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Combination recombinant simian or chimpanzee adenoviral vectors for vaccine development

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ABSTRACT

Adenoviral vector-based vaccines are currently being developed for several infectious diseases and cancer therapy, but pre-exising seroprevalence to such vectors may prevent their use in broad human populations. In this study, we investigated the potential of low seroprevalence non-human primate rAd vectors to stimulate cellular and humoral responses using HIV Env glycoprotein (gp) as the representative antigen. Mice were immunized with novel simian or chimpanzee rAd (rSAV or rChAd) vectors encoding HIV gp or SIV gp by single immunization or in heterologous prime/boost combinations (DNA/rAd; rAd/rAd; rAd/NYVAC or Ad/rLCM), and adaptive immunity was assessed. Among the rSAV and rChAd tested, SAV16 or ChAd3 vector alone generated the most potent immune responses. The DNA/rSAV regimen also generated immune responses similar to the DNA/Ad5 regimen. ChAd63 prime/ChAd3 boost and ChAd3 prime/NYVAC boost induced similar or even higher levels of CD4+ and CD8+ T-cell and IgG responses as compared to Ad28/Ad5, one of the most potent combinations of human rAds. The optimized vaccine regimen stimulated improved cellular immune responses and neutralizing antibodies against HIV compared to the DNA/rAd5 regimen. Based on these results, this type of novel rAd vector and its prime/boost combination regimens represent promising candidates for vaccine development.

1. Introduction

Adenoviruses are non-enveloped icosahedral double-stranded DNA viruses that can broadly infect vertebrates including humans and non-human primates. There are 68 known serotypes of human adenoviruses divided into seven groups from A to G [1]. These adenoviruses have been studied for their pathogenicity and more recently as vectors for gene therapy and genetic vaccines. The large numbers of naturally existing adenoviruses from different hosts with varied tropism and immune stimulating capabilities represent a rich repertoire for the construction of recombinant vectors that have broad clinical applications.

Recombinant adenoviral vectors used as viral vectors for vaccines have many advantages and are being tested in clinical trials for HIV, malaria, Ebola, HCV and TB. Among these viral vectors, Ad5 is known to induce the strongest CD8+ T-cell responses compared to other serotypes and other viral vectors. However, the high prevalence of anti-Ad5 neutralization antibodies in human populations may restrict the use of rAd5, especially for optimizing CD8+ T-cell immunity. In previous human clinical trials, neutralizing antibodies against Ad5 correlate with reduced immunogenicity of the vector [2–5]. Therefore, alternative adenoviral vectors with low seroprevalence are critical for advancing these vaccines into broad clinical applications. Many human adenoviruses have been

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shown to either have high seroprevalence or to induce weak primary immune responses to transgenes [6]. Currently only rAd35 and rAd26-based vectors are in clinical evaluations for HIV vaccines. Non-human primate adenovirus-based vectors are attractive alternatives to human adenoviruses because these viruses do not circulate in human populations and are rarely neutralized by human sera. Several simian adenoviruses have been also isolated from their hosts [7–10]. Those related to human adenoviruses are placed in the respective seven groups and the distantly related viruses are assigned to the distinct simian adenovirus A group. Based on phylogenetic analysis of genomic sequence, the chimpanzee adenoviruses ChAd3 and PanAd3 are classified in serotype C similar to Ad5, while ChAd63 and C68 are in serotype E. Recombinant ChAd3 vectors are reported to be similar to rAd5 since they induce similar levels of anti-tumor activity but have low seroprevalence in humans. Previous studies have focused on cellular immune responses generated with these vectors using gag as the antigen and neutralizing antibodies to HIV were not assessed [11]. Currently, rChAd3 and rChAd63 are being tested in clinical trials for the development of T-cell-based HCV, malaria, and Ebola vaccines [12,13]. Application of these vectors to HIV vaccines, especially in prime/boost combination regimens using HIV Env as the antigen, has not been systematically investigated. Efficacious HIV vaccines are likely to include HIV Env as the immunogen. Indeed, recent studies in an SIV NHP challenge model suggest that Env immune responses correlate with and are required for vaccine efficacy [14,15]. Combination of rAd with other genetic vectors including DNA, MVA/NYVAC and rLCMV in prime and boost regimen can further improve immune responses [12,15]. Although these vectors can be used alone, specific regiments, for example, rAd/MVA, rAd/rAd and rAd/rLCMV, are more potent [12,14,15].

In this study, we examined the ability of several novel simian and chimpanzee Ad vectors encoding HIV and SIV Env to generate cellular and humoral immune responses after single immunizations or in combination with DNA, NYVAC, or rLCMV. The optimized regimen outperformed the DNA/rAd5 prime boost combination and represents a candidate for further development.

2. Materials and methods

2.1. Animals

6-8 week-old female BALB/c mice were purchased from Jackson Laboratories (Bar Harbor, ME) and housed in the experimental animal facility of the Vaccine Research Center, NIAID, NIH (Bethesda, MD). All animal experiments were reviewed and approved by the Animal Care and Use Committee, VRC, NIAID, NIH and performed in accordance with all relevant federal and NIH guidelines and regulations.

2.2. DNA recombinant vaccines

The DNA plasmids expressing gp145ΔCFIΔV1V2 of HIV-1 clade B, gp145ΔCFI of HIV-1 clade A, gp145ΔCFI of HIV-1 clade C, or SIVmac239 gp140 were prepared by the methods described previously [16]. E1, E3, and E4-deleted rAd5 and E1-deleted simian and chimpanzee rAd were constructed and purified as described previously [10,17,18]. Simian adenoviruses SAV7, SAV11 and SAV16 were obtained from ATCC. The viruses were rescued and grown in an Ad5 E4 orf6-expressing HEK 293 cell line (293 orf6 cell line). Then, the E1 region of the viral genome was sequenced and deleted for the construction of recombinant vectors. The HIV or SIV Env gene under the control of a CMV promoter was inserted into the E1 region. Recombinant simian SAV and human rAd vectors were rescued in a 293 orf6 cell line. The chimpanzee rAd (rChAd3, rChAd63 and PanAd3) vectors containing Ad5 E4 orf6 in their E4 region as constructed previously were grown in HEK 293 cells [10]. Recombinant LCMV vectors were constructed as previously described [15]. NYVAC vectors were kindly provided by Dr. Tartaglia (Sanofi Pasteur, Allentown, PA) [19].

2.3. Verification of Env transgene expression

HEK 293 and A549 cells were transduced with rAd Env, 16 or 48 h later, respectively, the supernatants were collected and the cell-associated proteins were extracted with cell lysis buffer (Cell Signaling Technology, Danvers, MA). Samples were analyzed by SDS-PAGE and Western blotting using HIV-1 IIIB Strain Rabbit anti-gp120 Polyclonal (Advanced Biotechnology Inc., Columbia, MD) as a primary antibody and ECL Rabbit IgG, HRP-Linked Whole Ab (GE Healthcare, Piscataway, NJ) as a secondary antibody.

2.4. Immunization

For single immunization, mice were immunized with rAd by intramuscular routes. For DNA prime/rAd boost immunization, mice were intramuscularly primed with 15 μg of DNA three times at two-week intervals and intramuscularly boosted with rAd two weeks after the third DNA priming. For rAd prime/rAd intramuscular boost immunization, mice were intramuscularly primed with 10^7 to 10^9 VP of rAd and intramuscularly boosted with 10^7 to 10^9 VP of rAd three to four weeks later. The immunized mice were sacrificed three weeks after single immunization or two weeks after boost immunization. Five mice per group were used in the studies unless indicated otherwise.

2.5. Measurements of T-cell responses with Intracellular cytokine staining

Single cells from the spleens of immunized mice were stimulated with an HIV Env peptide pool for 5 h and the levels of effector cytokine-producing $CD4^+$ and $CD8^+$ T cells were examined by intracellular cytokine staining as described previously [20].

For measurement of IL-2-, IFN-γ- and TNF-producing $CD4^+$ or $CD8^+$ T cells, lymphocytes were plated with 2×10^6 cells/well and incubated with or without HIV gp140B peptide pool for 5 h in the presence of anti-CD28 (clone 37.51, BD Pharmingen, San Jose, CA), anti-CD49d (clone R1-2, BD Pharmingen) and 10 μg/mL brefeldin A (Sigma). Peptides used in this study were 15 mers overlapping by 11 amino acids that spanned the complete sequence of the protein. Cells were sequentially stained with VIVID dye (Invitrogen, Eugene, OR), Fc block (BD Pharmingen) and mAbs against surface markers, such as PerCP-Cy5.5-conjugated anti-CD3 mAb (clone 145-2C11, BD Pharmingen), Alexa Fluor 700-conjugated anti-CD4 mAb (clone RM4-5, BD Pharmingen) and APC-Cy7-conjugated anti-CD8α mAb (clone 53-6.7, Biolegend, San Diego, CA). The cells were permeabilized and fixed with Cytofix/Cytoperm (BD Pharmingen) and then stained with mAbs against cytokines, PE-conjugated anti-mouse IL-2 mAb (clone JES6-5H4), APC-conjugated anti-mouse IFN-γ mAb (clone XMG1.2) and PE-Cy7-conjugated anti-mouse TNF mAb (clone MP6-XT22, BD Pharmingen). The stained cells were examined by BD LSR-II (BD Pharmingen) and the data were analyzed by FlowJo software (Tree Star Inc., Ashland, OR).

2.6. ELISA for Env-specific antibodies

HIV or SIV Env-specific IgG were examined as follows: An ELISA plate with 96 wells was coated with 2 μg/mL recombinant HIV or SIV Env protein at 4 °C overnight and then blocked with PBS plus 1% BSA at 37 °C for an hour. 1:1000 diluted or serially diluted sera starting from 1:64 dilution from the immunized mice were added and

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