

HBVsvp-Pulsed Dendritic Cell Immunotherapy Induces Th1 Polarization and Hepatitis B Virus-Specific Cytotoxic T Lymphocytes Production

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Background: In chronic hepatitis B virus (CHB) patients, both dendritic cells (DCs) and T cells are functionally impaired and consequently the HBV-specific cellular immune responses are downregulated. The present study aims to investigate whether monocyte-derived DC (MoDCs)-pulsed-HBV subviral particles (HBVsvp) can polarize Th1 cells to induce HBV-specific cytotoxic T-lymphocytes (CTL) responses in CHB patients.

Methods and Materials: To this end, the human hepatoma HepG2.2.15 cell line was used to produce HBVsvp as a culturing system, and HBVsvp were concentrated for highly virus titer using the polyethylene glycol protocol. Peripheral blood mononuclear cells (PBMCs), collected from CHB patients and healthy donors, were differentiated into MoDCs and T cells. PBMCs-derived MoDCs were first pulsed with HBVsvp and then cultured with PBMCs-derived T cells. MoDCs and/or T subsets cells were identified for phenotypic activation by FACS analysis. The cytokine secretion of IL-4, IL-12, and IFN- γ in the culture supernatants was detected.

Results: The MoDCs were restored for their activation upon pulsing with HBVsvp in vitro, as identified by significantly overexpression of both CD86 and HLA-DR, and overproduction of IL-4 and IL-12. Furthermore, MoDCs-pulsed-HBVsvp induced Th1 frequencies and activated HBV-specific CTL to produce significantly highest amount of IFN- γ . Enhanced HBV-specific CTL led to strong cytolytic capacity against HepG2.2.15.

Conclusion: Overall, our data suggest that in vitro activation of MoDCs with HBVsvp overcomes the functionally impaired DCs and T cells in CHB patients offering a promising tool for therapeutic or vaccine-based approaches against HBV.

Keywords: CHB, MoDCs-pulsed-HBVsvp, HBV-specific CTL, immunotherapy

Introduction

HBV remains a major global public health problem, an estimated more than 400 million people are infected with HBV and one million people die annually of HBV liver diseases.^{1,2} Up to recent, it is unknown why individual HBV-infected people develop a chronic carrier state.³ The insufficient immune response of HBV patients probably has an important role in the chronicity of HBV infection.^{3,4} The immune system is specialized to recognize and eliminate foreign antigens. Therefore, if the immune response for any reason has become tolerant to HBV, the HBV-specific adaptive immune responses will be turned down to undetectable limits.⁵⁻⁷ Until now, it is not well determined how the virus escapes from immune protection leading to the chronic state characterized by absence of HBV-specific

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T-cell responses.^{3,5,8} In acute infection, patients exhibit a significant multi-specific CD8 CTL and CD4 Th1 cell responses.^{9,10}

Dendritic cells (DCs) are essential professionally antigen-presenting cells (APCs) and acting as key players initiating T cell activation against viral agents^{11,12} by strongly expressing different costimulatory and/or adhesion molecules.¹¹ An increasing titer of viruses, such as human immunodeficiency virus (HIV),¹³ hepatitis C virus (HCV),¹⁴ herpes simplex virus,¹⁵ measles virus,¹⁶ vaccinia virus,¹⁷ may induce functional paralysis of DC that suppresses antiviral immune responses. The basic role of DC in HBV infection is still unclarified.¹² However, the function of DC must be studied in relation to HBV infection to apply DC as an immunotherapeutic tool. There are many approaches that have been established for obtaining highly purified DCs from human and mice.^{18–22} DCs that generated from human peripheral blood progenitors in vitro are considered to be immature and possess the endocytosis capacity of soluble antigens (Ags) and present them to naïve T cells.²⁰ MoDCs isolated from CHB patients are functionally impaired.²³

The mature DCs can activate naïve T cells and determine their polarization into Th1 or Th2 cells. Th1 cells produce high amounts of type-1 cytokines (IFN- γ , TNF- α / β , and IL-2) which prime cell-mediated immunity against viruses.²⁴ Th2 cells produce significant levels of type-2 cytokines (IL-4, IL-5, IL-6, and IL-10) which induce humoral immunity. Only IL-12 or combined with both TNF- α / β and IFN- γ has been shown to be shared in the polarization of Th1 cells and the development of CTLs, T-memory cells, and humoral response.^{25,26} The HBV-specific CD4⁺ T cells are responsible for the induction and activation of HBV-specific CD8⁺ T cells. So, HBV particles-pulsed DC vaccine able to activate CD4⁺ T cells, B cells, and induce HBV-specific CD8⁺ T cells are considered important components of immunotherapy.²⁷

In our previous study, we demonstrated that DC can be activated in vitro specifically against HBV and initiate immune responses,^{21,22} and the tolerance of HBV transgenic mice was break-down by using DC-based immunization, which may also be applicable in vivo to treat CHB patients.²² Also, we showed that HBVsvp can stimulate DC for production a large array of cytokines.²⁸ The aim of the present study was to investigate whether MoDCs-pulsed-HBVsvp can polarize Th1 cells and induce an HBV-specific cytotoxic T-lymphocytes response in CHB patients and healthy donors, in vitro activation of MoDC with HBVsvp can overcome the

impaired DC and T-cell function in CHB, as well as vaccination with ex vivo activated MoDCs may be a promising tool for therapeutic or vaccine-based approaches against HBV.

Methods and Materials

Generation of HBVsvp

HBVsvp were prepared as described previously.^{21,22} In brief, the cell line human hepatoma HepG2.2.15 was kindly provided by Dr. Stefan Urban (Heidelberg University, Germany), where the cell lines have not been purchased from an accredited commercial source and was approved by the ethics committee at the CCHE. The cells were cultured in Williams' E medium (Sigma-Aldrich), supplemented with 100 U of penicillin/mL, 100 μ g of streptomycin/mL, 2 mL glutamine, and 10% (v/v) FCS (Invitrogen), 5 μ g/mL insulin and 5 μ g/mL of hydrocortisone (Sigma-Aldrich). The HepG2.2.15 cells were cultured at 37°C in a CO₂ incubator, which resulted in a strong production of HBsAg in the supernatant as shown in our previous results.²⁸ The HBVsvp was concentrated from the medium as described previously^{21,22} using polyethylene glycol (PEG) protocol. Briefly, HBVsvp containing supernatant was incubated with PEG (40%) overnight at 4°C and then centrifuged at 8000 rpm (4°C) for one hour. The pellets were resuspended in FCS/PBS (25%) followed by incubation at 4°C for overnight. The HBVsvp were harvested by centrifugation at 4000 rpm (4°C) for 20 min. Finally, the HBVsvp were quantified for HBsAg by ELISA technique and stored at -80°C.

Samples Collection

Peripheral heparinized blood samples were collected from 20 CHB patients and 20 healthy donors as control (Table 1). CHB patients who had received antiviral therapy within the past 6 months, or concurrent with HCV, HDV, or HIV infection or any immunological disorders were excluded.

For CHB patients, the median serum HBV DNA load was 65×10^5 geq/mL (range, 16×10^3 – 4×10^7) with mean 10.6×10^5 geq/mL, the median of alanine aminotransferase level (ALT) was 64 U/L (range, 17–156) with mean 67.35 U/L, the median of aspartate aminotransferase level (AST) was 67.5 U/L (range, 12–130) with mean 64.75 U/L, and the median of total bilirubin level was 1.25 (mg/dL) (range, 0.2–3.5) with mean 1.4 mg/dL (Table 1). The 20 patients were HBV surface antigen (HBsAg)-positive, and 18 patients were HBV

Table 1 Shows the Patients and Healthy Controls Clinical Characteristics

| Denotation | Patients (n=20) and Healthy Controls (n=20) Characteristics | | | | | | | | | |
|-----------------------------|---|-------------|--------------|------|--------------|------|-------------------------|------|-------------------------------------|------|
| | Age (y) | | ALT (U/L) | | AST (U/L) | | Total Bilirubin (mg/dL) | | HBV DNA (geq/mL) | |
| | CHB | HC | CHB | HC | CHB | HC | CHB | HC | CHB | HC |
| Mean | 44.7 | 34.35 | 67.35 | n.t. | 64.75 | n.t. | 1.4 | n.t. | 10.6×10^5 | n.a. |
| Median | 41.5 | 34.5 | 64 | n.t. | 67.5 | n.t. | 1.25 | n.t. | 65×10^5 | n.a. |
| 25th% - 75th% quartile | 33.75–61.5 | 28.25–40.75 | 39–79.5 | n.t. | 33.5–85.5 | n.t. | 0.725–1.9 | n.t. | 25×10^3 – 18×10^6 | n.a. |
| Non-outliers (min. to max.) | 22–68 | 21–46 | 17–156 | n.t. | 12–130 | n.t. | 0.2–3.5 | n.t. | 16×10^3 – 4×10^7 | n.a. |
| Standard Deviation (SD) | ± 14.220 | ± 7.400 | ± 37.764 | n.t. | ± 34.955 | n.t. | ± 1.006 | n.t. | $\pm 1,612,435.34$ | n.a. |
| Standard Error (SE) | ± 3.179 | ± 1.654 | ± 8.444 | n.t. | ± 7.816 | n.t. | ± 0.225 | n.t. | $\pm 2,820,226.27$ | n.a. |

Abbreviations: CHB, chronic hepatitis B virus; HC, healthy controls; n.t., not tested; n.a., not applicable.

envelope antigen (HBeAg)-positive. For controls, participants were negative to HBV, HCV, and HIV and free from any liver diseases. Control and CHB subjects were age/sex-matched.

All participants were consented to use their samples and clinical data (CHB patients) for this study. The consent form and the research proposal were approved by the ethical committee at the CCHE and the liver institute(s) that the CHB patients were recruited. After being consented, 30 mL of peripheral blood were collected from each participant using EDTA vacutainer.

Generation of Monocyte-Derived DCs (MoDCs)

PBMCs were prepared using a blood cell separator, then MoDCs were generated. Briefly, PBMCs (5×10^6 cells/mL) were suspended in 6-well plates in serum-free RPMI medium (Sigma-Aldrich) and incubated for 2 hours. Then, the non-adherent cells were gently collected and cryopreserved and used for autologous T cells. On the other hand, the adherent cells were incubated in complete RPMI medium supplemented with granulocyte-macrophage colony-stimulating factor (GM-CSF) (20ng/mL) and IL-4 (20ng/mL) (Sigma-Aldrich) at 37°C in CO₂ (5%) incubator. Fresh medium containing IL-4 and GM-CSF were replenished every 2 days. On day 7, the MoDC was harvested for phenotypic analysis by FACS analysis.

In vitro Activation of MoDCs with HBVsvp

On day 7, MoDCs (2×10^6 cells/mL) were placed in a 12-well culture plate and checked for their immaturity by FACS analyses. One day later, the MoDCs were activated by either HBVsvp, *E. coli* lipopolysaccharide (LPS) (1 µg/

mL), HBVsvp + LPS or adding nothing as a negative control. On day 9, MoDCs were harvested, extensively washed, and measured for expression of activating markers by FACS analyses.

Flow Cytometric Analysis for MoDCs

MoDCs cells (2×10^5) were resuspended in FACS buffer (0.5% BSA-PBS) and stained with either anti-CD11c, anti-CD86, or anti-HLA-DR (BD Bioscience) on for 30 min on ice in dark. Cells were centrifuged at 2000 rpm for 3 min and washed twice with FACS buffer. Unstained cells were used for determination of the fluorescence baseline. All analyses were performed on a BD FACS Calibur™ flow cytometer and analyzed using Cell Quest Pro software (Beckton Dickinson, USA).

Th1/Tc Polarization Assessment

Among the non-adherent PBMCs, autologous T cells were determined by FACS analysis using anti-CD4 or anti-CD8 (BD Bioscience). The T cell polarization capacity of the MoDCs was determined using autologous T cells as responder T cells, which were co-cultured with MoDCs. Briefly, MoDCs pulsed with HBVsvp were co-cultured with autologous T cells at a MoDCs: T cells ratio of 1:5 in 24-well plates in complete RPMI1640 medium and incubated for 3 days. T cells were cultured alone as a control. On day 4, T cells from each well were detected T cells subsets.

Detection of HBV-Specific-CTL Cytotoxicity

HBV-specific CTL obtained from CHB patients and healthy donors were used to evaluate cytotoxicity by FACS analysis.

Cells were counted and checked for viability by trypan blue exclusion. HBV-specific CTL was used as effector cells. Since the cell line HepG2.2.15 already has an HBV genome integrated into its chromosome was used as target cells. HepG2.2.15 was labelled with HEA125 FITC (5 μ L), as a specific antibody, for 30 min on ice and incubated in the dark. Target cells were washed twice in FACS buffer. Approximately 2×10^5 target cells and 1×10^6 or 2×10^6 effector cells (1:5 or 1:10 ratio) were resuspended in complete RPMI medium. Cells were centrifuged for 1 min at 800 rpm, incubated at 37°C in a CO₂ incubator for 5–6 hours, and then were washed twice with FACS buffer. Cells were resuspended in 200 μ L FACS buffer with 1.3 μ g/mL propidium iodide (PI) to stain dead cells. For instrument settings and compensations, individual FITC-labelled target cells and HBV-specific CTL were used. Analysis gates were set on the target cells and the percentage of FITC⁺/PI⁺ cells were determined using Cell Quest Pro software.

Detection of Cytokines Activity by ELISA

The cytokines (IL-4, IL-12, and IFN- γ) were measured in the culture supernatants by ELISA kits according to the manufacturer's instructions. The actual cytokine concentrations (pg/mL) were determined using standard reagents provided by the manufacturer.

Statistical Analysis

All data were analyzed by SPSS software (version 23) and summarized as the mean \pm SD. ANOVA analysis was used to test for differences in marker values between normal control and chronic patient groups at different treatments. *T*-test was

used to determine statistical differences ($P < 0.05$ is significant).

Results

MoDCs are Strongly Activated by HBVsvp and Secreted High Levels of Cytokines in vitro

After incubation for 7 days, approximately 79.5% of the cultured PBMCs were positive for CD11c, indicating the differentiation of monocytes towards MoDCs (Figure 1). Table 2 showed strong activation of MoDCs by HBVsvp and the co-stimulatory factor LPS in vitro, expressing higher levels of CD86 and HLA-DR than negative control. The MoDCs activation was significant ($p < 0.001$) increased in both CHB patients and healthy donors compared to the negative control.

Our results indicated that after 24 hours incubation of MoDCs of healthy donors or CHB patients with HBVsvp and LPS, CD86 activating marker of MoDCs was significantly ($p < 0.001$) increased than the negative control. Regarding the incubation with HBVsvp alone, CD86 activating marker of MoDCs was also increased significantly ($p < 0.001$) comparing with negative control (Figure 2).

For the HLA-DR activating marker, our results showed after 24 hours incubation of MoDCs of healthy donors or chronic patients with HBVsvp plus LPS, the HLA-DR activating marker of MoDCs was significantly ($p < 0.001$) increased than the negative control. Regarding the incubation with HBVsvp alone, HLA-DR activating marker of MoDCs was also increased

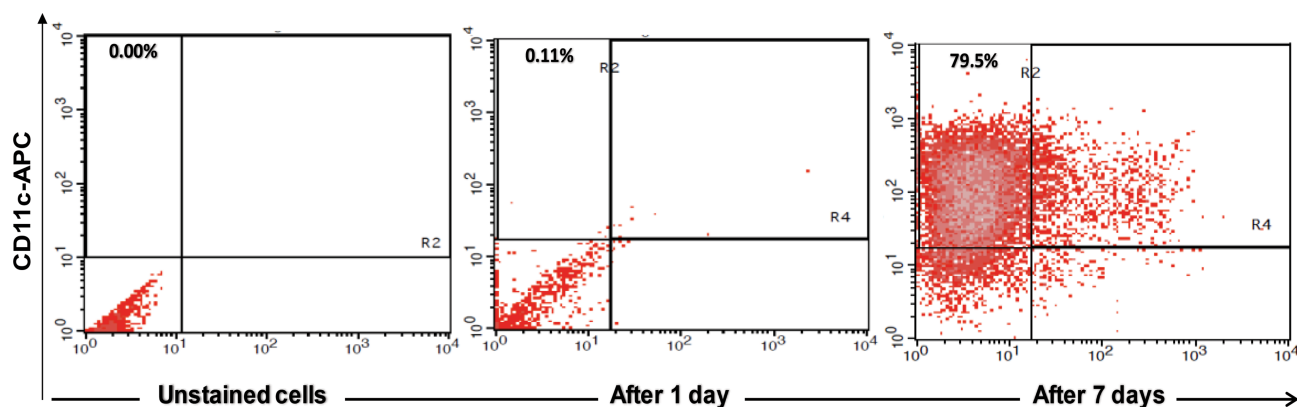


Figure 1 Showed FACS analysis of MoDCs, identified by expression of CD11c after 7 days of maturation, approximately 79.5% of the cultured cells were positive for CD11c, indicating the differentiation of monocytes towards MoDCs.

Table 2 Shows Strong Activation of MoDCs by HBVsvp in vitro, Which Expressed High Levels of CD86 and HLA-DR Than Negative Control. The MoDCs Activation Was Significantly ($p < 0.001$) Increased in Healthy Donors and Chronic Patients Comparing with the Negative Control for the Expression Levels of CD86 and HLA-DR Activation Markers

| Marker | Negative Control | | LPS | | HBVsvp | | HBVsvp+LPS | | p-value |
|-----------|------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|
| | HD | CP | HD | CP | HD | CP | HD | CP | |
| CD86-PE | 15.44 ±1.84 | 16.60 ±1.63 | 21.22 ±0.33 | 21.99 ±0.25 | 33.18 ±1.45 | 45.18 ±0.84 | 57.00 ±1.31 | 67.50 ±1.10 | <0.001 |
| HLA DR-PE | 21.47 ±3.40 | 32.03 ±0.55 | 32.03 ±0.55 | 44.71 ±1.19 | 44.71 ±1.19 | 51.37 ±0.26 | 63.48 ±0.74 | 75.42 ±1.50 | <0.001 |

Abbreviations: HD, healthy donors; CP, chronic hepatitis B patients.

significantly ($p < 0.001$) comparing with the negative control (Figure 3). Our results demonstrated that pulsing of MoDCs with HBVsvp alone or coupled with LPS upregulated expression of costimulatory molecules on MoDCs.

For cytokines secretions, our data showed that MoDCs pulsed with HBVsvp plus LPS produced significantly highest levels of IL-4 in both chronic HBV patients ($p < 0.05$) and healthy donors ($p < 0.001$), in vitro (Figure 4). Regarding IL-12, our results showed that MoDCs co-cultured with HBVsvp plus LPS produced significantly elevated amounts of IL-12 in chronic HBV patients ($p < 0.05$) and healthy donors ($p < 0.001$), in vitro (Figure 5). Our above-mentioned data indicated that pulsing of MoDCs with HBVsvp plus LPS upregulated expression of costimulatory molecules and enhanced the cytokine production ability of MoDCs.

MoDCs-Pulsed-HBVsvp Efficiently Induced Autologous T Cell Polarization into Th1/Tc in vitro

As shown in Figure 6, MoDCs-pulsed-HBVsvp were able to induce Th1 and Tc in vitro at a MoDCs: T cell ratio (1:5). The Th1 and Tc frequencies were highly induced by MoDCs-pulsed-HBVsvp, indicating the ability of MoDCs-pulsed-HBVsvp to induce an autologous Th1/Tc polarization response in vitro for both chronic HBV patients and healthy donors and confirmed the ability of HBVsvp to up-regulate the stimulatory capacity of the MoDCs. Our data indicated that MoDCs induced efficiently autologous T cell polarization into Th1 and Tc cells.

HBV-specific-CTL from chronic HBV patients and healthy donors secreted significantly elevated amounts of IFN- γ , the effector cytokine, into the culture medium upon co-culture with MoDCs-pulsed-HBVsvp. Our results showed that HBV-specific-CTL produced significantly

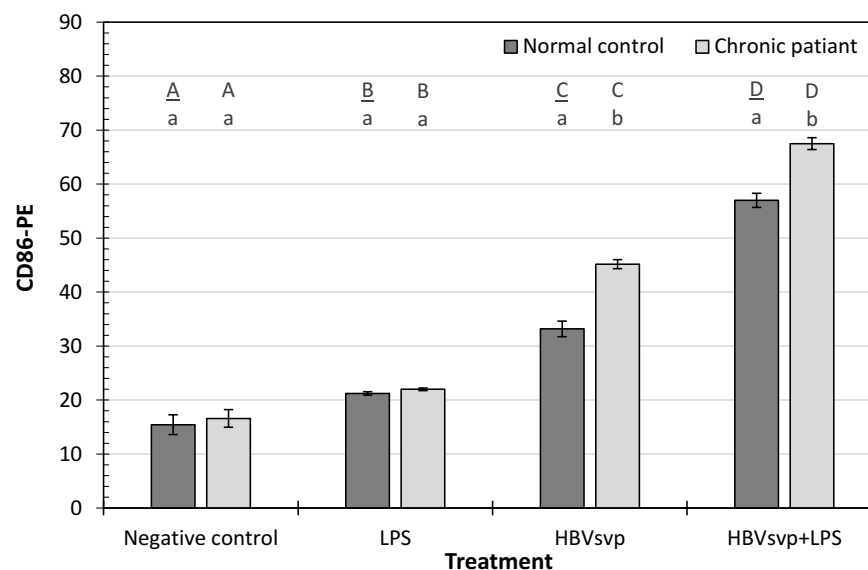


Figure 2 Showed CD86-PE activating marker of MoDCs detected in healthy donors and chronic patient groups at different treatment. Small letters represent the significance between normal control and chronic patient within each treatment. Capital letters represent the significance of normal control or chronic patient at different treatments. Error bars represent standard error (SE).

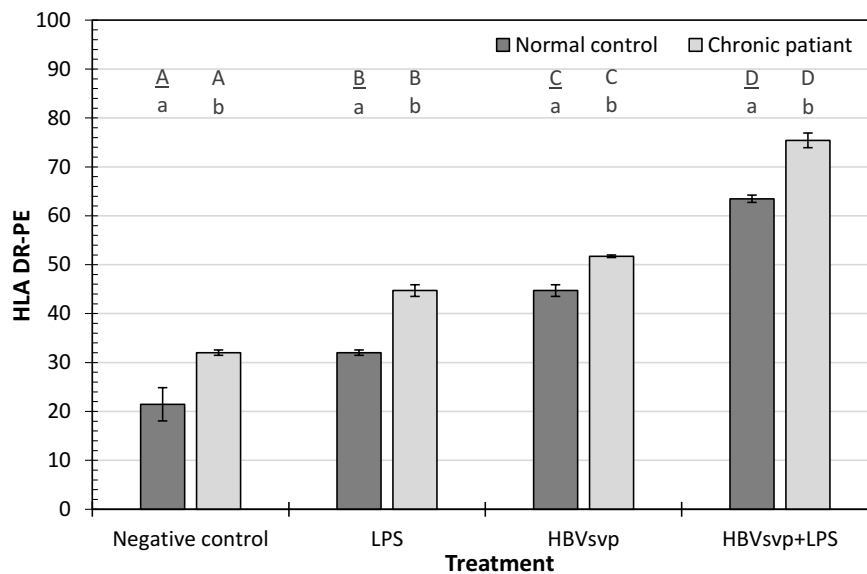


Figure 3 Showed HLA DR-PE activating marker of MoDCs detected in healthy donors and chronic patient groups at different treatment. Small letters represent the significance between normal control and chronic patient within each treatment. Capital letters represent the significance of normal control or chronic patient at different treatments. Error bars represent standard error (SE).

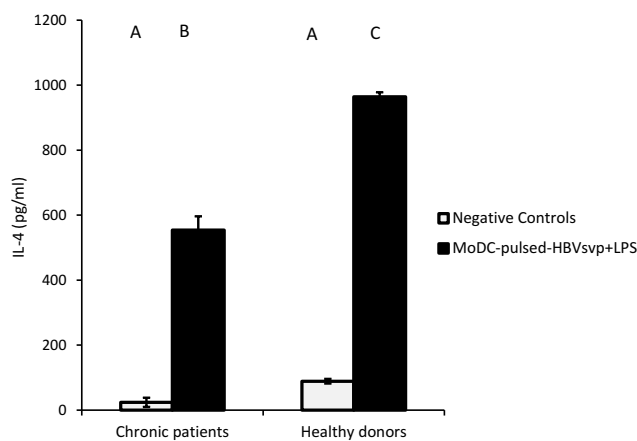


Figure 4 Showed MoDCs from healthy donors and chronic patients were secreted significantly larger amounts of the effector cytokine IL-4 into the culture medium when co-cultured with HBVsvp+ LPS. Capital letters represent the statistical significance difference between normal control and chronic patient at different treatments and/or within each treatment. Error bars represent standard error (SE).

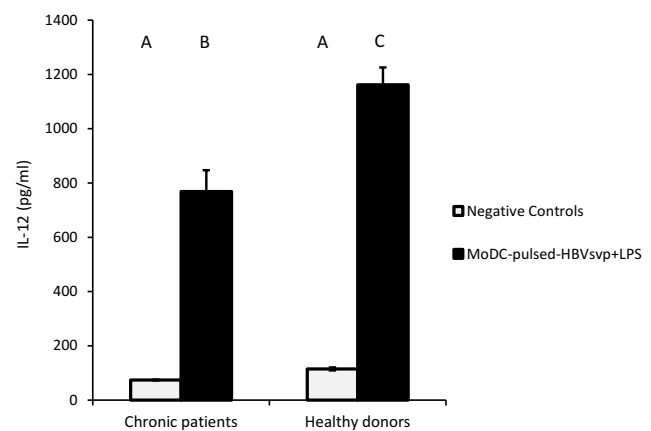


Figure 5 Showed MoDCs from healthy donors and chronic patients were secreted significantly larger amounts of the effector cytokine IL-12 into the culture medium when co-cultured with HBVsvp+ LPS. Capital letters represent the statistical significance difference between normal control and chronic patient at different treatments and/or within each treatment. Error bars represent standard error (SE).

elevated amounts of IFN- γ after co-culturing with MoDCs-pulsed-HBVsvp for chronic HBV patients ($p < 0.05$) and healthy donors ($p < 0.001$), in vitro (Figure 7).

MoDCs-Pulsed-HBVsvp More Efficiently Induced HBV-Specific CTL Responses in vitro

Our results showed that MoDCs-pulsed-HBVsvp were able to activate specific HBV-specific-CTL and led to very strong cytolytic capacity against HepG2.2.15, in vitro. As presented

in Figure 8, the cytolytic capacity of HBV-specific CTL against HepG2.2.15 at different ratios (5:1 or 10:1) was significantly increased for either chronic HBV patients ($p < 0.05$) or healthy donors ($p < 0.001$) compared to negative control. These data indicated that MoDCs-pulsed-HBVsvp efficiently induced an HBV-specific CTL which had specific cytotoxicity in healthy donors and CHB patients, in vitro.

Discussion

The molecular mechanisms required by viruses to escape host-specific immune responses and result in chronic states

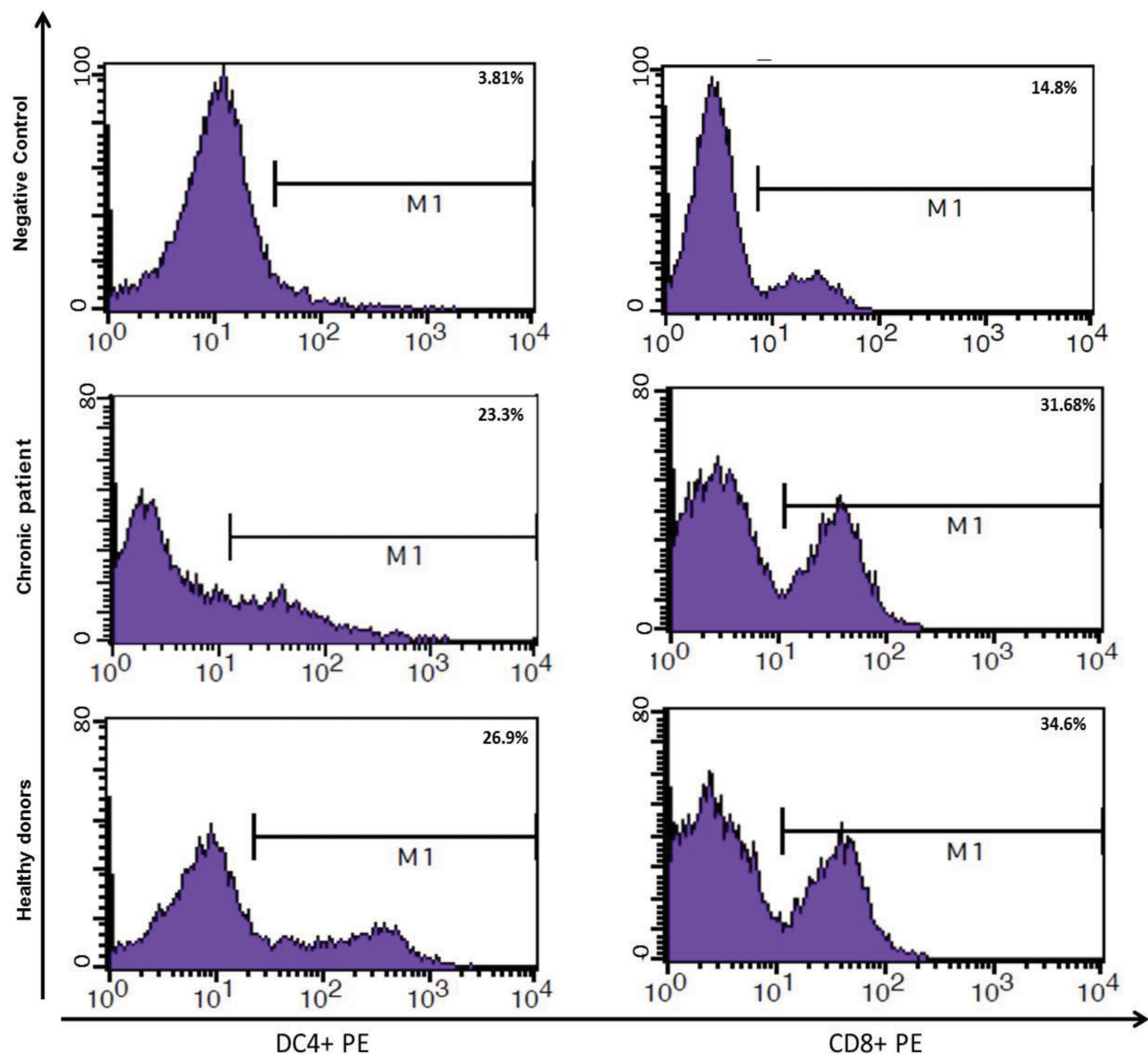


Figure 6 Showed ability of MoDCs-pulsed-HBVsvp to induce an autologous Th1/Tc polarization response in vitro for both healthy donors and chronic patients.

still need clarification. Many viruses have developed a broad range of molecular changes to escape the host-specific immune response.^{29,30} Many viral immune evade mechanisms target the function of DCs.^{13–17} Previous reports showed that in chronic HBV infection, DCs are more sensitive to HBV and the presence of HBV particles is associated with impaired functions of MoDCs.²³ Earlier studies confirmed that the response of CHB patients to antiviral treatments was correlated with the predominant Th1 immunity and associated with enhanced CTL activity, indicating Th1 immunity could be essential regulator for treatment of CHB patients.^{31,32} In the present study, we investigated the ability of MoDCs-pulsed-HBVsvp to

polarize Th1 cells and induce HBV-specific Cytotoxic T-Lymphocytes responses in both chronic HBV patients and healthy volunteers.

There is clear evidence that pathogens contribute to the production of defensive Th1 cells by their impacts on APCs.^{33–35} In our previous studies, we showed that mouse DC can specifically be activated in vitro against HBV and trigger immune responses, and the tolerance of HBV transgenic mice was broke down using DC-based immunization.^{21,22} In this study, we were able to prove that MoDCs can be triggered effectively with HBVsvp in vitro, which expressed high levels of costimulatory molecules such as CD86 and HLA-DR than negative

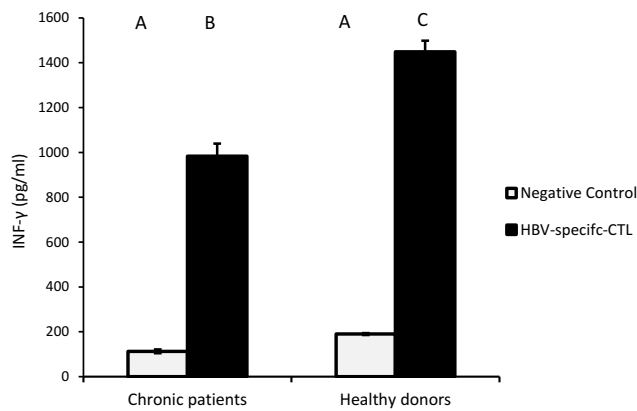


Figure 7 Showed HBV-specific-CTL from healthy donors and chronic patients were secreted significantly larger amounts of the effector cytokine IFN- γ into the culture medium when co-cultured with MoDCs-pulsed-HBVsvp. Capital letters represent the statistical significance difference between normal control and chronic patient at different treatments and/or within each treatment. Error bars represent standard error (SE).

control in healthy donors and chronic patients. Moreover, activation of MoDCs was highly significant in the presence of the co-stimulatory factor LPS. This finding was in consent with preceding studies that recorded higher levels of surface markers expression in HB-Ag-pulsed MoDCs,²⁴ and others have shown that the impaired DCs function could to some extent be restored by treating DC with pathogenic antigen in vitro.³⁶ In the present study, the HBsvp-pulsed DC vaccine developed much higher amounts of IL-4 and IL-12 than the DC control and was able to secrete IL-12 much more strongly and primarily

induce polarization of Th cells into Th1 and Tc cells. This result coincided with the previous study, showing that HB-Ag-pulsed MoDCs expressed higher cytokine levels.²⁴

It is known that naïve T cells can be primed by DC through three signals.^{37,38} The first signal results from antigen (peptide)-MHC complexes and assess immune antigen specificity, the second signal comes from costimulatory markers on the DC surface, the third signal is mediated by the cytokine balance or the OX40 ligand, and may decide polarization of naïve Th cells into Th1 or Th2 immune cells.^{37,38} HBV infection has been reported to have compromised MoDCs antigen-presenting function associated with Th1 immunity impairment, and that may play an important role in the processes of viral immune escape that results in persistent infection.¹² HBsAg pulsed DC vaccine has the potential to improve adaptive immunity against HBV, including a specific Th-cell and CTL response, to effectively control HBV infection. We found that both Th1 and Tc frequencies were strongly influenced by MoDCs-pulsed-HBVsvp, indicating the ability of MoDCs-pulsed-HBVsvp to induce an autologous Th1/Tc polarization response in vitro for both healthy subjects and chronic cases and confirmed the ability of HBVsvp to up-regulate the stimulatory capacity of the MoDCs. This finding agreed with previous studies that showed DC pulsed with HBV particles has been reported to effectively break CTL tolerance in transgenic HBV mice,³⁹⁻⁴¹ and inducing immune response among healthy donors.^{42,43}

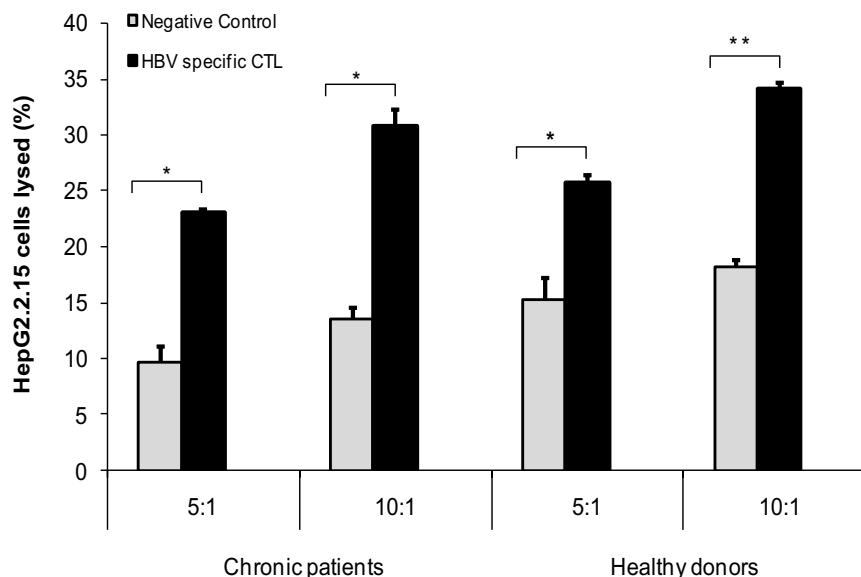


Figure 8 Showed the cytotoxicity assay for HBV-specific-CTL against HepG2.2.15 cell line in different ratio for different chronic patients and different healthy donors (** $p < 0.001$, * $p < 0.05$).

Preferably, HBV particles trigger a cellular response consisting of Th1 cells.⁴⁴ In the previous study, it was shown that a DC vaccine pulsed with HBcAg instead HBsAg efficiently induces HBV-specific CTLs in HBV-associated HCC patients;³⁶ therefore, in the present study, pulsation of MoDCs with HBVsvp from healthy donors and CHB patients developed an HBV-specific DC vaccine. In our study, MoDCs-pulsed-HBVsvp were effectively induced specific HBV-specific-CTL and led to very strong cytolytic capacity against target cell in vitro; however, the frequency of specific CTLs of CHB patients was lower than healthy donors. Further, MoDCs-pulsed-HBVsvp also induced the T cells to release high levels of IFN- γ to inhibit the HBV infection through their non-cytolytic action. Those findings indicate that priming of the CD8+ T cells from CHB patients and healthy donors with HBVsvp-loaded DCs recovered its cellular role of destruction of target cells infected with HBV. This result conformed to the previous study, which indicated that HB-Ag-pulsed MoDC can effectively induce specific CTL of HBV and recovered its function against the HBV-infected cells in vitro and secrete much more of IFN- γ from stimulated the T cells.²⁴

However, our data showed that MoDCs activation, Th1 and Tc frequencies, and cytokines secretion was significantly higher in the presence of LPS combined with HBVsvp. As adjuvant, LPS stimulant immune system to extremely trigger immune responses and could be indispensable to fully activate the mononuclear cells, in vitro.^{21,22,45,46} We had to choose LPS because it has been shown a potential in vaccination, wide application, easy storage, and with both carrier and adjuvant functions that activate MoDCs.⁴⁷ LPS frequently used in humans and different animal models with its safety consideration. Additionally, the use of LPS as a non-infectious stimulant adjuvant for activation of MoDCs is much less risk than using (adeno-) viral vectors.^{41,48}

To summarize, our findings demonstrate that MoDCs activation by HBVsvp can resolve the functionally impaired DCs and T cell in chronic HBV patients, suggesting that vaccination with ex vivo activated MoDCs could be a promising tool for therapeutic or prophylactic approaches against the HBV.

Abbreviations

Ags, antigens; APCs, antigen-presenting cells; CD86, cluster of differentiation 86; CHB, chronic hepatitis B virus; CTL, cytotoxic T-lymphocytes; DC, dendritic

cells; ELISA, enzyme linked immunosorbent assay; FACS, fluorescence-activated cell sorting; GMCSF, granulocyte macrophage colony-stimulating factor; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBVsvp, hepatitis B virus subviral particles; HLA-DR, human leukocyte antigen-DR isotype; IFN- γ , interferon gamma; IL-4, interleukin 4; IL-12, interleukin 12; LPS, lipopolysaccharide; MoDCs, monocyte-derived DCs.

Ethics Approval and Consent

Informed written consent was obtained from all participants included in the study. The consent form and the research proposal were approved by the ethical committee at the CCHE and the liver institute(s) that the CHB patients were recruited, and the research was conducted in accordance with the principles of the Declaration of Helsinki.

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Author Contributions

All authors contributed to study design, execution, acquisition of data, analysis and interpretations, drafting and revising the manuscript, giving final approval of the version to be published, and agree to be accountable for all aspects of the work and individually responsible for its content.

Disclosure

The authors report no conflicts of interest in this work.

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