Antiviral Research 90 (2011) 227–230

Contents lists available at ScienceDirect

Antiviral Research

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

Short Communication

Breaking B and T cell tolerance using cationic lipid–DNA complexes (CLDC) as a vaccine adjuvant with hepatitis B virus (HBV) surface antigen in transgenic mice expressing HBV

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ARTICLE INFO

Article history:
Received 25 January 2011
Revised 1 March 2011
Accepted 19 April 2011
Available online 23 April 2011

Keywords:
Hepatitis B virus
Transgenic mice
Therapeutic vaccine

ABSTRACT

Cationic lipid DNA complexes (CLDC), referred to here as JVRS-100, were evaluated as an adjuvant for hepatitis B surface antigen (HBsAg) for eliciting B and T cell responses in transgenic mice expressing hepatitis B virus (HBV). To confirm the immunogenicity of HBsAg + JVRS-1000, a study was conducted in C57BL/6 mice, the genetic background of the HBV transgenic mice used in the study. HBsAg + JVRS-100 elicited a T cell response and B cell response as evidenced by interferon-gamma (IFN-γ) secretion by re-stimulated splenocytes and anti-HBsAg IgG induction, respectively, whereas, HBsAg only elicited a B cell response. In HBV transgenic mice, HBsAg did not elicit either T or B cell responses, unlike the HBsAg + JVRS-100 that elicited both. Energix-B vaccine did perform better than the HBsAg by eliciting a B cell response in the transgenic mice, but it did not perform as HBsAg + JVRS-100 since it did not elicit a T cell response. The response by HBsAg + JVRS-100 was not sufficient to cause destruction of infected liver cells, but it did suppress HBV DNA non-cytolytically. From these results, JVRS-100 might be considered for further development as an adjuvant for HBV therapeutic vaccines.

1. Introduction

Treatment of chronic hepatitis B disease has substantially improved over recent years with the development of antiviral compounds that lower virus load. The weakness of antiviral therapy in chronically infected patients, however, is that the response is usually not durable, and patients relapse after treatment. These patients do not clear the virus that subsequently can result in hepatic flares, which may be severe (reviewed in Dienstag, 2008). The fundamental reason for this pathogenesis is a defect in an effective and properly coordinated adaptive immune response of cellular and humoral immunity mediated by complex cytokine interactions. Since antiviral therapy does not typically produce a sustained elimination of viral load either in the sera or the liver, therapeutic vaccines have been investigated to provide an effective and appropriate immunologic response that could eventually eliminate the virus without provoking serious hepatic flares (reviewed in Bertoletti and Gehring, 2009).

HBV transgenic mice, in some aspects, resemble chronically infected patients as they are both immunotolerant to the degree that they do not elicit an anti-HBV response sufficient to clear the virus or destroy infected cells – unlike acutely infected patients that clear the virus and infected cells, and resolve the disease (Guidotti et al., 1995). Consequently, transgenic mice containing the complete genome (Kakimi et al., 2002) or selected genes of HBV (Lobaina et al., 2010) have been used extensively as models for some aspects of chronic HBV infection and for the evaluation of therapeutic vaccines.

For this study, a transgenic mouse line (1.3.32) on a C57BL/6 background that produce HBV in the liver and measurable levels of HBV DNA in the serum (Guidotti et al., 1995, 1999) were used. These HBV transgenic mice have proven valuable for evaluating therapeutic substances (Iyer et al., 2004; Julander et al., 2002, 2003; Morrey et al., 1999), cytokines (Cavanaugh et al., 1997; Isogawa et al., 2005; Kimura et al., 2002), and vaccine strategies (Livingston et al., 1999).

This study describes the use of cationic lipid DNA complexes (CLDC), referred to here as JVRS-100, as an adjuvant for HBsAg vaccine. In earlier studies, CLDC reduced liver HBV DNA through the induction of cytokines (Morrey et al., 2008) using the same transgenic mouse model described herein, and has been evaluated pre-clinically and clinically as an immunostimulant or adjuvant (Bernstein et al., 2011; Hong et al., 2010). We show in this study that JVRS-100 combined with hepatitis B surface antigen (HBsAg) broke tolerance by stimulating significant B and T cell responses.

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0166-3542/$ - see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.antiviral.2011.04.006

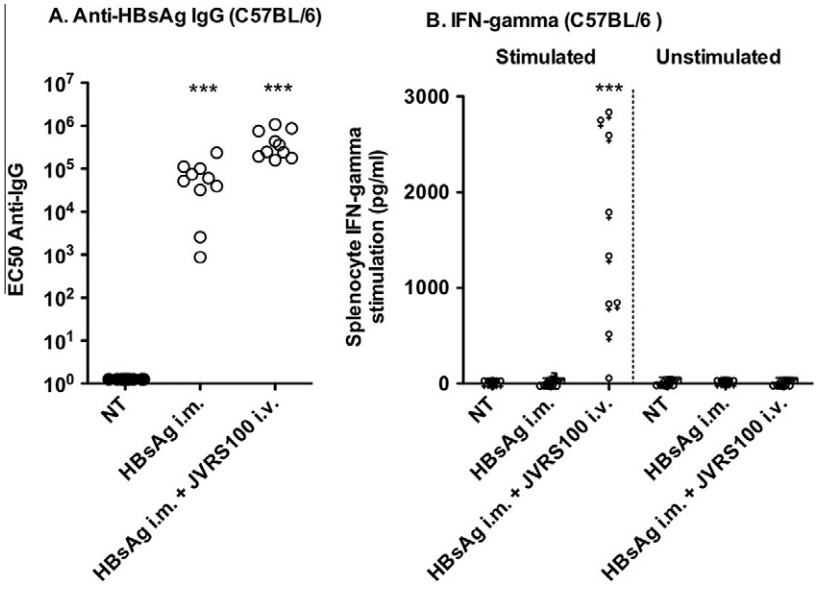

Fig. 1. Responses of (A) HBsAg-specific IgG and (B) IFN-γ to no treatment (NT), HBsAg (i.m., 5 μg), or HBsAg plus JVRS-100 (i.v., 10 μg) in female C57BL/6 mice (>6 weeks). Animals were treated on days 1, 22, and 43 and necropsied on day 57. Serum was assayed for HBsAg-specific IgG. The cell culture supernatants of splenocytes stimulated with HBsAg or unstimulated were assayed for IFN-γ by the same method shown in Fig. 2. Ten animals were included in each group. JVRS-100 was made as follows (Morrey et al., 2008). A sterile 10 mM solution of cationic liposomes composed of DOTIM [octadecenoyloxy (ethyl-2-heptadecenyl-3-hydroxyethyl) imidazolinium chloride] and cholesterol was prepared in a 1:1 M ratio as previously described (Dow et al., 1999; Gowen et al., 2006). A stock of 0.1 mg/mL was made by first dissolving the product in sterile water for injection to 1 mg/mL and then further diluting it into 5% dextrose (Baxter, Deerfield Ill.) to a final dextrose concentration of 4.5%. Prior to injection, cationic liposomes were gently mixed with erroneous plasmid DNA (pMB75.6 empty vector lacking the downstream HCMV promoter) at a ratio of 16 nmol lipid per 1 μg DNA in 10% sucrose in water at room temperature. Ten micrograms of JVRS-100 was administered intravenously (i.v.) or intramuscularly (i.m.), respectively. Five micrograms of HBsAg (Biodesigns International, Maine) in sterile PBS was administered i.m. in 0.05 mL to each animal. $^{***}P \leqslant 0.001$ using one-way analysis of variance. Prism 4, GraphPad Software, Inc. was used for all statistical analyses.

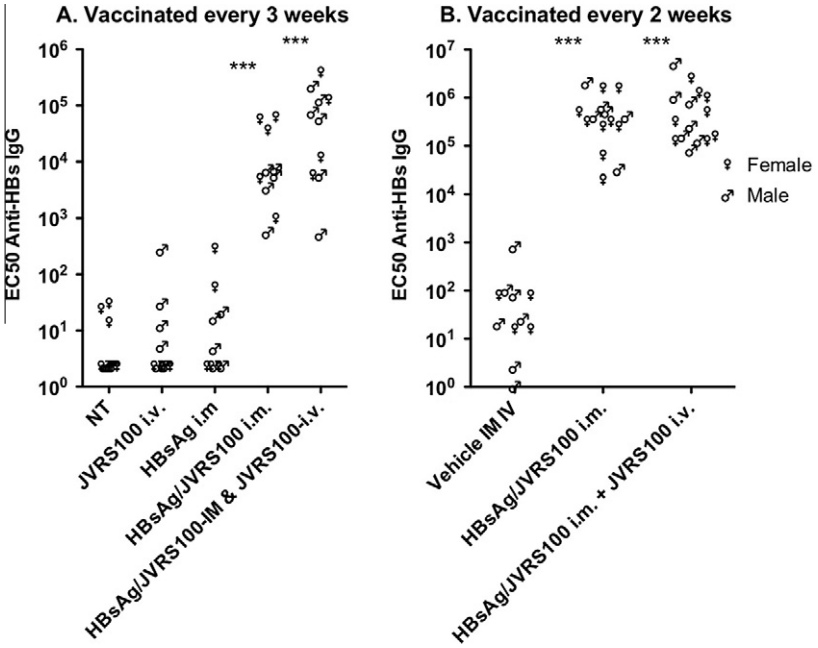

Fig. 2. HBsAg-specific IgG response to no treatment (NT), vehicle, JVRS-100 (IV, 10 μg), HBsAg (i.m., 5 μg), or HBsAg and JVRS-100 (i.m., 5 μg), 10 were treated (A) once every 3 weeks on days 1, 22, and 43 and necropsied on day 57, and (B) once every 2 weeks on days 1, 14, 28, 42, 56 and necropsied on day 70. Plasma was assayed for HBsAg-specific IgG. Ten animals were included in each group ($^{***}P \leqslant 0.001$ using one-way analysis of variance).

To confirm the immunogenicity, non-transgenic C57BL/6 mice vaccinated with HBsAg or HBsAg + JVRS-100 were shown to elicit a B cell response as indicated by increased levels of serum anti-HBsAg IgG (Fig. 1A). However, only the combination of HBsAg + JVRS-100 elicited a T cell response as indicated by increased levels of IFN-γ in splenocyte cell-culture supernatant (Fig. 1B). These results prompted experiments to determine if HBsAg + JVRS-100 could break B and T cell tolerance in transgenic mice expressing HBV.

Transgenic mice expressing HBV elicited a B cell response to vaccine containing both HBsAg and JVRS-100 administered once every A) 3 weeks or B) every 2 weeks as indicated by anti-HBsAg