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Boosting with an adenovirus-based vaccine improves protective efficacy against Venezuelan equine encephalitis virus following DNA vaccination

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Abstract

There is a requirement for a vaccine that protects against the alphavirus, Venezuelan equine encephalitis virus (VEEV). Previous work has shown that DNA vaccines encoding structural proteins of VEEV can elicit immune responses and protection against VEEV though this protection is incomplete against airborne VEEV. In this study, we demonstrate that particle-mediated epidermal delivery of a DNA vaccine encoding the E2 glycoprotein of VEEV can be boosted with a mucosally-delivered Ad-based vaccine encoding the same E2 glycoprotein. This results in an improved Th2-type IgG response, an increase in neutralising antibody and a significant increase in protection against airborne VEEV. This indicates that prime-boost may be a suitable immunisation regimen for providing protection against airborne VEEV.

Keywords: DNA vaccine; Prime-boost; VEEV; Venezuelan equine encephalitis virus; Adenovirus

1. Introduction

There are a number of strategies being developed to improve the performance of DNA vaccines. One of the most successful is the use of a prime-boost immunisation regimen. The prime-boost strategy involves priming the immune response to a target antigen delivered in one way and then selectively boosting this immunity by re-administration of the antigen using a different method. In this way, greater levels of immunity can be synergistically established compared to a single vaccine administration or homologous boost strategies [1]. The effectiveness of a prime-boost immunisation strategy has been demonstrated in a number of large animal models. For example, studies in macaques in which DNA vaccines against HIV were boosted with viral vector vaccines such as modified vaccinia virus Ankara (MVA), adenovirus (Ad) or Fowlpox, have elicited protection against challenge [2–4]. Furthermore, this strategy has proven effective in humans. DNA- and vaccinia-based vaccines for a pre-erythrocytic malarial antigen delivered in a prime-boost regimen induced T-cell responses five to ten-fold greater than each vaccine alone [5]. Additionally, following challenge with an alternative strain of parasite than that used for the vaccine antigen and at a higher level than might be expected in the field, partial protection was achieved against a large number of pre-erythrocytic *P. falciparum* heterologous parasites (for review see [6]).

The alphavirus Venezuelan equine encephalitis virus (VEEV) is an important human and equine pathogen. In one of the largest outbreaks of VEEV, which occurred in central Colombia during the 1960s, over 200,000 human cases and more than 100,000 equine deaths were estimated. In a more recent outbreak (Venezuela and Colombia, 1995) an estimated 75,000–100,000 people were affected. This and other recent outbreaks in Mexico and South America suggest that VEEV is a re-emerging disease [7]. Virulent strains of VEEV also appear to be readily transmissible to humans via the airborne route, causing infections in laboratory workers [8,9]. This feature of VEEV contributes to the threat of its use as a biological weapon [10–12] and VEEV has been weaponised by the former Soviet Union [13] and the USA [14].

A live-attenuated IA/B strain of VEEV, TC-83, is effective as an equine vaccine [15]. Although previously administered

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to humans under IND status, TC-83 has now been withdrawn for human use because of concerns over reactogenicity and poor immunogenicity [16]. A further live-attenuated vaccine of the IA/B strain of VEEV, V3526, has recently been developed and confers protection to cynomolgus monkeys against aerosol VEEV challenge [17], but as yet remains unlicensed.

DNA vaccines that encode structural proteins of VEEV have also been developed. Mice immunised with a DNA vaccine encoding the E2 glycoprotein, against which the majority of neutralising antibody is directed, developed significantly higher immune responses following particle-mediated epidermal delivery (PMED) compared to intramuscular immunisation [18]. A PMED DNA vaccine encoding the subgenomic 26S mRNA of VEEV also showed protective efficacy in mice. However, protection against aerosol challenge of 10^4 pfu VEEV was incomplete (80%) [19].

The present study demonstrates that the protective efficacy of PMED DNA vaccines against VEEV can be improved by a prime-boost regimen. In this case, a replication-defective adenovirus (Ad5) was used to boost the immune response following PMED of a DNA vaccine expressing the same VEEV truncated region (E3–E2–6K). This intranasally-delivered Ad-based VEEV vaccine is capable of generating mucosal immunity [20] which is important in protection against airborne VEEV [21].

The evaluation of a prime-boost immunisation regimen against VEEV may provide significant data towards the development of a VEEV vaccine. As well as enhancing protection afforded against aerosolised VEEV following PMED DNA vaccination, it may prevent the development of vector immunity, a concern following repeated use of Ad-based vaccines. Additionally, this immunisation regimen may prove valuable in a bioterrorist context where a general, safe, multiplexed priming system may be used prior to more specific information regarding the identity of the agent, which may only be available on short notice at a later date.

In the present study, VEEV-specific IgG responses are significantly improved with a PMED DNA vaccine prime, Ad-based vaccine boost immunisation regimen. The Ad-based vaccine significantly boosts the Th2-type response generated by the PMED DNA vaccine and results in the production of more neutralising antibody. These results correlate to a significant increase in protection against $100LD_{50}$ of airborne virulent VEEV.

2. Materials and methods

2.1. Construction and expression of DNA vaccine expressing E3–E2–6K of VEEV

The E3–E2–6K genes were PCR amplified from pVEEV #3 (in which the genes are based on the TC-83 strain of VEEV but with three mutations introduced to the E2 region [20]) using primers PB-E3-F1 (5′-ACTCTA*GCTAGC*TCACTAGGACCACCATGTGTCTG-3′) and PB 6K R1 (5′-TAT*GGATCC*TTAGGCGCCGGCTGCGGCGCCGGCCATGAC-3′). The amplified fragment was cloned into pJW4304 (a kind gift from Dennis Klinman, Food and Drug Administration (FDA), USA). Using the restriction sites *Nhe*I and *Bam*HI, the genes were fused in frame with the signal sequence for human plasminogen activator under the control of the CMV IE promoter. The resultant plasmid was verified by sequencing and named pSTU-TRDF.

African green monkey kidney COS-7 cells (European Collection of Animal Cell Cultures, Porton Down) were plated at 1×10^5 cells well^{-1} into Lab-Tek II Chamber Slides (Nalgene Nunc Inc., USA). Cells were transfected with 250 ng of plasmid DNA using the transfection reagent Polyfect (Qiagen) according to the manufacturer's guidelines. Forty-eight hours post-transfection cells were fixed in acetone at −20 °C for 15 min and dried at 37 °C, then reacted for 1 h at 37 °C with a 1/400 dilution of mouse polyclonal anti-VEEV (a kind gift from Dr. B. Shope of the Yale Arbovirus Research Unit, University of Texas, Austin, TX, USA). After three washes in PBS, cells were stained for 1 h at 37 °C with anti-mouse whole molecule IgG (Sigma) diluted 1/400 in PBS/1% FCS. The cell sheets were washed a further four times in PBS before mounting in 50% glycerol in PBS and examination under a UV microscope.

2.2. Vaccination of mice

Groups of 12 Balb/c mice (female, 6–8 weeks old, Charles River Laboratories, UK) were vaccinated with either three doses of DNA vaccine pSTU-TRDF followed by one dose of Ad-based vaccine, three doses of DNA vaccine only or one dose of Ad-based vaccine only. DNA vaccination was performed with 1 μg of DNA per dose coated onto gold particles and delivered using a Helios^{TM} gene gun (Bio-Rad) as previously described [18]. Ad-based vaccination was performed with the human adenovirus type 5 dl309 strain containing the genes for E3–E2–6K from the TC-83 strain of the virus and under the control of the CMV major immediate early promoter. The construction of this Ad-based vaccine, RAd/VEEV #3, has been previously described [20]. Mice were immunised with 1×10^7 pfu by the intranasal route under halothane anaesthetic. Mice were immunised with the DNA vaccines on day 0, 14 and 28 and Ad-based vaccine on day 42.

2.3. Measurement of anti-VEEV IgG by ELISA

Mouse sera obtained from the marginal tail vein were assayed for VEEV-specific total IgG, IgG1 or IgG2a. Microtitre plates were coated with β propiolactone-inactivated TC-83 antigen in carbonate buffer. Three columns on each plate were coated with anti-IgG (Fab) (Sigma) in order to produce a standard curve for quantification of IgG concentration. After washing three times with PBS containing 0.2% Tween-20, non-specific binding was blocked with