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Engineering enhancement of the immune response to HBV DNA vaccine in mice by the use of LIGHT gene adjuvant

Yali Zhang, Wenzheng Jiang*, Yan Fan, Jiejun Wen, Wenli Hao, Min Qian

School of Life Science, East China Normal University, Shanghai 200062, People's Republic of China

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DNA vaccines could induce protective immune responses in several animal models. Many strategies have been employed to improve the effect of nucleic acid vaccines. LIGHT is a member of the TNF superfamily and functions as a co-stimulatory molecule for T cell proliferation. In the study, the immunogenicity in the induction of humoral and cellular immune responses by HBV DNA vaccine and the adjuvant effect of LIGHT were studied in a murine model. The eukaryotic expression plasmid pcDNA-L was constructed by inserting mouse LIGHT gene into the vector pcDNA3.1(+). In vitro expression of LIGHT was detected by RT-PCR and indirect immunofluorescence assay in transfected HeLa cells. MLR assay showed that LIGHT-transfected DCs induced markedly higher allogeneic lymphocyte proliferation than pcDNA-transfected DCs and untreated DCs at all dilutions. After BALB/c mice were immunized by three intramuscular injections of the HBV DNA vaccine plasmids alone or in combination with LIGHT expression plasmids, the different levels of anti-HBV immune responses were measured comparable to the control groups immunized with parent plasmid pcDNA or PBS. The HBsAg-specific splenocytes proliferation and specific cytotoxic activities of splenic CTLs in the coinoculation group were both significantly higher than those in the HBV DNA single inoculation group, and an enhancement of antibody response was also observed in the coinoculation group compared with the single inoculation group. Taken together, coimmunization of HBV DNA vaccine plasmids and LIGHT expression plasmids can elicit stronger humoral and cellular immune responses in mice than HBV DNA vaccine plasmids alone, and LIGHT may be an effective immunological adjuvant in HBV DNA vaccination.

1. Introduction

Chronic HBV infection is one of the most common infectious diseases worldwide and leads to a high morbidity and mortality due to the development of liver cirrhosis and cancer. Studies showed that genetic immunization, i.e. in vivo transfection of somatic cells with antigen-encoding DNA, effectively induces major histocompatibility complex (MHC) class I-restricted cell-mediated immunity in CD8+ cytotoxic T lymphocytes (CTLs) and elicits humoral immune reactions which are dependent on MHC class II-restricted activation of T helper (Th) cells. So DNA-based vaccination appears to be a particularly pertinent approach for chronic hepatitis B therapy.

DNA vaccines contain gene(s) for an antigenic portion of a virus, such as the core protein or the envelope protein, usually under the transcriptional control of a viral promoter (Ulmer et al., 1996; McDonnell and Askari, 1996). Direct injection of the plasmid DNA in vivo results in prolonged expression of viral proteins in the host and may thus mimic the action of attenuated vaccines. Many animal models of infectious diseases have been reported (Loirat et al., 2000; Dunham et al., 2002; Huang et al., 2001) which showed DNA vaccine induced a broad range of protective immunities against challenge with the pathogen. Application of this genetic vaccination approach has been extended to the treatment of cancers (Gavarasana et al., 2000; Thirdborough et al., 2002) and autoimmune disease (Garren et al., 2001). It has been demonstrated that plasmid DNA-encoding HBV surface antigen (HBsAg) elicited vigorous humoral and cellular responses (Zhao et al., 2000; Huang et al., 2001; Xing et al., 2005). However, the immune responses induced by DNA vaccines in large outbred animals are weak and short-lived (Chaplin et al., 1999), looking for an effective way to promote DNA immune efficacy is an urgent case (Du et al., 2003; Chen et al., 2004).

Accumulating evidence demonstrate that two distinct signals are necessary for the optimal activation of T cell response, a first signal delivered from T-cell receptor and a second signal from co-stimulatory molecules such as CD80 and CD86 (Lenschow et al., 1996). Co-stimulation is known to play important roles in numerous immune responses both in vitro and in vivo and co-stimulatory pathways can be manipulated for therapeutic purposes. LIGHT

* Corresponding author. Tel.: +86 21 6223 3574; fax: +86 21 6223 3295.
E-mail address: wzjiang@bio.ecnu.edu.cn (W. Jiang).

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(homologous to lymphotoxins, exhibits inducible expression, and competes with HSV glycoprotein D for herpes virus entry mediator (HVEM), a receptor expressed by T lymphocytes, also named HVEM-L or TNFSF14) is a member of TNF superfamily (Mauri et al., 1998). Studies showed that LIGHT is expressed as a homotrimer on activated T cells (Mauri et al., 1998; Morel et al., 2000) and immature dendritic cells (Tamada et al., 2000a,b). Three receptors with distinct cellular expression patterns were described to interact with LIGHT. HVEM is detected on T-, B-lymphocytes, NK cells, monocytes, and endothelial cells (Morel et al., 2000; Harrop et al., 1998a,b; Kwon et al., 1997). Lymphotoxin β receptor (LTβR), in contrast, expressed on stromal cells (Browning et al., 1997; Murphy et al., 1998) but absent from lymphocytes. Decoy receptor 3 (DcR3), a TNFR family member lacking a transmembrane region, competes with LTβR and HVEM for LIGHT engagement, thereby acting as a negative regulator. So far, in vitro studies suggested a role of LIGHT as a co-stimulatory molecule providing T cells with the "second signal" in addition to "signal one" delivered by the T cell receptor. For some other members of the TNF superfamily such co-stimulatory functions in T cell activation have been established (Watts and DeBenedette, 1999). Tamada et al. reported that LIGHT is a CD28-independent co-stimulatory molecule for T-cell growth and differentiation. Other studies have also confirmed that LIGHT-HVEM signaling plays a co-stimulatory role in TCR-mediated T cell proliferation (Scheu et al., 2002; Tamada et al., 2000a,b).

Relying on the knowledge above, pcDNA-L, an eukaryotic vector expressing mouse LIGHT, was constructed and the potential to induce hepatitis B surface antigen (HBsAg)-specific immune responses with HBV DNA vaccine alone and coimmunization was compared. The results showed that the efficacy of HBV DNA vaccine can be greatly improved by coimmunization with LIGHT as an immunoadjuvant for immune responses.

2. Materials and methods

2.1. DNA vaccine plasmid and molecular cloning of mouse LIGHT

The eukaryotic expression vector pcDNA3.1(+), purchased from Invitrogen Corporation (Invitrogen, San Diego, CA), was used as the parental plasmid for constructing the expression vectors. The HBV DNA vaccine plasmid pcDNA-S encoding the major (S) envelope protein of HBV was constructed previously by the author's laboratory (data not shown).

Mouse LIGHT was cloned from bone marrow (BM)-derived immature DCs and the construction of the eukaryotic expression plasmid pcDNA-L was performed according to the routine method. In brief, BM cells prepared from femora and tibiae of normal BALB/c mice were depleted of red blood cells with 0.84% ammonium chloride and plated in DC culture medium (RPMI 1640 plus 10% FCS, 10 ng/ml GM-CSF (Genzyme, Cambridge, MA) and 1 ng/ml IL-4 (Genzyme, Cambridge, MA)). On day 3, the nonadherent granulocytes and T and B cells were gently removed and fresh media were added. On day 6, the nonadherent cells were harvested for extraction of RNA. RNA was isolated from 4×10^5 cells using 1 ml TRIzol (Invitrogen) following the manufacture's protocol. First-strand cDNA synthesis was performed using a RT-PCR Kit (TOYOBO, Japan) with the supplied oligo(dT)20 primer under the condition of 42 °C for 20 min and 99 °C for 5 min. PCR primers used for the mouse LIGHT cDNA sequence were the sense (5′-GGAATTCATGGAGAGTGTGGTACAGCCTTCAGTGT-3′, underlined portion denotes EcoR| restriction site) and antisense (5′-CCGCTCGAGTCAGACCATGAAAGCTCCGAAAT-3′, underlined portion denotes Xho| restriction site). The protocol employed for PCR amplification comprised: 95 °C for 2 min, 30 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min and 72 °C for 10 min. Following amplification, products were analyzed by agarose (1%) gel electrophoresis and DNA bands were detected by ethidium bromide staining and photographed under UV trans-illumination. The PCR products of LIGHT gene was cleaved with restriction endonucleases EcoR| and Xho| (TaKaRa, China) and was cloned into the restriction sites of pcDNA digested with the same endonucleases.

2.2. Antibodies and synthetic peptide

Goat anti-mouse LIGHT multiclonal antibody was bought from Genetimes Technology Inc. (Shanghai, China). Rabbit anti-goat IgG antibody labeled by FITC was bought from Tiangen Co. Ltd. (Beijing, China). The HBsAg-specific peptide (IPQSLDSWWTSL, 28–39) and HIV-1 IIIB gp120 epitope peptide (RGPGRAFVTI, 311–320) were synthesized by GL Biochem Co. Ltd. (Shanghai, China).

2.3. Cell line and transfection

HeLa cells (human cervical epithelial carcinoma cell line), obtained from Shanghai Institute of Biochemistry and Cell Biology of Chinese Academy of Sciences, were propagated in RPMI 1640 supplemented with 10% FBS, 10 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin. Lipofectamine reagent (Beyotime, China) was used to transfect HeLa cells according to the manufacturer's instruction. The day before transfection, cells were seeded in a six-well plate at 3×10^5 cells per well and incubated at 37 °C in a 5% CO_2 humidified atmosphere incubator until the cells were 70–80% confluent. For each well for transfection, 4 μg of plasmid DNA and 6 μl of lipofectamine in 70 μl of serum-free medium were mixed gently in tube and incubated for 15 min at room temperature to form DNA–liposome complexes. Then the complexes were overlayed onto the cells. After incubated for 6 h, the medium was replaced with 2 ml of fresh complete culture medium. Cells were assayed for transient gene expression 48 h after the start of transfection.

2.4. Extraction of total RNA and RT-PCR analysis of the transfected cells

Total RNA was prepared from the transfected cells by TRIzol reagent as before. The extracted RNA was used as a template and first-strand cDNA was synthesized. PCR amplifications for the mouse LIGHT were performed and the product was subjected to electrophoresis on 1% agarose gel in 0.5× TBE buffer (45 mM Tris–boric acid, 1 mM EDTA, pH 8.0) and DNA bands were detected by ethidium bromide staining.

2.5. Indirect immunofluorescence assay of the transfected cells

Two days after transfection, cells grown on slides were washed three times by phosphate buffer saline (PBS) and then incubated with 3% BSA for 1 h at room temperature. Then cells were incubated with goat anti-mouse LIGHT multiclonal antibody at a dilution of 1:800 at 4 °C for 16 h. The slides were maintained with rabbit anti-goat IgG antibody labeled by FITC diluted at 1:80 for 40 min without light. After being washed in the dark, the fluorescence microscope was employed to observe the transcription of LIGHT gene in transfected cells.

2.6. Mixed lymphocyte reaction (MLR)

The BM-derived immature DCs from BALB/c mice (H-2K^b, Center for Experimental Animals of the Second Military Medical University, Shanghai, China) for genetic modification were prepared as before. The harvested DCs were transfected with pcDNA-L and

pcDNA3.1 by Lipofectamine reagent and incubated with RPMI 1640 supplemented with 10% FBS, 10 ng/ml GM-CSF and 1 ng/ml IL-4 for 40 h in a humidified atmosphere of 5% CO_2 at 37 °C. Then the DCs were treated with 30 μg/ml Mitomycin C (Sigma, USA) for 45 min. After being washed four times by RPMI 1640, they were used as stimulators. T cells were purified from C57BL/6 (H-2K^d, SIPPR/BK Animal Co. Ltd., Shanghai, China) mouse splenocytes using T cell Enrichment Columns (R&D Systems, Minneapolis, MN) and were used as responders (2×10^5/well).

For the induction of allogeneic MLR, purified T cells were cocultured with transfected or untreated allogeneic DCs at the DC/T cell ratio of 1:1, 1:5, 1:10, 1:20 and 1:50 in triplicate in flat-bottom 96-well microplates. Negative control received T cells and medium. The plates were incubated at 37 °C in a humid atmosphere with 5% CO_2 and the result was measured by MTT Cell Proliferation Kit (Beyotime, China). After 72 h, 10 μl of MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide) solution (5 mg/ml) was added to each well and incubated for further 4 h. 100 μl of Formazan solvent was added to each well and incubated until all Formazan was soluted and the absorbance was evaluated by a microplate reader at 570 nm. The results were expressed as stimulation index (SI, SI = OD_{570} of DCs plus T cells/OD_{570} of negative control), which indicates significant proliferation if SI > 2.

2.7. Nucleic acid immunization in mice

Eight-week-old female BALB/c mice (Center for Experimental Animals of the Second Military Medical University) were divided into four groups, eight mice of each group were immunized with one of the following regiments dissolved in 100 μl of PBS. (1) 100 μg of pcDNA-S; (2) mixture of 50 μg of pcDNA-S and 50 μg of pcDNA-L; (3) 100 μg of pcDNA3.1 vector; the mice in the fourth group served as negative control received 100 μl of PBS only. All mice received intramuscular injections into the left thigh quadriceps muscle at 0, 21 and 41 days and were killed 20 days after the last immunization.

2.8. Splenocyte proliferation assay

To determine whether LIGHT takes effect on HBsAg-specific lymphoproliferation, splenocytes from the immunized mice were obtained for the proliferative assay. RBC were removed by incubation in ammonium chloride (0.83%, w/v) for 5 min at 37 °C. After being washed three times in PBS, the cells were resuspended in complete medium (RPMI 1640 supplemented with 10% FBS). Cell numbers were counted with a haemocytometer by trypan blue dye exclusion technique and cell viability exceeded 95%. To perform the lymphoproliferative assay, splenocytes were grown in triplicate in 96-well flat-bottom microtiter plates (2.0×10^5 cells per 100 μl complete medium). Stimulated wells received HBsAg-specific peptide at 40 μg/ml; HIV-1 IIIB gp120 epitope peptide (40 μg/ml) served as irrelevant control. Blank control wells received splenocytes and medium. The splenocytes were incubated at 37 °C in a humid atmosphere with 5% CO_2. After 72 h in culture, 10 μl of MTT solution (5 mg/ml) was added to each well and incubated for further 4 h. Hundred microliters of Formazan solvent was added to each well and incubated until all Formazan was soluted and the absorbance was evaluated in an ELISA reader at 570 nm. The results were expressed as a stimulation index (SI, SI = OD_{570} of peptide stimulation/OD_{570} of blank control), which indicates significant proliferation if SI > 2.

2.9. CTL assay

The mice were killed 20 days after the last immunization. Spleens removed from the immunized mice were compressed through sterile stainless steel mesh and then washed twice with RPMI 1640. After the red blood cells were removed with NH_4Cl solution, the splenocytes were resuspended in complete culture medium (RPMI 1640 medium supplemented with 10% fetal bovine serum, 50 μM 2-mercaptoethanol, 10 mM HEPES, 2 mM L-glutamine, 100 U of penicillin per milliliter, and 100 μg of streptomycin per milliliter) with 5 μg/ml Concanavalin A (Dingguo Biotech, Beijing, China) and 10 U/ml of IL-2 (Pepro Tech, UK) and cultured in vitro for 1–2 days as the effector cells. The stimulator cells, harvested from naive mice, were pulsed with final concentration of 20 μg/ml of HBV-specific peptide for 4 h at 37 °C in 5% CO_2 and then treated with 80 μg/ml Mitomycin C at 37 °C in 5% CO_2 for another 2 h. The cells were washed extensively with RPMI 1640 medium. The effector cells (4×10^7 cells) were incubated with stimulator cells at an effector-stimulator ratio of 10:1 for 5 days in culture medium containing 10 U/ml recombinant IL-2 at 37 °C in 5% CO_2. The target cells were prepared by P815 cells (mouse mastocytoma cell line, Shanghai Institute of Biochemistry and Cell Biology of Chinese Academy of Sciences) pulsed with HBV-specific peptide for 4 h at 37 °C in 5% CO_2.

The lactate dehydrogenase (LDH) release assay was employed to measure the ability of in vitro-stimulated responder cells to lyse P815 target cells. This assay yields results similar to those obtained with the standard chromium release assays but does not require the use of radioisotopes. The assays were performed in triplicate with 1×10^4 target cells/well incubated with effector cells at various effector cell/target cell (E:T) ratios of 100:1, 50:1, 25:1 and 12.5:1 in 96-well round-bottom plates for 4 h in phenol red-free RPMI 1640 containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U of penicillin per milliliter, and 100 μg of streptomycin per milliliter. Following a 6 h incubation, 100 μl of the supernatant per well was then transferred to new 96-well plates, and lysis was determined by measuring LDH release according to Non-Radioactive Cytotoxicity Assay Kit (Promega, USA). The released LDH converts the added substrate tetrazolium salt into a red Formazan product, and the amount of color is proportional to the number of lysed cells. The absorbance values from supernatants were recorded at 490 nm on an ELISA microplate reader. The percentage of specific lysis of target cells for a given effector cell sample was calculated using the following relationship: % specific lysis = (optical density (OD) of experimental LDH release − OD of effector cell spontaneous LDH release − OD of target cell spontaneous LDH release)/(OD of maximum target LDH release − OD of target spontaneous LDH release) × 100%. All determinations were performed in triplicate.

2.10. Measurement of HBsAg-specific antibody

Serum samples were collected by tail bleeding at different times, beginning at 2 weeks after the first immunization, and the presence of HBsAg-specific antibody was analyzed by an enzyme linked immunosorbent assay (ELISA) kit (Kehua Bio-Engineering Co. Ltd., China) for the anti-HBsAg with ELISA plate reader at 450 nm and assays were performed according to the manufacturer's instructions. In brief, if the sample contained an anti-HBsAg antibody that reacted with the HBsAg, the antibody would bind the HRP-labeled HBsAg and a color reaction would occur. If the sample did not contain an antibody that reacted with HBsAg, the HRP-labeled HBsAg would not bind and no color reaction would occur. Titers were expressed as the highest dilution while OD_{450} of experimental group/OD_{450} of negative control ≥ 2.

2.11. Statistical analysis

Data were reported as $x \pm s$ and were analyzed by professional statistical computer software SPSS. Significance was set at $p < 0.05$.

3. Results

3.1. Construction and identification of the recombinant plasmid

A 725 bp of full-length cDNA of mouse LIGHT was obtained from BALB/c mouse bone marrow-derived immature DCs. Amplified cDNA was ultimately digested with EcoR| and Xho| and cloned into pcDNA3.1(+). The sequence of the inserted mouse LIGHT cDNA was confirmed by restriction enzyme digestion and direct sequencing (data not shown) and the plasmid were designed as pcDNA-L. The DNA sequence of mouse LIGHT was found to have a homology of 99.86% compared to that of mouse LIGHT strain (GenBank accession no. NM019418).

3.2. In vitro expression of the recombinant plasmid

In order to determine whether the gene of interest in recombinant plasmid was expressed in vitro, HeLa cells were transiently transfected with pcDNA-L or pcDNA3.1 and the expression at the transcriptional and protein level was detected by RT-PCR and indirect immunofluorescence assay, respectively. After extracting the total RNA from the transfected cells, RT-PCR was performed with the specific primers of LIGHT, a specific DNA band of 725 bp for mouse LIGHT gene (lane 2 in Fig. 1) was amplified. As expected, LIGHT was not detected in pcDNA-transfected HeLa cells (lane 1 in Fig. 1). Indirect immunofluorescence assay indicated that the protein of interest was detected in recombinant plasmid-transfected HeLa cells but not in pcDNA-transfected cells (data not shown).

3.3. MLR

To determine the effect of LIGHT gene transfection on DC's ability to stimulate T cells, their allostimulatory capacity in MLR was tested. The bone marrow-derived DCs from BALB/c mice were untreated or transfected with pcDNA-L and vector plasmid pcDNA3.1, respectively. DCs were used as stimulators and the purified T cells from C57BL/6 mice were used as responders. The stimulation index was shown in Fig. 2. The result demonstrated that LIGHT-transfected DCs induced markedly higher allogeneic proliferation than pcDNA-transfected DCs at all dilutions ($p < 0.01$).

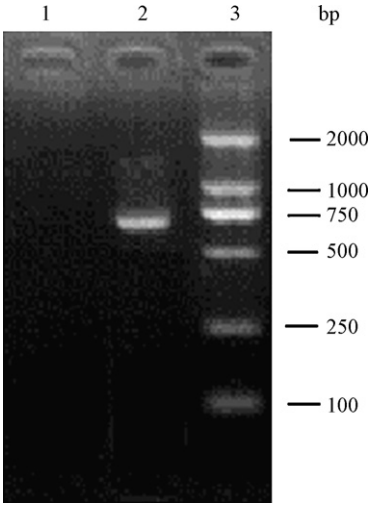

Fig. 1. RT-PCR analysis of the transfected cells. There were specific DNA bands of 725 bp for full-length LIGHT (lane 2). DNA molecular marker was indicated in base pairs (lane 3). No specific DNA bands of LIGHT were amplified in pcDNA-transfected cells (lane 1).

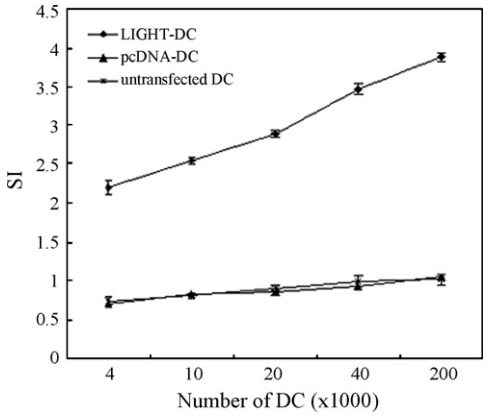

Fig. 2. LIGHT enhanced DC allostimulatory ability. The BALB/c-derived DCs were transfected with pcDNA-L or the vector plasmid pcDNA3.1 for 40 h. AllogeneicT cells (2×10^5/well) from C57BL/6 mice were incubated with untreated or transfected DCs at the graded DC numbers for 72 h. Proliferation was determined by MTT Cell Proliferation Assay Kit.

3.4. Effect of LIGHT on splenocyte proliferation in HBV immunized mice

To determine whether splenocyte proliferation response to the DNA vaccine encoding HBsAg may be boosted by LIGHT, splenocytes from the vaccinated mice were examined for proliferation. As shown in Fig. 3, the most significant proliferation was exhibited in the group of mice immunized with pcDNA-S alone compared to pcDNA3.1 and PBS groups ($p < 0.01$). A relatively higher SI was observed in the group immunized with pcDNA-S plus pcDNA-L compared to pcDNA-S alone ($p < 0.01$). No specific stimulation with the plasmid pcDNA or PBS was observed. T cell proliferation assays with the presence of irrelevant peptide were also performed as negative controls, and no distinct proliferation was observed, demonstrating that the T cell proliferation was in an HBsAg-specific fashion.

3.5. Induction of CTL responses in mice immunized with DNA vaccine and LIGHT

In order to analyze the capacity of recombinant LIGHT to enhance the HBsAg-specific CTL response, splenic cells, derived from the immunized mice 20 days after the last inoculation, were restimulated specifically by naive mice splenocytes pulsed with HBsAg-specific peptides in vitro for 5 days. P815 cells pulsed with HBsAg-specific peptides were used as target cells. The cytotoxic

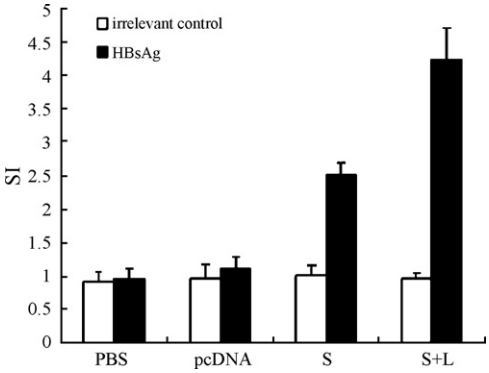

Fig. 3. Splenocyte proliferation assay. Splenocytes were obtained from immunized mice 20 days postimmunization and were co-cultured with HBsAg peptide or irrelevant peptide control (40 μg/ml) in RPMI 1640 for 72 h before addition of MTT.

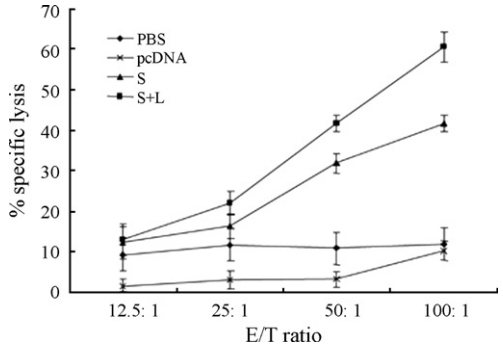

Fig. 4. CTL activities in splenocytes from the immunized mice. BALB/c mice were injected three times with HBV DNA vaccine or coadministered with HBV DNA vaccine plus LIGHT expression plasmid at 20 days' intervals. The parent plasmid pcDNA3.1 or PBS were inoculated as the controls. Splenocytes from the mice ($n = 8$) in each group were harvested and CTL responses were measured by using autologous P815 cells incubated with HBsAg-specific peptide as the target cells. Effector cell-target cell ratios are indicated on the abscissa. The percent specific lysis is reported on the vertical axis.

activity was tested by non-radioactive LDH release assay and the specific lysis rates were shown in Fig. 4. HBsAg-specific CTL was detectable in the mice immunized with the HBV DNA vaccine plasmids compared with pcDNA or PBS group at the E/T ratio of 100:1 and 50:1 ($p < 0.01$). The specific CTL activities increased significantly in the presence of LIGHT compared with single immunization of the HBV DNA vaccine plasmids and the strongest CTL responses was detected at the E/T ratio of 100:1 ($p < 0.01$). The results demonstrated that cellular immunity was markedly enhanced by coimmunization of HBV DNA vaccine plasmid and LIGHT expression plasmid.

3.6. Measurement of HBsAg-specific antibody

The blood samples were collected by tail bleeding every 2 weeks after the first immunization and the sera were isolated. The presence of anti-HBsAg-specific antibodies in sera was analyzed by ELISA. As shown in Fig. 5, specific antibody response was detectable in both the group immunized with HBV DNA vaccine plasmid pcDNA-S alone and the group co-administered with pcDNA-S plus pcDNA-L compared with control groups immunized with parent plasmid pcDNA or PBS, and the enhanced antibody response was observed in the coimmunized group compared with the single

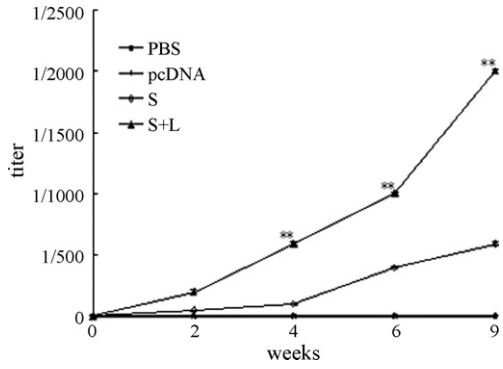

Fig. 5. ELISA analysis of the level of anti-HBV antibodies. Female BALB/c mice at the age of 6–8 weeks were immunized with HBV DNA vaccine or coimmunized with HBV DNA vaccine plus LIGHT expression vector, respectively. Parent plasmid pcDNA and PBS were inoculated as the controls. Sera obtained from mice ($n = 8$) were detected at different dilutions by ELISA. The results were expressed as the highest dilution while OD_{450} experimental group/OD_{450} negative control 2 (**$p < 0.01$ compared with the group immunized with pcDNA-S alone).

DNA vaccine injection group 4 weeks after the first immunization ($p < 0.01$).

4. Discussion

HBV infection is very common in China (He et al., 2005; Xu et al., 2005; Zhang et al., 2006; Gu et al., 2007). It is estimated that there are 400 million carriers of hepatitis B virus (HBV) by the year 2000 according to the WHO. These people are at risk of developing chronic hepatitis leading to liver cirrhosis and hepatocellular carcinoma (Yuen and Lai, 2001; Jung et al., 2002). Thus, effective preventive and therapeutic strategy to chronic HBV infection has been a major exploration (Bocher et al., 2001; Couillin et al., 1999). Up to now, vaccination is a main way in prevention HBV infection and there are no specific therapies to cope with it. DNA-based vaccination is an efficient new technique to stimulate immune responses and HBV-specific DNA vaccine could induce strong humoral and cell-mediated immunity against HBV infection (Michel and Loirat, 2001; Davis et al., 1998). HBV DNA vaccination can induce CD8+ T cells as well as a dominant Th1 phenotype among the splenic lymphocytes, so eliciting strong CTL and protective levels of antibody (Roy et al., 2000; Roh et al., 2001; Chen and Li, 2006). HBV (HBsAg, HBc/eAg) DNA vaccine has been popularly studied for prophylaxis or therapy against HBV infection and the effect was improved by many different ways (Wu et al., 2001; Musacchio et al., 2001; Lee et al., 2001; Kwon and Park, 2002; Daryani et al., 2007). Compared with conventional protein-based vaccines, persistent expression of the encoded Ag by DNA vaccines would be expected to produce long-lived immunity. Although the genetic vaccination approach has been applied to the development of DNA vaccines in various diseases, the efficacy of different DNA vaccines has varied widely (Kim et al., 2001; Nobiron et al., 2001). Thus, improvement of vaccine efficacy has become a critical issue for the acceptance of DNA vaccines as a standard vaccination technology and many approaches have been tried.

Co-stimulation plays important roles in immune responses and antigenic stimulation of T cells in the absence of co-stimulation induces an unresponsive state known as anergy (Mueller et al., 1989). Co-stimulatory molecules B7-1 and B7-2 are expressed on professional APCs, particularly on dendriticcells (DCs), and play a critical role in the initiation and regulation of primary T cell response (Banchereau and Steinman, 1998). Studies showed that several molecules belonging to the TNF superfamily function as co-stimulatory molecules for the induction, differentiation, and survival of the immune cell (Smith et al., 1994). LIGHT is a member of the TNF family, with its protein expressed on activated T cells and immature dendritic cells. It is a ligand for TR2/HVEM, LTβR, and TR6/DcR3, all of which are TNF receptor (TNFR) family members. Studies showed that LIGHT plays a critical role in T cell development and the dysregulation of T cell-derived LIGHT leads to altered T cell homeostasis and autoimmune disease. LIGHT is expressed on human immature DC and is required to initiate primary allogeneic T cell responses induced by DC. LIGHT has been proved to be a co-stimulatory molecule for TCR-mediated T cell proliferation (Scheu et al., 2002; Tamada et al., 2000a,b). Although it is generally proved that LIGHT has co-stimulatory activities, there are few reports on its adjuvant effect for DNA vaccine.

In order to study the effect of LIGHT as an HBV DNA vaccine adjuvant, mouse LIGHT gene was cloned and inserted into pcDNA3.1(+) vector to construct pcDNA-L. Restriction enzyme digestion and sequencing analysis of pcDNA-L indicated that the LIGHT gene was inserted correctly into the vector. The expression of LIGHT in vitro was confirmed in transiently transfected HeLa cells by RT-PCR analysis and indirect immunofluorescence assay. Several studies showed that MLR can be enhanced by inclusion of soluble LIGHT

(Harrop et al., 1998a,b) and can be inhibited by neutralization of LIGHT (Kwon et al., 1997; Harrop et al., 1998a,b). In present study, the allostimulatory activities of DC were measured in MLR, the results showed that modification of DC with LIGHT enhanced the allostimulatory effects. It is widely acknowledged that, in humans, T cells play a critical role in clearing HBV infections and in inducing liver lesions associated with persistent HBV infections (Chisari and Ferrari, 1995). Mancini et al. reported that T cell-mediated immunity induced by a HBV DNA vaccine resulted in the complete clearance of circulating HBsAg and in the long-term control of transgene expression in hepatocytes using a transgenic model (Mancini et al., 1996). Guidotti et al. has shown that adoptively transferred HBsAg-specific CTL can abolish HBV gene expression and replication in HBV transgenic mice. Antibodies against viral pathogens also play a critical role in preventing infection (Guidotti et al., 1994).

To determine whether the LIGHT plasmid has effect on the cellular and humoral immune responses induced by HBV DNA vaccination in BALB/c mice, four groups of mice were immunized in the study. Splenocytes from the mice coadministered by pcDNA-L and HBV DNA vaccine plasmid pcDNA-S were evaluated for antigen-specific proliferation as well as CTL component of immune response by the specific killing of syngeneic target cells pulsed with a recognized CTL epitope peptide and anti-HBsAg antibodis in sera were detected at each time point. The strongest HBsAg-specific proliferative activity in coimmunized group was well consentaneous with the highest level of CTL activity and HBsAg-specific antibody production among the four groups, suggesting the augmented responses were based on antigen-specific immunological memory in T lymphocytes. The results indicated that the plasmids expressing LIGHT can enhance the specific cellular and humoral immune responses in mice elicited by HBV DNA vaccine plasmid. In conclusion, LIGHT is a possible candidate adjuvant for HBV DNA vaccine.

However, the CTL activity and HBsAg-specific antibody production in the study are low. It is reported that the end-point titers of anti-HBc reached the highest 1/97 200, 4 weeks after the third immunization and the specific CTL killing with the highest specific lysis reached 73.25% at effector:target ratio of 20:1 in mice (Xing et al., 2005). Chow et al. constructed a plasmid-encoding HBV middle envelope and IL-2 fusion protein and a bicistronic plasmid encoding the envelope protein and IL-2 separately and compared their efficacy in inducing immune responses to plasmids encoding the HBV envelope protein alone. The results showed that coexpression of IL-2 and the HBV envelope protein within the same plasmid vector resulted in at least a 100-fold increase in its ability to induce humoral and cellular immune responses to HBsAg (Chow et al., 1997). Coexpression of genetic adjuvant and HBV DNA vaccine might be a better choice. Maybe we can improve the genetic adjuvant of LIGHT by coexpressing with HBsAg and increase the dose as well.

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