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Lentiviral vector encoding ubiquitinated hepatitis B core antigen induces potent cellular immune responses and therapeutic immunity in HBV transgenic mice

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ABSTRACT

Predominant T helper cell type 1 (Th1) immune responses accompanied by boosted HBV-specific cytotoxic T lymphocyte (CTL) activity are essential for the clearance of hepatitis B virus (HBV) in chronic hepatitis B (CHB) patients. Ubiquitin (Ub) serves as a signal for the target protein to be recognized and degraded through the ubiquitin-proteasome system (UPS). Ubiquitinated hepatitis B core antigen (Ub-HBcAg) has been proved to be efficiently degraded into the peptides, which can be presented by major histocompatibility complex (MHC) class I resulting in stimulating cell-mediated responses. In the present study, lentiviral vectors encoding Ub-HBcAg (LV-Ub-HBcAg) were designed and constructed as a therapeutic vaccine for immunotherapy. HBcAg-specific cellular immune responses and anti-viral effects induced by LV-Ub-HBcAg were evaluated in HBV transgenic mice. We demonstrated that immunization with LV-Ub-HBcAg promoted the secretion of cytokines interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), generated remarkably high percentages of IFN-γ-secreting $CD8^+$ T cells and $CD4^+$ T cells, and enhanced HBcAg-specific CTL activity in HBV transgenic mice. More importantly, vaccination with LV-Ub-HBcAg could efficiently decreased the levels of serum hepatitis B surface antigen (HBsAg), HBV DNA and the expression of HBsAg and HBcAg in liver tissues of HBV transgenic mice. In addition, LV-Ub-HBcAg could upregulate the expression of T cell-specific T-box transcription factor (T-bet) and downregulate the expression of GATA-binding protein 3 (GATA-3) in spleen T lymphocytes. The therapeutic vaccine LV-Ub-HBcAg could break immune tolerance, and induce potent HBcAg specific cellular immune responses and therapeutic effects in HBV transgenic mice.

1. Introduction

Chronic hepatitis B (CHB) infection remains a global health concern, and approximately 600,000 individuals die from liver cirrhosis or hepatocellular carcinoma complicating CHB (Liaw and Chu, 2009). Hepatitis B virus (HBV) is a small enveloped DNA virus which is non-cytopathic and cannot cause liver damages and HBV-related complications by itself. The clinical outcomes and viral persistence in CHB patients are mainly determined by viral replication and host immune responses (Bertoletti and Ferrari, 2013; Chisari et al., 2010). However, for over 350 millions CHB patients, current treatment options based on antiviral drugs such as interferon and nucleostide/nucleotide analogues offer limited efficacy, have side effects and seldom achieve cure (Yang and Bertoletti, 2015). Studies have reported the restoration of host immunity of CHB patients is crucial to control HBV replication and contain the liver damages (Boni et al., 2012; Koziel, 1999). However, host immunity especially the HBV-specific T cell immune responses have been proved to be weak in CHB patients who have sustained HBV replication and liver damages (Chisari et al., 2010). Therefore, strategies to enhance HBV-specific T cell responses may have therapeutic implications in CHB individuals.

T helper (Th) 1-type immune responses and HBV-specific cytotoxic T lymphocyte (CTL) activity have been shown to play vital roles in clearing HBV (Balmasova et al., 2014; Yang et al., 2006). Investigators have shown that virus specific immune responses, especially HBV core antigen (HBcAg)-specific CTL can control the replication of HBV and the progress of liver damages (Akbar et al., 2012; Bertoletti and Maini, 2000). HBcAg should be an essential component of the therapeutic vaccine against HBV (Akbar et al.,

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2012). Ubiquitin (Ub) is a small highly conserved protein in eukaryotic cells and the function of ubiquitin in proteolysis is to serve as a signal for the target protein to be recognized and degraded through the ubiquitin-proteasome system (UPS) (Gao and Luo, 2006). Ubiquitinated antigen, that is fusion of Ub to the antigen, has been demonstrated to be efficiently degraded into antigen peptides, which could be presented by MHC classIresulting in significantly enhanced antigen-specific CTL responses (Wang et al., 2009; Zhang et al., 2005).

The ability to activate vigorous and sustained HBV-specific T cell responses is crucial to the success of an anti-HBV therapeutic vaccine. Dendritic cells (DCs) are the most efficient antigen-presenting cells to initiate such responses, so current HBV vaccines are performed to target DCs in vitro (Akbar et al., 2012). These approachs involve ex vivo pulsing DCs with antigenic peptides or transducing DCs with antigen-recombinant viral vectors before transferring them back to the patient (Jiang et al., 2014; Wei et al., 2015). However, the preparation and in vitro modification of DCs is laborious, time-consuming and costly. As a kind of gene transfer carrier, the lentiviral vector (LV) has been developed to achieve stable gene transduction in the target cell (Cockrell and Kafri, 2007; Naldini, 2009). Investigators have demonstrated that vaccination with in vivo administration of lentiviral vectors carrying antigen gene can induce more potent CTL response than with transfer of transduced DCs or peptide/adjuvant (Esslinger et al., 2003). Direct administration of lentiviral vectors encoding the antigen gene in mice has been shown to cause transduction of antigen-presenting cells in the spleen (VandenDriessche et al., 2002) and dendritic cells (DCs) in draining lymph nodes (Esslinger et al., 2003).

Our previous work has reported that lentiviral vectors encoding the ubiquitinated HBcAg (LV-Ub-HBcAg) can promote DC maturation, enhance Th1-type immune responses, and induce HBcAg-specific CTLs in vitro (Chen et al., 2012). Moreover, we previously showed that direct injection of LV-Ub-HBcAg induced potent HBV-specific immune responses, similar to those elicited by *in vitro* LV-Ub-HBcAg-transduced DCs in BALB/c mice (Dai et al., 2015). In the present study, we aimed to assess whether LV-Ub-HBcAg has therapeutic efficacy in the presence of HBV. Therefore, we investigated whether LV-Ub-HBcAg could induce HBcAg-specific T cell responses and anti-viral immunity in vivo in HBV transgenic mice.

2. Materials and methods

2.1. Cell lines and mice

The mastocytoma cell line P815/*c* cell (*H-2K*^{d}) expressing the HBcAg, was kept by our lab. Briefly, P815 cells were transduced by recombinant lentiviruses encoding HBcAg and Puromycin resistance gene, and 2 μg/ml puromycin was applied for screening for 10 days after 48 h of transduction. The surviving cells were resistant to puromycin, and named P815/c cells were successfully transduced with HBcAg gene. The expression of HBcAg in the cells was further identified by western blot analysis. Human Embryonic Kidney 293T cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Gaithersburg, MD, USA) containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

H-2K^d HBV-transgenic BALB/c mice (half mail and half female, 6–8 weeks old) were purchased from the Key Liver Army Laboratory (The No.458 Hospital, Guangzhou, China). They contained 1.3 copies of the HBV genome (subtype *ayw*). The level of serum HBsAg and HBV DNA was high, and the expression of HBsAg and

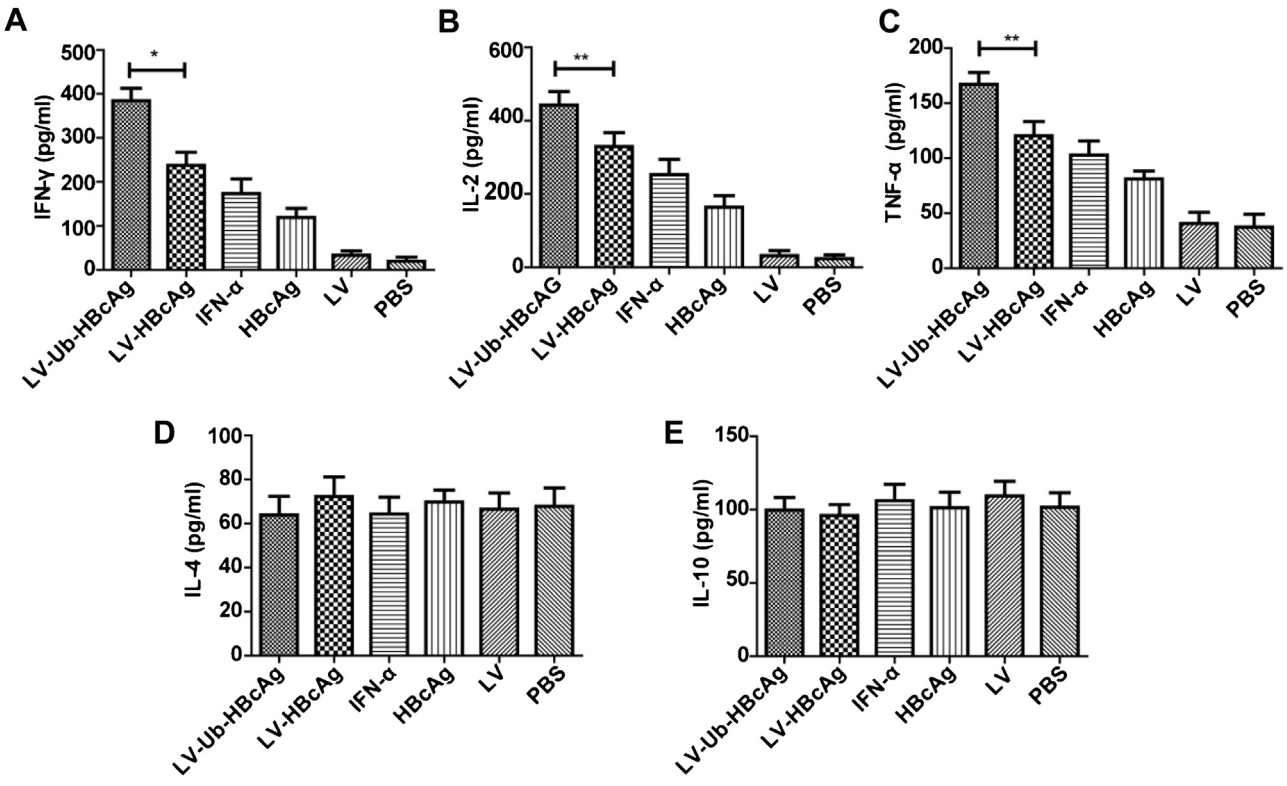

Fig. 1. Productions of the cytokines IFN-γ, IL-2, TNF-α, IL-4, and IL-10. (A, B, C) The secretion of IFN-γ, IL-2 and TNF-α in the supernatants of T cells from the group of LV-Ub-HBcAg immunized HBV transgenic mice was significantly higher than that in the other groups. $^*P < 0.05$, $^{**}P < 0.01$. (D, E) The secretion of IL-4 and IL-10 in the supernatants of T cells from immunized mice. There was no statistical difference among all of the groups. The data are from at least three independent experiments, and the data are represented as mean ± SD ($n = 6$).