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# Immunization with lentiviral vector-modified dendritic cells encoding ubiquitinated hepatitis B core antigen promotes Th1 differentiation and antiviral immunity by enhancing p38 MAPK and JNK activation in HBV transgenic mice

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**Abstract.** Hepatitis B virus (HBV) infection is a global public health problem. T helper (Th)1-associated cytokines are involved in HBV clearance during acute and persistent infection. In our previous study, it was demonstrated that lentiviral vectors encoding ubiquitinated hepatitis B core antigen (LV-Ub-HBcAg) effectively transduced dendritic cells (DCs) to induce maturation, which promoted T cell polarization to Th1 and generated HBcAg-specific cytotoxic T lymphocytes (CTLs) *ex vivo*. In the present study, HBV transgenic mice were immunized with LV-Ub-HBcAg-transduced DCs and HBcAg-specific immune responses were evaluated. Cytokine expression was analyzed by ELISA. T lymphocyte proliferation was detected with a Cell Counting Kit-8 assay and HBcAg-specific CTL activity was determined using a lactate dehydrogenase release assay. The expression levels of p38-mitogen-activated protein kinase (p38-MAPK), phosphorylated (p)-p38MAPK, c-Jun N-terminal kinase (JNK) and p-JNK were detected by western blot analysis. The results demonstrated that LV-Ub-HBcAg-transduced DCs significantly increased the Th1/Th2 cytokine ratio, and effectively reduced the levels of serum hepatitis B surface antigen (HBsAg), HBV DNA, and liver HBsAg and HBcAg. Furthermore, the LV-Ub-HBcAg-transduced DCs upregulated the expression of p-P38-MAPK and p-JNK in T lymphocytes. In conclusion, the present study indicated that LV-Ub-HBcAg-transduced DCs generated predominant Th1 responses and enhanced CTL activity in HBV transgenic mice. Activation of the P38-MAPK/JNK signaling pathway may be involved in this induction.

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*Key words:* dendritic cell, hepatitis B core antigen, lentiviral vector, P38-mitogen-activated protein kinase/c-Jun N-terminal kinase signaling pathway, ubiquitin

## Introduction

It is reported that ~250,000,000 individuals worldwide currently have chronic hepatitis B (CHB) infection and are at risk of developing liver cirrhosis, hepatic decompensation and hepatocellular carcinoma (1-3). Furthermore, CHB infection can be life-threatening in certain cases (1-3). Viral replication and host immune responses have been shown to determine the clinical outcomes and viral persistence in patients with CHB (4,5). The eradication of HBV infection and life-long anti-HBV immunity can be monitored following the acute infection period of HBV, which occurs through the development of a robust innate or adaptive immune response (6,7). $CD4^+$ T cell priming and subsequent development of effective $CD8^+$ cytotoxic T lymphocyte (CTL) responses contribute to viral clearance (8). However, patients with CHB have weak or functionally impaired $CD4^+$ and $CD8^+$ T cell immune responses (4). Consequently, novel immunotherapeutic strategies are required to enhance HBV-specific T cell responses.

Dendritic cell (DC)-based therapeutic vaccines are essential in linking DCs and T lymphocytes and provide approaches to reactivate HBV-specific host immunity against HBV. As the most dominant antigen-presenting cells (APCs) (9), DCs are the main cells involved in antigen presentation and activation of T cells, which are important in the development of adaptive immune responses. DCs can prime naïve T helper (Th) cells to polarize into Th1 or Th2 cells. Various methods have been designed to load DCs with viral antigen genes in order to induce specific immune responses, for example, peptide- or protein-pulsed DCs (10,11). However, there are certain limitations of these types of vaccine strategies, including the short duration in antigenic epitope presentation and the limited effect of the resulting immune responses. Lentiviral vectors (LVs) are mostly based on human immunodeficiency virus-l and represent effective gene carriers. LVs can stably integrate large antigenic genes into DCs, leading to the continuous

expression of the related genes. Gene-modified DCs may mediate enhanced antigen-specific immune responses (12).

Therapeutic vaccines that are based on hepatitis B core antigen (HBcAg) can polarize Th1 cells and elicit high levels of HBcAg-specific CTLs in HBV transgenic mice (13,14). HBcAg-specific CTLs have been shown to suppress HBV replication and alleviate liver damage (13,14). Ubiquitin (Ub) is a conserved protein in eukaryotic cells, which serves as a signal for the target protein to be recognized and degraded through the ubiquitin-proteasome system (UPS) during proteolysis (15). In our previous study, LVs were used to encode the ubiquitinated HBcAg (LV-Ub-HBcAg), and it was demonstrated that LV-Ub-HBcAg promoted the maturation of DCs, which induced T cell polarization to Th1 cells and the production of HBV-specific CTLs *in vitro* (16). The present study aimed to investigate whether DCs transduced with LV-Ub-HBcAg can enhance the polarization of Th1 cells and elicit HBV-specific T cell responses in HBV transgenic mice.

## Materials and methods

*Mice*. H-2K$^d$ HBV-transgenic BALB/c mice (half male and half female) were obtained from the Key Liver Army Laboratory of No.458 Hospital (Guangzhou, China). They were 6-8 weeks old (20-23 g) and their characteristics were as described previously (17,18). All mice were housed under specific pathogen-free conditions (22-24˚C; humidity 50-55%; 12 h light/dark cycle), with free access to food and water. in the Experimental Animal Centre of the Sixth Hospital affiliated to Shanghai Jiao Tong University (Shanghai, China). The study protocol was performed according to the guidelines established by the Laboratory Animal Ethics Committees of Shanghai Jiao Tong University.

*Recombinant lentiviral vector preparation and DC transduction*. The recombinant lentiviral vectors encoding Ub-HBcAg and/or HBcAg were constructed as described previously (19). The lentiviral particles (LV-Ub-HBcAg and LV-HBcAg or LV) were prepared in 293T cells (American Type Culture Collection, Manassas, VA, USA) and tittered as described previously (19). Briefly, the recombinant pLOV.UBC.Ub-HBcAg.EGFP.3FLAG vector plasmid was constructed by inserting the Ub-HBcAg fragment into the *Bam*HI and *Nhe*I sites of the pLOV.UBC.EGFP.3FLAG plasmid (Obio Technology Corp., Ltd., Shanghai, China). The control plasmid was constructed by inserting the HBcAg fragment. Lentiviral particles of LV-Ub-HBcAg, LV-HBcAg or LV were produced by triple transfection of 80% confluent 293T cells with pLOV.UBC.Ub-HBcAg.EGFP.3FLAG, pLOV.UBC.HBcAg.EGFP.3FLAG or pLOV.UBC.EGFP.3FLAG using the pHelper 1.0 and pHelper 2.0 helper plasmids (Obio Technology Corp., Ltd.) using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Murine DCs were generated according to the protocol described by Chen *et al* (16). The DCs were transduced by the LVs (LV-Ub-HBcAg, LV-HBcAg, or LV) at a multiplicity of infection of 20 as described in our previous study (19).

*Mice immunization*. The HBV transgenic mice were randomly divided into six groups, with six mice in each group. Subcutaneous immunization of the mice was performed twice at an interval of 2 weeks with 5x10$^5$ recombinant lentiviral-transduced DCs (LV/DC, LV-HBcAg/DC, or LV-Ub-HBcAg/DC), 20,000 IU interferon (IFN)-α (Roche Diagnostics, Basel, Switzerland) or 50 $\mu$g HBcAg (CalBioreagents, Inc., San Mateo, CA, USA). Mice injected with PBS and/or untransduced DCs served as the controls (Fig. 1).

*Assessment of the levels of the cytokines*. The splenocytes were harvested 2 weeks following the final immunization, and were seeded in 24-well plates (2x10$^6$ cells/ml) in the presence of 10 $\mu$g/ml HBcAg. Following 72 h of incubation in RPMI 1640 culture medium (Invitrogen; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 g/ml streptomycin at 37˚C in a humidified atmosphere with 5% $CO_2$, the levels of IFN-γ, interleukin (IL)-2, tumor necrosis factor (TNF)-α, IL-4 and IL-10 in the supernatants were measured using commercial mouse cytokine ELISA kits (cat. nos. MIF00, M2000, MTA00B, M4000B and M1000B; R&D Systems, Inc., Minneapolis, NN USA), according to the manufacturer's protocols. The results are expressed as pg/ml.

*Detection of T lymphocyte proliferative activity and CTL response*. The T lymphocytes were separated from the splenocytes using nylon wool columns (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) according to the method described previously by Chen *et al* (20). The Cell Counting Kit-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was utilized to detect T lymphocyte proliferation. The T lymphocytes were seeded at a density of 4x10$^5$ cells/well in a final volume of 200 $\mu$l in 96-well culture plates. The cells were subsequently co-cultured with 1 $\mu$g of ConA solution at 37˚C with 5% $CO_2$. Following 48 h of incubation, 20 $\mu$l of Cell Counting Kit-8 reagent was added to the plates, and the plates were incubated at 37˚C with 5% $CO_2$ for 4 h. The absorbance was recorded at 450 nm.

P815/c cells (expressing HBcAg; Nanjing Medical University, Nanjing, China) were maintained in our laboratory (Department of Infectious Disease, Shanghai Jiao Tong University Affiliated Sixth People's Hospital) and used as target cells. T lymphocytes were used as the effector cells and incubated (RPMI1640 culture medium containing 10% FBS, 100 U/ml penicillin, and 100 g/ml streptomycin) with the P815/c cells at different effector and target (E/T) ratios (5:1,10:1, and 20:1) at 37˚C with 5% $CO_2$ for 4 h. HBcAg-specific CTL activity was determined using a lactate dehydrogenase release assay according to the protocol of the CytoTox 96® Non-radioactive cytotoxicity kit (Promega Corporation, Madison, WI, USA). The absorbance values of the sample supernatants were recorded at 490 nm. The cytotoxicity (%) was calculated as follows: [(experimental release-effector spontaneous release-target spontaneous release)/(target maximum release-target spontaneous release)] x100% (16,21).

*Serological analysis*. The serum was harvested 2 weeks following the first and final immunization. Serum HBsAg and HBV DNA levels were measured respectively using Abbott kits (Abbott Diagnostics, Chicago, IL, USA) and quantitative polymerase chain reaction (qPCR; Terra PCR Direct

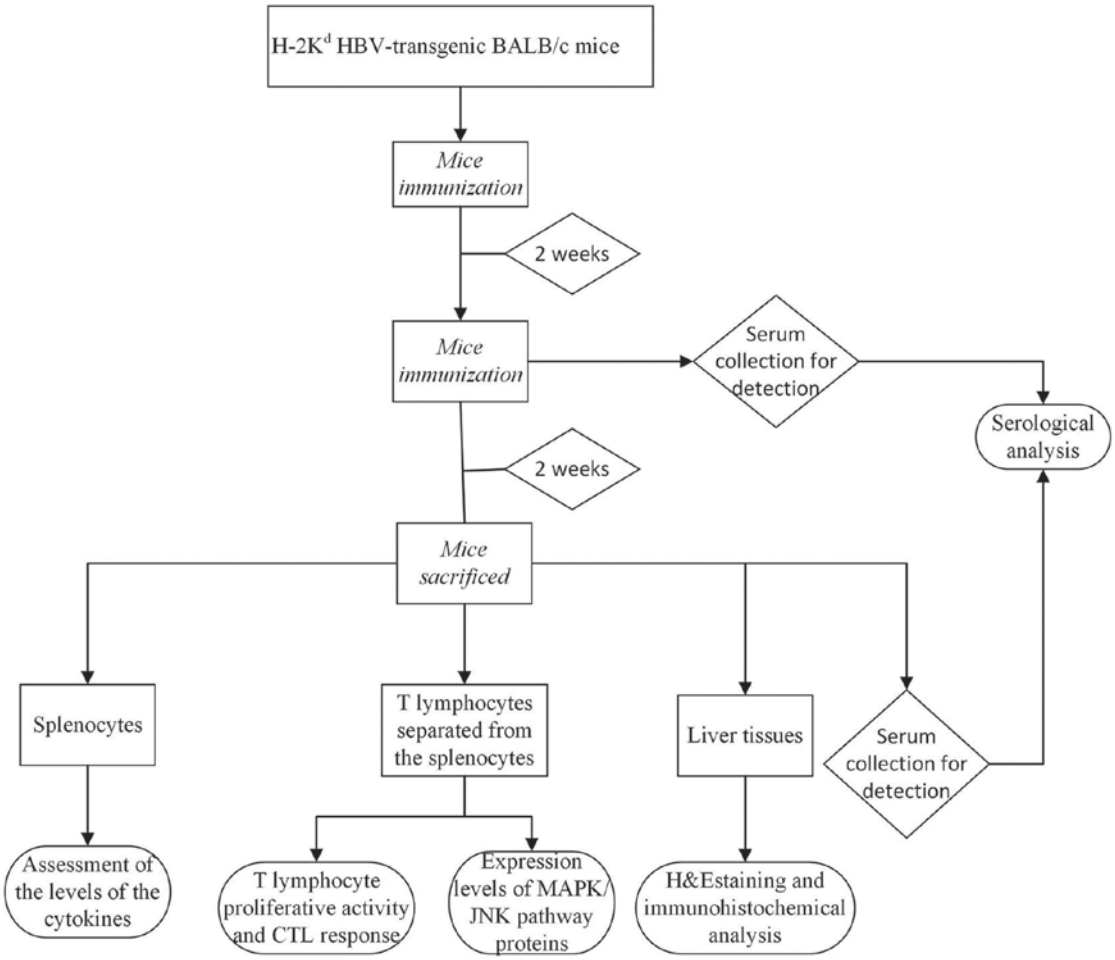


Figure 1. Flowchart of the overview of the experiment. CTL, cytotoxic T lymphocyte; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; H&E, hematoxylin and eosin.

Polymerase mix, Clontech Laboratories, Inc., Mountainview, CA, USA) (22). Primer sequences (sequences unavailable) were synthesized and provided by DaAn Gene Co., Ltd. of Sun Yat-sen University (Guangzhou, China). Fluorescence was measured using a LightCycler 600 real-time fluorescence quantitative PCR instrument (Roche Diagnostics, Basel, Switzerland). The thermocycling conditions were as follows: 93˚C for 7 min; 10 cycles of 93˚C for 45 sec and 55˚C for 1 min; 30 cycles of 93˚C for 30 sec and 55˚C for 45 sec; then 40˚C for 20 sec. The $2^{-\Delta\Delta Cq}$ method was used to quantify the results (23). Furthermore, the levels of alanine aminotransferase (ALT) and aspartate transaminase (AST) in the serum were detected using the ARCHITECT Automatic Biochemistry Analyzer (Abbott Diagnostics, Abbott Park, IL, USA).

*Hematoxylin and eosin (H&E) staining and immunohistochemical analysis of the liver.* The liver tissues were fixed in paraformaldehyde solution (4%), embedded in paraffin and sectioned (5 $\mu$m). For histological analysis, the de-paraffinized sections were stained with H&E (Beyotime Institute of Biotechnology, Jiangsu, China). For immunohistochemical analysis, the de-paraffinized sections were treated with 0.3% $H_2O_2$ for 10 min in order to inactivate the endogenous peroxidase. The sections were blocked with 2% goat serum (Beyotime Institute of Biotechnology) for 30 min at room temperature and washed with PBS. Mouse anti-HBsAg (cat. no. NB110-62652; 1:500 dilution) and/or anti-HBcAg (cat. no. NB100-64452; 1:500 dilution) monoclonal antibodies (Novus Biologicals, LLC, Littleton, CO, USA) were added overnight at 4˚C. Following washing with PBS, the sections were stained with secondary antibodies (cat. no. BA1001; 1:1,000 dilution; Boster Biological Technology, Wuhan China) for 30 min at 37˚C and then with streptavidin-biotin-peroxidase complex for 30 min. The sections were subsequently visualized with diaminobenzidine (Boster Biological Technology) and counterstained with hematoxylin under a Nikon light microscope (Nikon Corporation, Tokyo, Japan).

*Detection of expression levels of MAPK/JNK pathway proteins.* The T lymphocytes were lysed in RIPA lysis buffer containing protease inhibitor mixture (Beyotime Institute of Biotechnology, Jiangsu, China). A Pierce BCA protein assay reagent kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to assess the protein concentration levels. The protein lysates (30 $\mu$g/ml) were separated by 10% SDS-PAGE and subsequently transferred onto a PVDF membrane (EMD Millipore, Bedford, MA, USA). Rabbit anti-p38MAPK (cat. no. 8690; 1:1,000 dilution), phosphorylated (p)-p38MAPK (cat. no. 4511; 1:1,000 dilution), JNK (cat. no. 9258; 1:1,000 dilution) and p-JNK (cat. no. 4668; 1:1,000 dilution) monoclonal

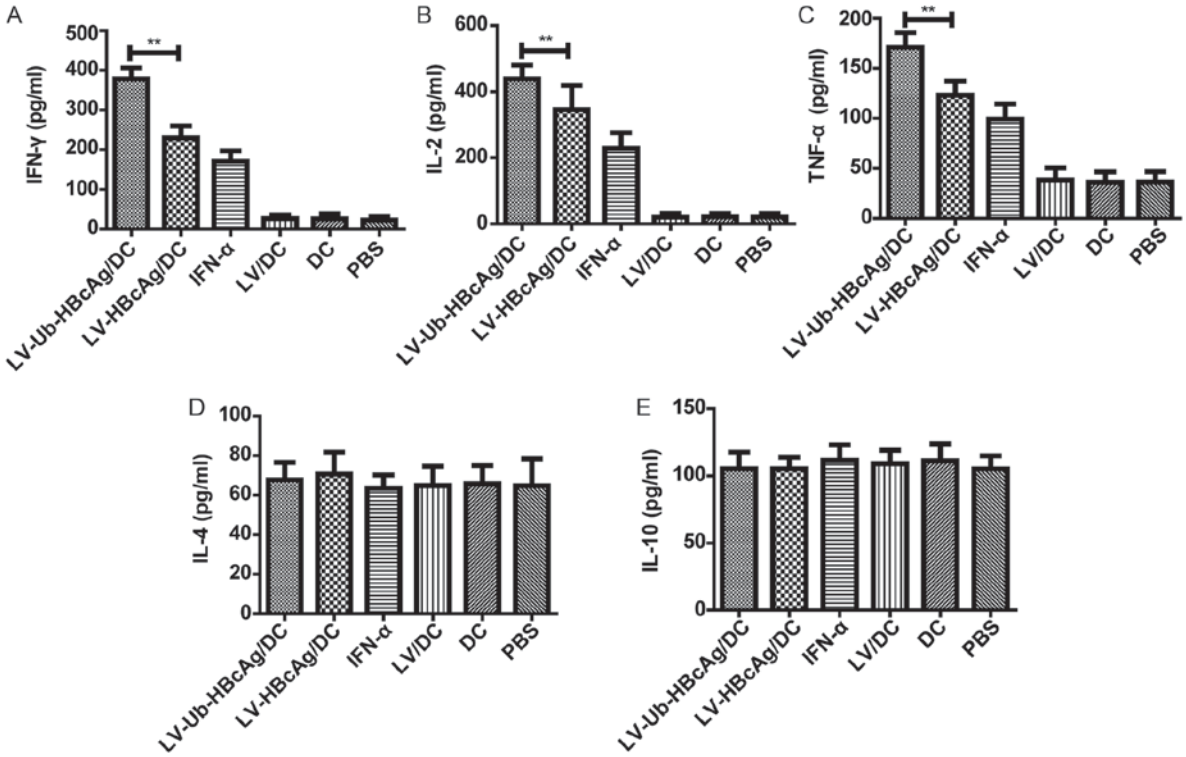


Figure 2. Expression levels of the IFN-γ, IL-2, TNF-α, IL-4, and IL-10 cytokines. The levels of (A) IFN-γ, (B) IL-2 and (C) TNF-α in the supernatants of the splenocytes from the group of LV-Ub-HBcAg/DC-immunized HBV transgenic mice were significantly higher than those detected in the other groups ($^{**}$P<0.01). The levels of (D) IL-4 and (E) IL-10 in the supernatants of splenocytes from immunized mice did not differ significantly among the groups. The data are representative from at least three independent experiments, and presented as the mean ± standard deviation (n=6). IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; LV, lentivirus; HBcAg, hepatitis B core antigen, Ub-HBcAg, ubiquitinated HBcAg; DC, dendritic cell; PBS, phosphate-buffered saline.

antibodies were used as primary antibodies. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin-G (cat. no. 7074; 1:2,000 dilution) was used as secondary antibody. All antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The protein bands were visualized by enhanced chemiluminescence (Beyotime Institute of Biotechnology) and analyzed using Image Pro Plus version 6.0 (Media Cybernetics, Inc., Rockville, MD, USA).

*Statistical analysis*. The data are presented as the mean ± standard deviation and each value was obtained from at least three independent experiments. One-way analysis of variance and a post-hoc least significant difference test were used to determine the statistical significance compared with the control samples. The data were analyzed using SPSS 19.0 software (IBM SPSS, Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

## Results

*LV-Ub-HBcAg/DC stimulates the secretion of Th1-like cytokines*. The present study assayed the splenocytes from immunized animals for the secretion of cytokines, namely IFN-γ, IL-2, TNF-α (Th1-like), IL-4 and IL-10 (Th2-like), upon restimulation with HBcAg (10 μg/ml). T cells from the LV-Ub-HBcAg/DC groups produced higher levels of IFN-γ (369.71±13.04 pg/ml), IL-2 (422.85±10.91 pg/ml) and TNF-α (171.02±14.68 pg/ml), compared with the other groups (Fig. 2A-C). However, no significant difference was found in the production of IL-4 or IL-10 (Th2-like) between the groups examined (Fig. 2D and E).

*LV-Ub-HBcAg/DC enhances T cell proliferation and the specific CTL response*. The activity of T lymphocyte proliferation from the immunized animals was examined. T lymphocyte proliferative activity in the LV-Ub-HBcAg/DC group was higher compared with that in the other groups (P<0.01; Fig. 3A). The CTL response was further assessed in the different groups. The LV-Ub-HBcAg/DC group induced higher percentages of specific cytolysis at E:T ratios of 20:1, 10:1 and 5:1, respectively, compared with the other groups (P<0.05; Fig. 3B).

*LV-Ub-HBcAg/DC decreases the serum HBsAg and HBV DNA levels and enhances ALT and AST levels*. Serum was collected from the immunized mice 2 weeks following the first and the final immunization. The aim of the subsequent experiments was to evaluate whether LV-Ub-HBcAg/DC was able to clear HBV in the mice. The LV-Ub-HBcAg/DC group exhibited decreased levels of serum HBsAg and HBV DNA titer compared with the other groups (Fig. 4A and B). Furthermore, the ALT and AST levels were assessed in the serum 2 weeks following the final immunization of the mice, and the levels of serum ALT and AST in the LV-Ub-HBcAg/DC group were higher compared with those in the other groups (Fig. 4C and D).

*LV-Ub-HBcAg/DC increases the inflammatory reaction and reduces the expression of HBsAg and HBcAg in liver tissue*. The

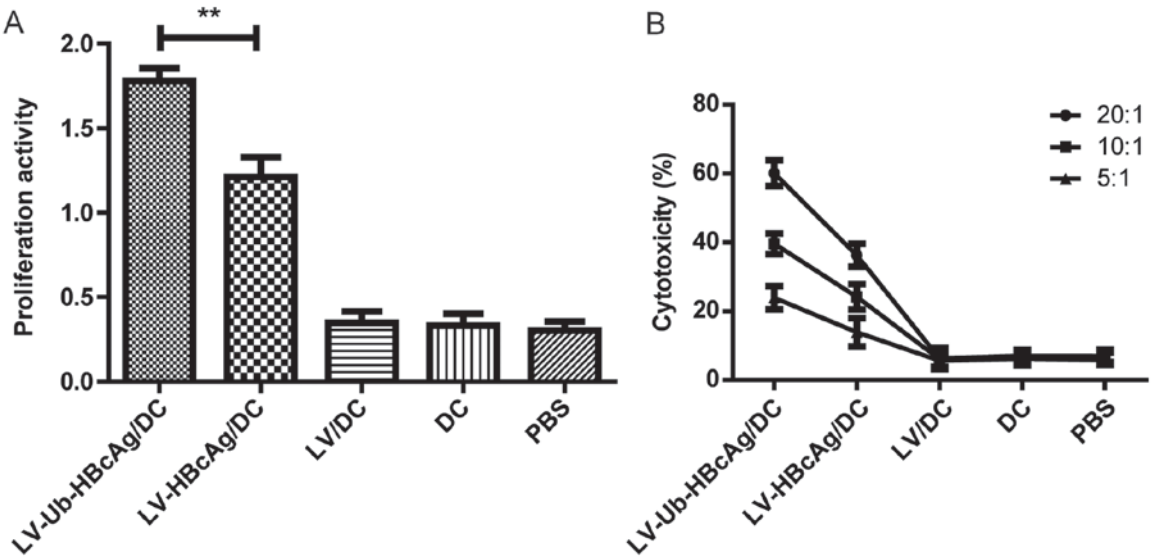


Figure 3. T lymphocyte proliferative activity and CTL response assay. (A) Proliferative activity of T lymphocytes was evaluated using the Cell Counting Kit-8. The absorbance values were recorded at 450 nm. (B) Specific CTL activity was detected using the lactate dehydrogenase release assay; CTL activity is indicated as the percentage of specific lysis at different effector: target ratios (20:1, 10:1, and 5:1). The data are representative of at least three independent experiments and presented as the mean ± standard deviation (n=6). **P<0.01. CTL, cytotoxic T lymphocyte; LV, lentivirus; HBcAg, hepatitis B core antigen, Ub-HBcAg, ubiquitinated HBcAg; DC, dendritic cell; PBS, phosphate-buffered saline.

H&E-stained sections of liver tissues were observed in order to evaluate the histological changes. A higher level of lymphocyte infiltration was observed in the liver of mice immunized with LV-Ub-HBcAg/DC compared with the other groups (Fig. 5A). To further evaluate whether LV-Ub-HBcAg/DC resulted in viral clearance in transgenic mice, the therapeutic effects of this agent were detected by immunohistological analysis in the liver tissues from the immunized mice. The results suggested that LV-Ub-HBcAg/DC immunization reduced the expression levels of HBsAg in the cytoplasm and the expression levels of HBcAg in the nuclei (stained brownish yellow) compared with those noted in the other groups (Fig. 5B and C).

*LV-Ub-HBcAg/DC enhances the expression levels of p-P38-MAPK and p-JNK.* LV-Ub-HBcAg/DC affected the MAPK/JNK signaling pathway. To investigate the underlying mechanism, the expression levels of P38-MAPK, p-P38-MAPK, JNK and p-JNK were analyzed in the T cells derived from the LV-Ub-HBcAg/DC, LV-HBcAg/DC, LV/DC and PBS mice groups. The results demonstrated that p-P38-MAPK and p-JNK were significantly upregulated in the LV-Ub-HBcAg/DC group compared with the corresponding protein expression in the other groups (P<0.01; Fig. 6).

## Discussion

In the present study, DCs transduced with LV-Ub-HBcAg were used to immunize HBV transgenic mice. Th1-type (IFN-γ, IL-2 and TNF-α) and Th2-type (IL-4 and IL-10) cytokines were then assessed as an indicator of the Th1/Th2 immune balance. The T cell proliferation and specific CTL activities were also assessed. In addition, to evaluate the specific anti-HBV activity *in vivo*, the levels of HBsAg and HBV DNA in the serum were detected, and the expression levels of HBcAg and HBsAg in liver tissues were examined. Furthermore, the protein expression levels of the P38-MAPK/JNK pathway were analyzed by western blot analysis to examine the mechanism of Th cell differentiation. The results indicated that LV-Ub-HBcAg/DC induced Th1 cell differentiation and potent HBV-specific T cell responses in mice. The data also suggested that the P38-MAPK/JNK pathway may be associated with the differentiation of Th cells.

The control of HBV replication and the clinical outcome of HBV infection depend on the immunity of the host. The resolving infection of HBV results in multiple HBV antigen-specific $CD8^+$ and $CD4^+$ T cell responses (24,25) which are associated with viral clearance (26). However, patients with CHB have impaired and dysfunctional HBV-specific T cell responses (27-29). The specific T cell responses are mediated by APCs, and DCs are considered the most potent APCs of the immune system (30).

DCs are important in inducing immunity against viral infections (31). DC-based therapeutic vaccines pulsed with HBV antigens can boost HBV-specific adaptive immunity, including specific $CD4^+$ and $CD8^+$ T cell immune responses (31). LVs are efficient delivery systems that are used as vaccine vectors to elicit protective T cell immune responses in infectious diseases and cancer (32). LVs have the ability to transduce antigens into DCs and generate specific immune responses (32). Accumulated data have shown that patients with CHB have impaired function of DCs and are at an immunocompromised state of immune tolerance (33-35). Our previous study demonstrated that LV-Ub-HBcAg can induce DC maturation and improve DC function *in vitro* (16). Our previous studies have shown that the production of Ub-HBcAg can promote HBcAg degradation into antigenic peptides by the UPS (16,19). These peptides are presented by DCs and are readily recognized by $CD8^+$ T cells, whereas the activation of HBV-specific $CD8^+$ T cells is critical for HBV control (36,37). In the present study, it was demonstrated that treatment with

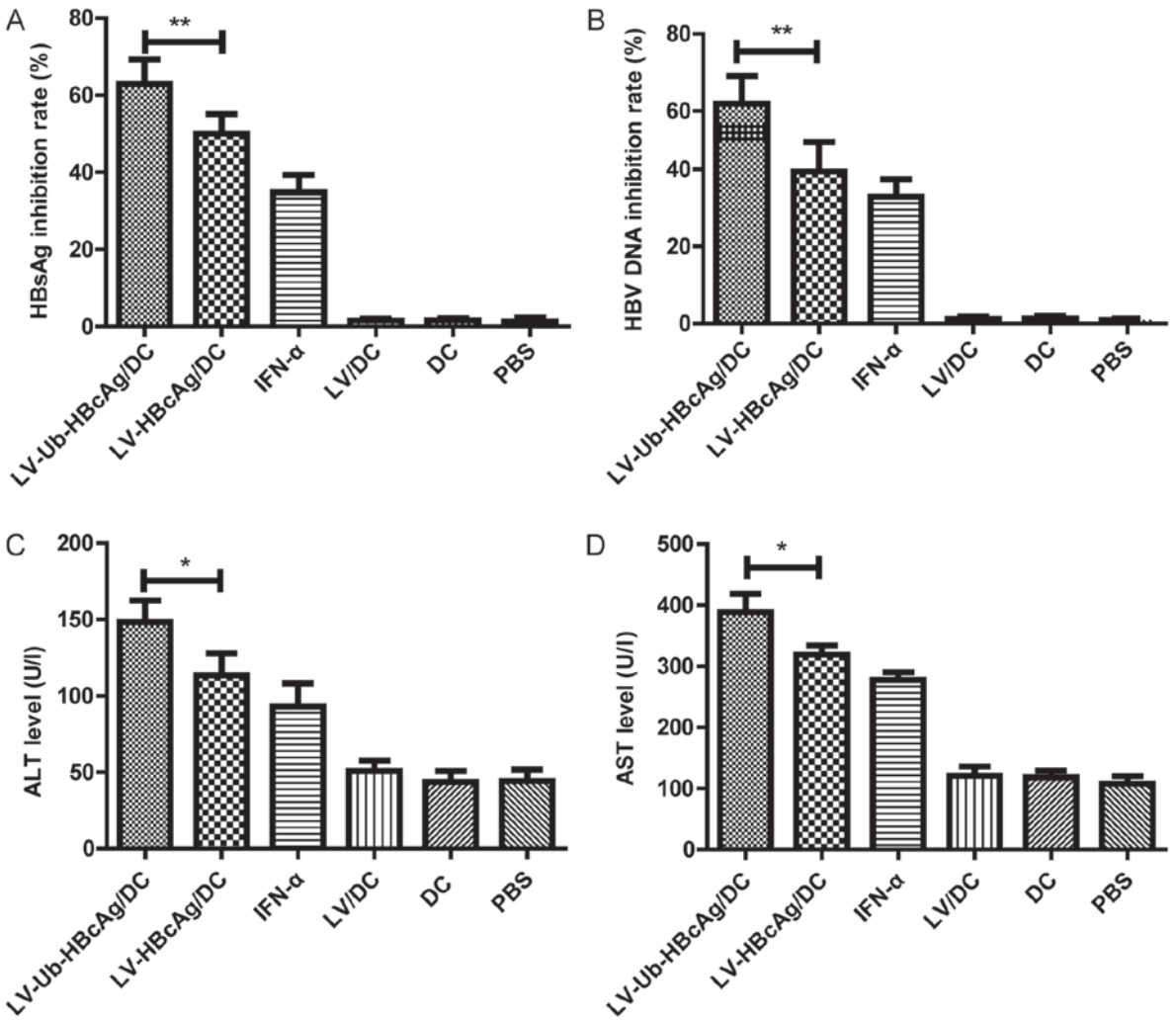


Figure 4. Suppression of serum levels of HBsAg, HBV DNA, ALT and AST in immunized HBV transgenic mice. (A) HBsAg and (B) HBV DNA inhibitory rates in the serum samples from immunized mice were evaluated. (C) ALT and (D) AST levels in the mice were detected 2 weeks following the final immunization. The data are presented as the mean ± standard deviation (n=6). $^{*}$P<0.05, $^{**}$P<0.01. LV, lentivirus; HBcAg, hepatitis B core antigen, HBsAg, hepatitis B surface antigen; Ub-HBcAg, ubiquitinated HBcAg; HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate transaminase; DC, dendritic cell; IFN, interferon; PBS, phosphate-buffered saline.

DCs transduced by LV-Ub-HBcAg increased the secretion of Th1-like cytokines (IFN-γ, IL-2 and TNF-α) and induced HBcAg-specific CTL activity.

The present study further evaluated whether LV-Ub-HBcAg/DC immunization can generate antiviral immunity. HBV transgenic mice are used as a model of chronic HBV infection, appropriate to assess the efficacy of treatment strategies. The findings of the present study demonstrated that the inflammatory reaction in the liver was consistent with specific CTL activity induced by LV-Ub-HBcAg/DC. LV-Ub-HBcAg/DC reduced serum HBsAg and HBV DNA levels in the HBV transgenic mice and the expression of HBsAg and HBcAg in the liver tissue. These data indicated that LV-Ub-HBcAg/DC induced anti-HBV activity *in vivo*.

A number of studies have demonstrated the presence of predominant Th1 responses and increased CTL activity in patients with CHB who respond positively to antiviral therapy (37); therefore, the activation of Thl responses may be critical for the successful treatment of HBV (37,38). The findings of the present study highlighted that immunization with DCS that were modified by LV-Ub-HBcAg effectively promoted the secretion of IFN-γ, IL-2 and TNF-α (Th1-like). Preferential priming of anti-HBcAg Th1 immunity was evident. Several signaling pathways are required for Th1 cell differentiation. Certain studies have established that the P38 and JNK MAPK pathways are selectively induced during the activation of Th1 effector cells and are required for Th1 immune responses (39,40). MAPK pathways have an important regulatory role in the proliferation and migration of T cells (41,42). In the present study, LV-Ub-HBcAg/DC increased the production of IFN-γ, which activated MAPK signaling. The protein expression of the P38MAPK/ JNK pathway was analyzed by western blot analysis. The results revealed that the expression levels of p-P38MAPK and p-JNK were significantly upregulated in the LV-Ub-HBcAg/DC-immunized mice group compared with those in the other groups. It was noted that the difference in the production of IL-4 and IL-10 (Th2-like) between these groups was not statistically significant. Cell metabolism and the cellular environment may be artificially altered during the processes of cell stimulation or isolation, whereas the levels of the cytokines in these cells measured using ELISA may not

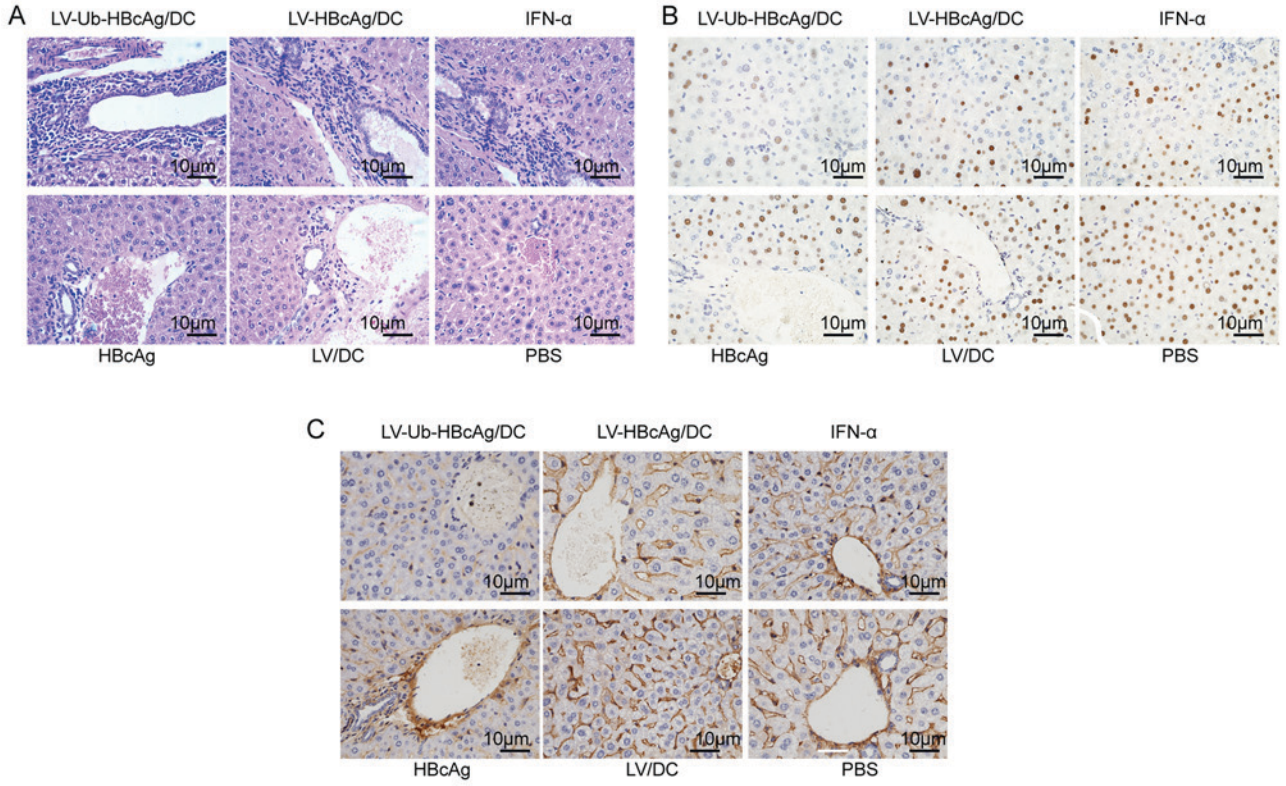


Figure 5. Histological analysis and immunohistochemical staining for HBcAg and HBsAg in liver sections of HBV transgenic mice. The mice were treated with PBS, LV/DC, HBcAg, IFN-α, LV-HBcAg/DC or LV-Ub-HBcAg/DC. (A) Liver sections were stained with hematoxylin and eosin and inflammatory infiltrates were observed by light microscopy. Immunohistochemical staining for (B) HBcAg and (C) HBsAg was performed. Representative images are shown (original magnifications, x400). LV, lentivirus; HBcAg, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; Ub-HBcAg, ubiquitinated HBcAg; DC, dendritic cell; IFN, interferon; PBS, phosphate-buffered saline.

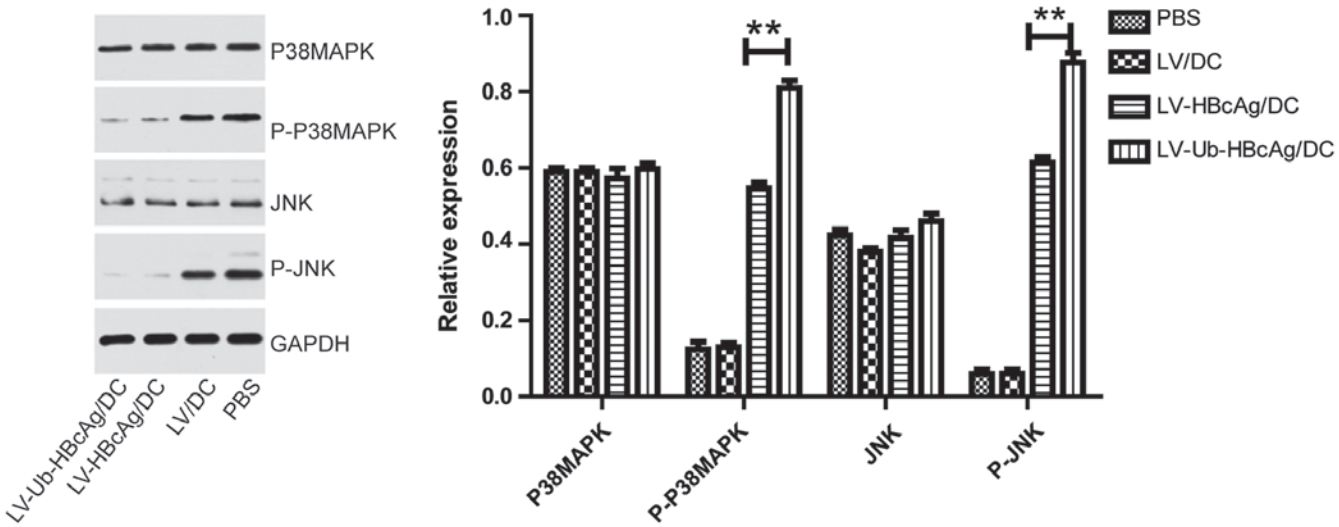


Figure 6. Expression levels of MAPK/JNK pathway proteins. The expression levels of p-P38MAPK and p-JNK were significantly upregulated in mice immunized with LV-Ub-HBcAg/DC. The data are presented as the mean ± standard deviation (n=6, $^{**}$P<0.01). LV, lentivirus; HBcAg, hepatitis B core antigen, Ub-HBcAg, ubiquitinated HBcAg; DC, dendritic cell; IFN, interferon; PBS, phosphate-buffered saline; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; p-, phosphorylated.

reflect the apparent biological levels *in vivo* (43). Further investigations may use more effective detection methods of these biomarkers. The present study did not conclude that Th2-like cytokines were associated with the P38MAPK/JNK pathway. The results indicated that the P38MAPK/JNK pathway may be associated with the differentiation of Th cells, which requires additional confirmation using specific inhibitors of the signaling pathway examined.

Despite the emergence of novel antiviral drugs for the treatment of patients with CHB, sustained off-treatment responses have rarely been achieved. In addition, the maintenance of antiviral therapy with currently available

antiviral drugs in patients with CHB is associated with the long-term risk of viral resistance and drug toxicity (44,45). The data obtained in the present study indicated that immunization with DCs modified with LV-Ub-HBcAg may be a promising candidate for the treatment of CHB. In conclusion, immunization with DCs modified with LV-Ub-HBcAg induced predominant Th1 responses and antiviral immunity in HBV transgenic mice. The activation of the P38-MAPK/JNK signaling pathway may be involved in this induction. These results support the conclusion that vaccination with LV-Ub-HBcAg/DC may be a potential therapeutic strategy for HBV clearance.

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**Availability of data and materials**

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

**Authors' contributions**

GZ and ZT conceived and designed the study. SD, XC and YY performed the experiments. SD wrote the paper. GZ, ZT, XC and YY reviewed and edited the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The study protocol was performed according to the guidelines established by the Laboratory Animal Ethics Committees of Shanghai Jiao Tong University (Shanghai, China).

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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