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ScienceDirect
Virology 364 (2007) 245–255
VIROLOGY
www.elsevier.com/locate/yviro

Immunogenicity of hybrid DNA vaccines expressing hepatitis B core particles carrying human and simian immunodeficiency virus epitopes in mice and rhesus macaques

Deborah Heydenburg Fuller [a,b,*,1], Tim Shipley [a], Todd M. Allen [c,2], James T. Fuller [a,3], Mary S. Wu [a], Helen Horton [c,4], Nancy Wilson [c], Georg Widera [a,5], David I. Watkins [b,c]

[a] *PowderJect Vaccines, Inc., Madison, WI 53562, USA*
[b] *Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI 53715, USA*
[c] *Wisconsin Regional Primate Research Center, Madison, WI 53715, USA*

Received 27 July 2006; returned to author for revision 20 December 2006; accepted 20 February 2007
Available online 11 April 2007

Abstract

An effective HIV vaccine will likely need to induce broad and potent CTL responses. Epitope-based vaccines offer significant potential for inducing multi-specific CTL, but often require conjugation to T helper epitopes or carrier moieties to induce significant responses. We tested hybrid DNA vaccines encoding one or more HIV or SIV CTL epitopes fused to a hepatitis B core antigen (*HBcAg*) carrier gene as a means to improve the immunogenicity of epitope-based DNA vaccines. Immunization of mice with a HBcAg-HIV epitope DNA vaccine induced $CD8^+$ T cell responses that significantly exceeded levels induced with DNA encoding either the whole HIV antigen or the epitope alone. In rhesus macaques, a multi-epitope hybrid HBcAg-SIV DNA vaccine induced CTL responses to 13 different epitopes, including 3 epitopes that were previously not detected in SIV-infected macaques. These data demonstrate that immunization with hybrid HBcAg-epitope DNA vaccines is an effective strategy to increase the magnitude and breadth of HIV-specific CTL responses.

Keywords: Cytotoxic T lymphocyte response; Epitope; Hybrid vaccine; Hepatitis B core antigen; Human immunodeficiency virus; Simian immunodeficiency virus; nonhuman primate

Introduction

A vaccine capable of controlling or preventing HIV infection is needed to stem the AIDS epidemic. An effective vaccine will likely need to induce $CD8^+$ cytotoxic T lymphocytes (CTL) (McMichael and Rowland-Jones, 2001) and protective immunity against different viral variants may require induction of CTL responses against a broad range of epitopes, including subdominant and conserved sequences.

DNA immunization effectively induces T cell responses, including CTL (Donnelly et al., 1997), making this strategy an attractive approach for vaccination against HIV. Studies in nonhuman primates have shown that DNA vaccines afford various levels of protection against challenge with avirulent or pathogenic AIDS viruses (Amara et al., 2001; Barouch et al., 2000; Boyer et al., 1997; Kent et al., 1998; Robinson et al., 1999; Rosati et al., 2005; Singh et al., 2005). However, in most studies, boosting with a viral vaccine vector, numerous booster immunizations, or high doses of DNA was required to induce strong CTL responses and suppress infection, suggesting that new strategies are needed to improve DNA vaccine potency.

* Corresponding author. Department of Molecular Genetics and Biochemistry, University of Pittsburgh, School of Medicine, RIDC Park, 260 Kappa Drive, Pittsburgh, PA 15238, USA. Fax: +1 412 967 6568.
E-mail address: dfuller@pitt.edu (D.H. Fuller).
[1] Present address: Department of Molecular Genetics and Biochemistry, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15238, USA.
[2] Present address: Partners AIDS Research Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA.
[3] Present address: Recombiworks, Cranberry, PA 16066, USA.
[4] Present address: Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA.
[5] Present address: ALZA Corporation, Drug-Device Research, Mountain View, CA 94039-7210, USA.

0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.02.024

Epitope-based vaccines offer advantages for induction of CTL. Unlike whole antigens, epitope vaccines can be designed to include the relevant epitopes while excluding sequences outside epitope domains that can negatively influence immunogenicity (Collins et al., 1998; Gratton et al., 1998; Maggi et al., 1994; Romagnani and Maggi, 1994). Epitope-based vaccines can also induce more potent immune responses than whole antigen vaccines (Ishioka et al., 1999; Restifo et al., 1995). The combination of an epitope-based strategy in the context of a DNA vaccine may, therefore, provide a highly effective strategy to elicit HIV-specific CTL.

Epitopes often require conjugation to a longer, immunogenic T helper peptide (Restifo et al., 1995; Shirai et al., 1994; Vitiello et al., 1995) or a carrier moiety (Griffiths et al., 1993; Layton et al., 1993; Michel et al., 1988; Schlienger et al., 1992; Schodel et al., 1994a) to achieve significant immunogenicity. The hepatitis B virus nucleocapsid antigen (HBcAg) has been used as an efficient carrier moiety for peptide or protein vaccines (Milich et al., 1995; Schodel et al., 1994a; Ulrich et al., 1998), and immunization with purified hybrid HBcAg-B cell epitope particles enhances antibody responses against the carried epitope (Milich et al., 1995; Schodel et al., 1994a; Tindle et al., 1994; Ulrich et al., 1998). This prompted us to investigate HBcAg as a carrier for CTL epitopes in the context of a DNA vaccine as a method to augment CTL responses. Here, we demonstrate hybrid DNA vaccines encoding HIV or SIV epitopes fused to a *HBcAg* carrier gene increase CTL epitope immunogenicity in mice and can be used to induce broad CTL responses against multiple epitopes in nonhuman primates.

Results

Generation of HBcAg-epitope DNA vaccines

Heterologous epitopes can be inserted into either the immunodominant antibody binding or the C-terminus regions of the *HBcAg* gene without disrupting the core particle (Borisova et al., 1996). To test this concept as a DNA vaccine, an H-2D^d-restricted HIV CTL epitope, RGPGRAFVTI (Takeshita et al., 1995) recognized in Balb/c mice, was inserted into the immunodominant region of a plasmid encoding the *HBcAg* carrier gene (*pHBc*) (Fig. 1), resulting in the hybrid DNA vaccine, pHBc-V3-10. To determine if the hybrid vaccine expressed core particles, Vero cells were transfected with pHBc-V3-10 and the supernatants were subjected to sedimentation in a 20% glycerol cushion to pellet the core particles. For comparison, supernatants from Vero cells transfected with either the parent HBcAg vector (positive control) or DNA encoding irrelevant antigen (negative control) were also analyzed. Core particles were measured by ELISA detection of a hepatitis B e antigen (HBeAg) epitope that is exposed on particles expressed by the HBc plasmids due to deletion of the C-terminal arginine-rich region of the *HBcAg* gene. HBeAg was readily detected in the fraction from the parent HBcAg carrier vector (pHBc) and the hybrid pHBc-V3-10 vector, but not from DNA encoding the HIV envelope gene

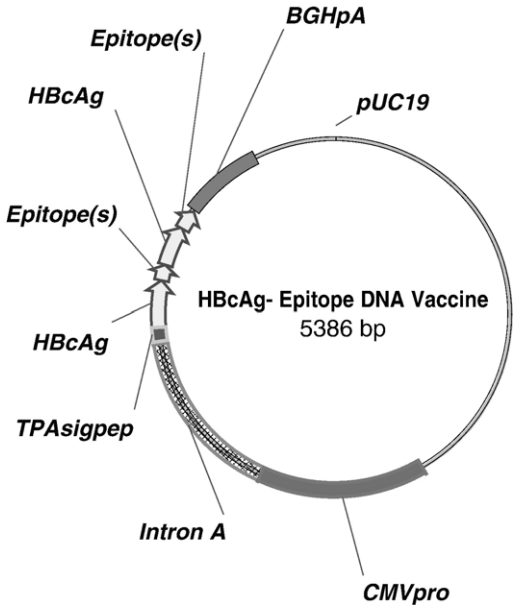

Fig. 1. Schematic of the chimeric hepatitis B core antigen-epitope plasmid design. Vector elements include the human cytomegalovirus immediate early promoter (CMVpro) with intron A sequences, the tissue plasminogen activation signal peptide (TPAsigpep), the coding sequence for hepatitis B core antigen (HBcAg), the bovine growth hormone polyadenylation signal (BGHpA), and the pUC19 origin of replication. One to three CTL epitopes were inserted into either the immunodominant loop or at the C-terminus of the *HBcAg* gene.

(*pHIVgp120*) (Fig. 2), confirming that the hybrid vector expressed hepatitis B core particles.

A multi-epitope SIV DNA vaccine encoding19 SIV_{mac239}-specific CTL epitopes that bind the Mamu-A*01 MHC class I molecule (Allen et al., 2001) was also generated. The epitopes were inserted into either the internal region or C-terminus of the *HBcAg* gene. Separation of epitopes onto different plasmids reduces competition between epitopes (Rodriguez et al., 2002). Therefore, the two immunodominant CTL epitopes, $Gag_{181–189}$ CM9 and $Tat_{28–35}$ SL8 (Allen et al., 2001), were inserted into 2 separate HBcAg vectors, and the remaining 16 epitopes were separated among 7 additional vectors resulting in a cocktail of 9 hybrid HBc-SIV vectors, each carrying 1–3 epitopes (Table 1) separated by two alanines. All 9 HBc-SIV vectors expressed variable, but comparable, levels of core particles *in vitro* (data not shown).

Hybrid HBcAg-epitope DNA vaccines increase epitope immunogenicity

We investigated HBcAg for the ability to enhance the immunogenicity of the HIV epitope. Groups of 8 Balb/c mice were immunized with three 1 μg DNA doses of either the hybrid vector (pHBc-V3-10) or a control vector encoding the same epitope without carrier (pV3-10) using a gene gun to deliver the DNA directly into the cells of the epidermis. A 3rd group of mice was immunized with a co-delivery of the parent HBcAg carrier and the epitope (pHBc+pV3-10) to determine if expression of the *HBcAg* carrier gene on a separate plasmid is sufficient to enhance epitope immunogenicity, and a 4th group