Immunology Letters 122 (2009) 44–51

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/

A preliminary and comparative evaluation of a novel Ad5 [E1-, E2b-] recombinant-based vaccine used to induce cell mediated immune responses

Elizabeth S. Gabitzsch[a,*], Younong Xu[a], Lois H. Yoshida[a], Joseph Balint[a], Richard B. Gayle[a], Andrea Amalfitano[b], Frank R. Jones[a]

[a] Etubics Corporation, Seattle, WA, United States
[b] Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, United States

ARTICLE INFO

Article history:
Received 11 August 2008
Received in revised form
13 November 2008
Accepted 18 November 2008
Available online 13 December 2008

Keywords:
Adenovirus 5
Vaccine
Cell mediated immunity

ABSTRACT

Adenovirus vectors have been shown to be highly effective as vaccine platforms capable of inducing both humoral and cell mediated immune (CMI) responses. An Ad serotype 5 vector containing unique deletions in the E2b region (Ad5 [E1-, E2b-]) has been reported to have several advantages over conventional Adenovirus serotype 5 (Ad5) vectors deleted in only the E1 region (Ad5 [E1-]), including increased carrying capacity and diminished viral late gene expression. Here, we evaluated a novel Ad5 [E1-, E2b-] vector utilizing the E.C7 cell line for viral packaging. Its' effectiveness as a potential vaccine platform as compared to the currently utilized Ad5 [E1-]-based platform was assessed in both Ad5 naïve and Ad5 immune mice. We employed the HIV-1 Gag gene as the antigenic transgene expressed by the novel vector. Cellular expression of the Gag was confirmed by Western Blot analysis. Dose response studies using three intradermal immunizations of 10^7 to 10^{10} virus particles (VP) of each construct revealed that immunization with 10^{10} VP resulted in the maximum immunological response. Multiple immunizations of Ad naïve BALB/c mice with an Ad5 [E1-, E2b]-gag vaccine resulted in higher ELISpot CMI responses as compared to mice immunized with an Ad5 [E1-]-gag vaccine. More importantly, multiple immunizations of Ad5 immune BALB/c mice with an Ad5 [E1-, E2b]-gag vaccine resulted in significant increases in ELISpot CMI responses when compared to Ad5 immune mice vaccinated with an Ad5 [E1-]-gag vector. Preliminary studies in three Ad5 immune non-human primates (NHP) demonstrated that vaccination with Ad5 [E1-, E2b-]-gag-induced elevated levels of interferon-γ and IL-2 secreting lymphocytes as assessed by ELISpot assays. These studies indicate that the novel Ad5 [E1-, E2b-] viral vector can be utilized as a potential vaccine platform to induce elevated CMI responses as compared to current generation Ad5 [E1-] viral vectors even in the presence of pre-existing Ad5 immunity.

1. Introduction

Important diseases such as HIV/AIDS, malaria, tuberculosis and Leishmaniasis afflict hundreds of millions of people and are potentially candidates for vaccine-based preventative or treatment strategies. These vaccines need to be capable of not only large scale, cGMP compliant production, but also provide for safe and effective induction of both antigen specific humoral and cell mediated immune (CMI) responses. Vaccination using current generation recombinant Adenovirus serotype 5 (Ad5) vector vaccines deleted at the E1 or the E1 and E3 regions (Ad5 [E1-]) have been reported to have promise as vaccine platforms for the prevention and treatment of diseases. Ad5 viruses are ideal for vaccine applications because of their propensity to induce robust humoral and CMI responses, which has been demonstrated in murine, canine and non-human primate (NHP) models as well as in human clinical trials [1–6]. A barrier to the widespread use of current generation Ad5 vector platforms is pre-existing Ad5 immunity present in a high percentage of potential vaccinees. It has been reported that 40–60% of humans have detectable levels of neutralizing antibody (NAb) against Ad5 [7].

The adverse effect of Ad5 immunity on the level of B- and T-cell responses to transgene products expressed by Ad5 [E1-] vectors has been reported in animal models and in humans [8–11]. Results from the Merck STEP HIV-1 vaccine trial demonstrated that the Ad5 [E1-] vector containing HIV-1 transgene inserts (MrkAd5) failed to significantly inhibit trended HIV-1 infection. This trial involved immunization of approximately 3000 healthy uninfected volunteers with MrkAd5, which consisted of three current generation vectors each expressing an HIV-1 gene: gag, pol, or nef, respectively. Vaccinees who had high titers of Ad5 antibodies (Ad5 > 1:200) prior

* Corresponding author at: 1124 Columbia St, Suite 718, Seattle, WA 98116, United States. Tel.: +1 206 838 5110; fax: +1 206 838 2978.
E-mail address: beth@etubics.com (E.S. Gabitzsch).

0165-2478/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2008.11.003

to immunization with MrkAd5 tended to have a higher incidence of HIV-1 infection than those without pre-existing Ad5 immunity (Ad5 < 1:18). The robustness of the CMI to the HIV-1 antigens was also weaker in the Ad5 immune individuals [8]. Vaccinees with pre-existing Ad5 immunity from exposure to wild-type Ad5 could have activated a memory immune response against the Ad5 vectors. This immune reactivation may have cleared the vaccine, eliminating the development of a robust CMI response against the inserted Gag, Pol and Nef products.

The presence of Ad5 immunity has resulted in a variety of immunization protocols designed to overcome this limitation. Although there is evidence that increasing vaccine dosage can increase induction of desired CMI responses in Ad-immune animals [12], it can result in unacceptable adverse effects and also increased immunity against the vector. Investigators have explored development of Ad-based vaccines derived partially or completely from other serotypes or species, such as chimpanzee AdC68 and AdC1 [10,13,14], under the premise that NAb and CMI against the serotype or non-human primate viruses are not present in the human vaccinee. Several important issues with these approaches includes that it has been demonstrated that Ad5 specific NAb and CD8+ T cells cross-react between alternate serotypes of human and chimpanzee Ad vectors [9,10,15]. Once an alternate serotype is utilized the vaccinee would mount a serotype specific immune response, a condition that would terminate the future use of that Ad vector for boosting or re-immunization. Furthermore, it has been confirmed that a number of alternative serotype Ad vectors have novel innate and adaptive immune response profiles which may increase safety concerns with their use [15–17]. Such concerns prompt continued efforts to improve the properties of the safe, well-characterized Ad5 platform.

Minimizing the number of viral genes in an Ad5 vaccine vector can reduce the number of Ad5 viral proteins expressed, possibly reducing the potential of Ad5 encoded viral proteins from impacting host immune responses [18]. An advanced generation of Ad5 vectors with unique deletions of the E1, E2b and E3 regions (Ad5 [E1-, E2b-]) has previously been described and utilized to allow for improved gene transfer in a number of clinically relevant applications [19–23]. The E2b region encodes the viral DNA polymerase (pol) and the pre-terminal protein (pTP) genes, which are required for Ad-based genome replication, as well as late gene expression which encode all the viral structural proteins. Lack of E2b functionality completely prevents Ad5 replication even in the presence of supra-normal levels of the E1 transactivator [19–23]. Removal of the E2b region results in a 10,000-fold reduction in the expression of Ad late genes occurs due to the cis-activation of the Ad major late promoter [20]. As a result of this unique biology, it has been demonstrated *in vivo* that Ad5 [E1-, E2b-] vectors lead to an increased quantity and/or allow for sustained transgene expression in a number of animal models [23]. Ad5 [E1-, E2b-] vectors also display reduced acute toxicities when directly compared to Ad5 [E1-] vectors [24,25]. Specifically, Ad5 [E1-, E2b-] vectors were reported to have decreased toxicity in multiple cell types, including hepatocytes, myocytes, and cochlear hair cells found in the inner ear [26–29].

We hypothesized that the reduction of toxic Ad5 gene expression and/or induction of immune responses to these gene products would result in improved CMI responses to Ad5 [E1-, E2b-] expressed antigenic targets. Furthermore, this might allow for improved vaccine efficacy in the presence of Ad5 immunity since Ad5 [E1-, E2b] deleted vectors may provoke a decreased Ad5 specific immune response in this context. This would allow for focused CMI responses to the desired transgene antigen. We report herein the capability of an Ad5 [E1-, E2b-] vector to be utilized as a vaccine platform that induces CMI responses. Studies were performed to directly compare Ad5 [E1-, E2b-] and Ad5 [E1-] vectors' propensities to induce CMI responses in Ad5 naïve and Ad5-immune mice. Ad5 [E1-, E2b-] and Ad5 [E1-] vectors were constructed containing the HIV-1 Gag gene insert as a transgene. We choose the HIV-1 model due to the importance of CMI responses in controlling the disease [30–32] and also because of the use of recombinant Ad5 viral vectors as a vaccine platform in HIV-1 clinical trials [31,32]. CMI was assessed by total number of T cells secreting IFN-γ and IL-2 as determined by ELISpot analysis. We found that the novel Ad5 [E1-, E2b-] viral vector can induce increased levels of antigen specific CMI responses following immunization when compared to Ad5 [E1-] vectors in both Ad5 naïve and Ad5 immune animals despite the presence of high levels of Ad5 neutralizing activity. The propensity of this vector to illicit robust CMI in Ad5-immune animals also enabled for homologous boosting. Based upon the numerous improvements noted, we undertook a pilot study in which Ad5-hyper immune non-human primates were immunized with Ad5 [E1-, E2b-]-gag. The immunogenicity of this novel vaccine platform in Ad5 immune vaccinees was confirmed in the NHP model.

2. Materials and methods

2.1. Animals

Specific pathogen-free, BALB/c mice (Jackson Laboratory, Bar Harbor, Maine) ages 6–8 weeks were housed in animal facilities at the Infectious Disease Research Institute (Seattle, WA) and all procedures were conducted according to IACUC approved protocols. Cynomolgus macaques were housed at the Southern Research Institute (SRI, Frederick, MD) and all protocols were reviewed and approved by appropriate animal care and biosafety committees before initiation of the study. NHP peripheral blood mononuclear cells (PBMC) and serum samples were collected by SRI and shipped overnight to Etubics Corporation (Seattle, WA) for analysis.

2.2. Vaccine vectors

Using the HXB Gag gene (Genbank Accession # K03455) derived from pVRC3900 (kindly provided by the Vaccine Research Center, NIAID) [33] the Ad5 [E1-]-gag and [E1-, E2b-]-gag vector platforms were constructed, and vaccines were purified according to previously published procedures [19]. Briefly, the HXB Gag cDNA was sub-cloned into the E1 region of the Ad5 [E1-] or Ad5 [E1-, E2b-] vectors using a previously described homologous recombination-based system and Gag protein production was placed under the expressional control of a cytomegalovirus (CMV) enhancer/promoter element. The replication deficient virus was then propagated in the HEK-293 (Ad5 [E1-]) or E.C7 (Ad5 [E1-, E2b-]) packaging cell lines, $CsCl_2$ purified, and titered as described previously [26–29]. The infectious unit and particle numbers of the Ad5 [E1-]-gag and Ad5 [E1-, E2b-]-gag were compared. Infectious titers were determined on 293 cell monolayers which had plaque-forming titers of 3.0×10^{10} and 5.0×10^{10} PFU/mL for the Ad5 [E1-]-gag and Ad5 [E1-, E2b-]-gag virus preparations, respectively. The manufacturing particle concentrations were determined spectrophotometrically and were 1.1×10^{12} virus particles/mL for both viral lots. Thus, the ratios of particle number to PFU were similar for both virus lots, 36 VP/PFU versus 22 VP/PFU, respectively.

2.3. Immunization

Mice were injected with Ad5 [E1-]-gag or Ad5 [E1-, E2b-]-gag intradermally into a hind footpad and NHP were immunized intradermally in a hind leg below the knee. Ad5 vector constructs were delivered at a dose of 10^{10} virus particles (VP) to both mice and NHP