnon, S.Y. Yang, A. Sette, D.A. Scheinberg, Specific human cellular immunity to bcr-abl oncogene-derived peptides, Blood 87 (1996) 3587–3592.
[24] M. Shi, S. Qian, W.W. Chen, H. Zhang, B. Zhang, Z.R. Tang, Z. Zhang, F.S. Wang, Hepatitis B virus (HBV) antigen-pulsed monocyte-derived dendritic cells from HBV-associated hepatocellular carcinoma patients significantly enhance specific T cell responses in vitro, Clin. Exp. Immunol. 147 (2007) 277–286.
[25] A. Penna, F.V. Chisari, A. Bertoletti, G. Missale, P. Fowler, T. Giuberti, F. Fiaccadori, C. Ferrari, Cytotoxic T lymphocytes recognize an HLA-A2-restricted

epitope within the hepatitis B virus nucleocapsid antigen, J. Exp. Med. 174 (1991) 1565–1570.
[26] S. Néron, A. Roy, N. Dumont, Large-scale in vitro expansion of polyclonal human switched-memory B lymphocytes, PLoS One 7 (2012) e51946.
[27] S.H. Yoon, H.I. Cho, T.G. Kim, Activation of B cells using Schneider 2 cells expressing CD40L for the enhancement of antigen presentation in vitro, Exp. Mol. Med. 37 (2005) 567–574.
[28] T. Reichert, M. DeBruyère, V. Deneys, T. Tötterman, P. Lydyard, F. Yuksel, H. Chapel, D. Jewell, L. Van Hove, J. Linden, L. Buchner, Lymphocyte subset reference ranges in adult Caucasians, Clin. Immunol. Immunopathol. 60 (1991) 190–208.
[29] G. Raimondi, I. Zanoni, S. Citterio, P. Ricciardi-Castagnoli, F. Granucci, Induction of peripheral T cell tolerance by antigen-presenting B cells. I. Relevance of antigen presentation persistence, J. Immunol. 176 (2006) 4012–4020.
[30] K.J. Cho, P.A. Roche, Regulation of MHC class II-peptide complex expression by ubiquitination, Front. Immunol. 4 (2013) 369.
[31] A. Bertoletti, M. Maini, R. Williams, Role of hepatitis B virus specific cytotoxic T cells in liver damage and viral control, Antiviral Res. 60 (2003) 61–66.
[32] F.V. Chisari, M. Isogawa, S.F. Wieland, Pathogenesis of hepatitis B virus infection, Pathol. Biol. (Paris) 58 (2010) 258–266.
[33] D. Kim, K.S. Lyoo, D. Smith, W. Hur, S.W. Hong, P.S. Sung, S.K. Yoon, S. Mehta, Number of mutations within CTL-defined epitopes of the hepatitis B Virus (HBV) core region is associated with HBV disease progression, J. Med. Virol. 83 (2011) 2082–2087.
[34] J. Nyström, A. Chen, L. Frelin, G. Ahlén, S. Koh, A. Brass, D.L. Peterson, M. Fons, D.R. Milich, C. Hultgren, M. Sällberg, Improving on the ability of endogenous hepatitis B core antigen to prime cytotoxic T lymphocytes, J. Infect. Dis. 201 (2010) 1867–1879.