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Construction and immunogenicity of replication-competent adenovirus 5 host range mutant recombinants expressing HIV-1 gp160 of SF162 and TV1 strains

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ABSTRACT

An HIV Env immunogen capable of eliciting broad immunity is critical for a successful vaccine. We constructed and characterized adenovirus 5 host range mutant (Ad5hr) recombinants encoding HIV_{SF162} gp160 (subtype B) and HIV_{TV1} gp160 (subtype C). Immunization of mice with one or both induced cellular immunity to subtype B and C peptides by ELISpot, and antibody responses with high binding titers to HIV Env of subtypes A, B, C, and E. Notably, Ad5hr-HIV_{TV1} gp160 induced better cellular immunity than Ad5hr-HIV_{SF162} gp160, either alone or following co-administration. Thus, the TV1 Env recombinant alone may be sufficient for eliciting immune responses against both subtype B and C envelopes. Further studies of Ad5hr-HIV_{TV1} gp160 in rhesus macaques will evaluate the suitability of this insert for a future phase I clinical trial using a replication-competent Ad4 vector.

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1. Introduction

Recently, the Centers for Disease Control and Prevention released the first estimates of HIV incidence in the United States from the nation's new HIV incidence surveillance system [1]. This study revealed that 56,300 new HIV infections occurred in the United States in 2006, far higher than the previous estimate of 40,000 new infections annually. Whether this result reflects an actual increase, or simply better methods of prediction, it highlights the urgent need to halt the spread of the HIV/AIDS epidemic.

The most effective method for preventing new infections is vaccination. However, in the case of the HIV/AIDS pandemic, despite over 2 decades of research there are no prospects in the near future for a vaccine that would induce "sterilizing immunity". The development of an Env immunogen able to elicit broad neutralizing antibody which completely blocks HIV entry and initial infection, thus providing sterilizing immunity, is ongoing and elusive. In the absence of potent neutralizing antibody, it is believed that protection against HIV will require both humoral and cellular immune responses at systemic and mucosal sites. Non-neutralizing antibodies (NNAbs) may contribute to control of infected cell foci in the brief window of time following HIV transmission prior to systemic spread of the virus [2]. By binding to Fc receptors on effector cells and targeting Env antigens on the surface of virus-infected cells, NNAbs can mediate several activities that eliminate the infected cells or inhibit viral replication, including antibody dependent cellular cytotoxicity (ADCC), antibody dependent cell-mediated viral inhibition (ADCVI), and opsonization [3–8]. In fact, vaccine-elicited NNAbs have been shown to correlate with reduced viral burdens following challenge in macaques [6–8]. On the other hand, T-cell responses also contribute to control of virus replication and progression to AIDS. Nevertheless, the elicited cellular immunity must be broadly reactive in order to protect against infection by the spectrum of HIV isolates across the diverse subtypes. Thus, additional vaccine components, including more conserved viral genes such as *gag*, *pol*, and *nef*, will likely be necessary. For our replicating Ad-recombinant approach, these are incorporated into vaccine regimens as separate recombinants. Here, we have investigated only the *env* component.

Both naked DNA and viral vectors encoding HIV genes of interest have been exploited in HIV vaccine design. Adenovirus vectors are among the most widely tested HIV vaccine vehicles. They are non-enveloped, double-stranded DNA viruses with an icosahedral structure made up of an array of a dozen different proteins, and are able to infect dividing and non-dividing cells. Their genome of approximately 36-kb is easily manipulated to generate recombinant

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constructs and can be grown to high titer. We have pursued a replication-competent Ad-recombinant vaccine approach [9,10] with the rationale that a replicating vector will best mimic the highly successful live attenuated vaccines which give essentially life-long protection against diseases such as polio, yellow fever, smallpox, and measles. In fact, in the HIV field, a live attenuated SIV vaccine has exhibited the best efficacy in protecting macaques against SIV infection to date [11] yet due to eventual reversion to virulence is deemed too unsafe for use in people [12]. In addition to mimicking live attenuated SIV (or HIV) a replicating Ad-recombinant should also target mucosal inductive sites leading to the induction of mucosal immune responses.

Adenoviruses are very species specific, with replication of human Ad restricted to people and chimpanzees. However, use of an Ad5 host range mutant vector (Ad5hr) which can replicate in cells of non-human primates [13] allows study of candidate replicating Ad-recombinant vectored vaccines in SIV or SHIV rhesus macaque monkey models. Replication competence is maintained by preserving the Ad E1 region and replacing the Ad E3 region with a transgene of interest for expression in target cells [14]. The Ad E3 region encodes proteins involved in evading host immunity and is non-essential for viral replication. We have shown that replication-competent Ad-HIV recombinants elicit better cellular immune responses and prime higher antibody titers compared to animals immunized with non-replicating Ad-HIV recombinants [15]. Further, in rhesus macaques, priming with replicating Ad-recombinants and boosting with envelope protein has resulted in strong immune responses [16,17] and potent protection against both SIV [18,19] and SHIV [20–22] challenges.

Selection of an appropriate *env* gene for insertion into a candidate Ad-HIV vaccine is critical. In order for the vaccine to be applicable to a global population, the ideal insert should elicit broad immune responses and recognize a spectrum of HIV isolates across subtypes. In addition, several lines of evidence have shown that initial HIV infections are established by macrophage tropic HIV isolates that utilize primarily CCR5 (R5) rather than CXCR4 (X4) co-receptors [23–25]. Hence, R5 strains of HIV are preferred for prophylactic HIV vaccine development. Previously, our preclinical vaccine studies evaluating HIV envelope immunogens made use of subtype B X4-tropic *env* inserts, including Ad4-, Ad5-, or Ad7-HIV_{IIIB} or -HIV_{MN} gp160 [15,26,27]. Similarly, for evaluating HIV Env in rhesus macaques we have used an Ad5hr-recombinant expressing the subtype B dual-tropic $HIV_{89.6P}$ gp140 [20–22]. For this study we constructed replication-competent Ad5hr-HIV recombinants expressing the gp160 glycoprotein of subtype B (HIV_{SF162}) and subtype C (HIV_{TV1}) R5 strains. Subtype B HIV isolates are prevalent in North America, Latin America, the Caribbean, Europe, Japan, and Australia, while subtype C HIV isolates are prevalent in India and South Africa. Overall subtype C isolates are the most prevalent worldwide. The immunogenicity of both R5-tropic subtype B and C recombinants was evaluated in mice as a first step in selecting one or both *env* inserts for use in future clinical development of a replicating Ad-HIV vaccine. The Ad5hr-HIV_{TV1} recombinant was shown to be more immunogenic than Ad5hr-HIV_{SF162} and therefore a logical choice as an initial candidate immunogen. Recently initiated immunogenicity and protective efficacy studies in rhesus macaques will validate this selection.

2. Materials and methods

2.1. Ad5hr-HIV recombinants

Replication-competent Ad5hr-recombinants carrying the HIV_{TV1} and HIV_{SF162} gp160 genes were constructed. The shuttle plasmid, pBRAd5ΔE3 containing the Ad5 sequence from 59.5 to 100 map units (mu) with a 78.8–85.7 mu deletion in the E3 region, and the plasmid carrying the Ad5 tripartite leader (pAd5tpl-18RD2) were obtained from Wyeth-Lederle Vaccines under a Cooperative Research and Development Agreement. The HIV_{TV1} and HIV_{SF162} gp160 genes, optimized for expression in mammalian cells, were obtained from Chiron Corporation, Emeryville, CA, as pCMVlink160mod-TV1 and pCMVlink160mod-SF162 plasmids, respectively.

A Kozak sequence (GCCACC) was inserted immediately upstream of the start codon of the HIV_{TVI} gp160 gene. This was done by incorporating the sequence into the TV1.EcoRI.Kz.S primer (Table 1) which together with the TV1.DraIII.AS primer were used to amplify the first ~200 bp of the gene. The synthetic PCR products were then digested with EcoRI and DraIII and used to replace the corresponding fragment in the original plasmid.

The tPA leader sequence in the pCMVlink160mod-SF162 plasmid was changed to an optimized wild type SF162 leader. Briefly, 11 oligonucleotides (5-wt L01 to 5-wt L06 and 3-wt L01 to 3-wt L05; Table 1) were phosphorylated with T4 polynucleotide kinase (Invitrogen) and boiled for 90 s. The nucleotide mixture was allowed to cool down slowly to room temperature and subsequently ligated to the pCMVlink160mod-SF162 plasmid from which the EcoRI–DraIII fragment was already excised.

A BamHI site present in the HIV_{SF162} gp160 sequence was removed without changing the expressed amino acid sequence using the Gene Tailor Site-Directed Mutagenesis System (Invitrogen) according to the manufacturer's instructions with some modification. Briefly, the target plasmid was methylated, followed by PCR amplification using AccuPrime Pfx Supermix (Invitrogen) with a pair of overlapping primers (SF162.BamHI.KO.S and SF162.BamHI.KO.AS; Table 1). The sense primer contained a single C → T nucleotide change. The amplification products were then transformed into One-Shot MAX Efficiency DH5α-T1 (Invitrogen) in which the methylated template DNA is digested by the host. Expression of gp160 by both TV1 and SF162 plasmids was confirmed by Western blotting.

Using pCDNA3.1(−) plasmid (Invitrogen) as a template, the Bovine Growth Hormone (BGHpA) poly-A signal sequence was PCR-cloned using BGHpA.XhoI.S and BGHpA.XbaI.AS primers (Table 1) and inserted at the XhoI/XbaI site downstream of the HIV gp160 gene in both the TV1 and SF162 plasmids. The ~2.8 kb SalI/XbaI fragments of the resulting plasmids were subsequently cloned downstream of Ad5tpl in the pAd5tpl-18RD2 plasmid to generate pAd5tpl-TV1-BGHpA or pAd5tpl-SF162-BGHpA plasmids, respectively. Finally, after removing the vector portion of these plasmids by NheI/XbaI digestion, the constructs were subcloned into the pBRAd5ΔE3 shuttle plasmid at the XbaI site. The resulting shuttle plasmids were confirmed by DNA sequencing.

Ad5hr-HIVgp160 recombinants were generated by homologous recombination as previously described [28]. In brief, DNA isolated from wild-type Ad5hr was digested with SpeI and separated on a 0.8% agarose gel. The larger fragment (~27 kbp) representing 0–75 mu was recovered from the gel using a Qiaex II Gel Extraction Kit (Qiagen). The shuttle plasmids were also digested with BamHI, separated on a 0.8% agarose gel and the larger fragments (~14 kbp) representing 59.5–100 mu and containing the HIV gp160 gene were isolated using the same method. Both Ad5hr and shuttle plasmid fragments were co-transfected into QBI 293 cells with lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Plaques were observed within 10–14 days. Ad5hr-HIVgp160 recombinant candidates were screened by PCR, and gp160 expression was evaluated by Western blotting. Potential contamination with full length Ad5 wild-type virus was excluded by the absence of the Ad5 E3 gene in PCR analysis using Ad5E3-P1 and Ad5E3-P2 primers (Table 1). The Ad5hr-HIVgp160