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Adenoviruses as Vectors for HIV Vaccines

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Abstract

The tropism of adenoviruses (Ad) for mucosal epithelium makes them ideal vectors for the development of recombinant Ad-HIV vaccines. Currently, several Ad-HIV vaccine candidates are being tested in clinical and preclinical trials. Here, we review the progress on the safety, immunogenicity and efficacy of replication-competent and replication-defective Ad-HIV and Ad-SIV vaccines in animal models, including non-human primates. Replication-defective Ad-SIV*gag* vaccines have elicited cellular responses that control intravenous infection with an HIV/SIV chimeric immunodeficiency virus (SHIV), while replication-competent Ad-SIV*env/rev/gag/nef* vaccines have stimulated cellular and humoral responses and protected rhesus monkeys from a mucosal challenge with pathogenic SIV. The composition and advantages of these and other Ad vaccines are described, with particular emphasis on strategies to increase the immunogenicity of the replication-defective vaccines and the safety and efficacy of the replication-competent approach. The overall efficacy of Ad-based vaccines in non-human primates should encourage further evaluation of additional replication-competent and replication-defective Ad-HIV candidates in human trials.

Key words

AIDS vaccine. HIV. SIV. Adenovirus. Non-human primates. Humoral immunity. Cellular immunity. Mucosal immunity.

Introduction

Like HIV, human adenoviruses (Ads) were discovered almost simultaneously by two independent research teams: one searching for the "common cold" virus in cultured human tonsils and adenoids[1], and the other searching for the etiologic agent of an "influenza-like" Acute Respiratory Disease (ARD) of army recruits[2] in the 1950's. Since then, at least 51 different human Ad serotypes have been identified as the etiologic agents of respiratory, gastrointestinal, urinary and ocular infections[3]. These human Ad serotypes can be further grouped into six species (A-F, formerly called subgenera) based on their hemagglutinating properties and biophysical and biochemical criteria[4].

Ad virions are larger than HIV; their structure is that of a non-enveloped icosahedron, measuring approximately 880 angstroms in diameter[5]. The inner core contains double-stranded viral DNA, which accounts for up to 13.5% of the virion's mass. The 12 vertices on the outer shell are made

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up of penton capsomeres, which are complexes of two oligomeric proteins: a pentameric penton-base anchored in the capsid, and a protruding, antenna-like trimeric fiber extending outwards. The penton capsomere plays an essential role in Ad infection. While the fiber component interacts with the host's coxsackie-adenovirus receptor (CAR)[6], expressed by a wide array of human cell types lining the respiratory tract and gastrointestinal epithelium (Fig. 1), the penton base binds cellular integrins, facilitating viral entry[7].

Why choose adenoviruses as vectors for HIV vaccines?

In the pursuit of an AIDS vaccine capable of inducing mucosal immunity, the tropism of Ad for mucosal epithelium has made it an extremely attractive virus for use as a vector for HIV vaccine development. Ad vectors target mucosal inductive sites leading to the generation of specific immunity at effector sites via the common mucosal immune system (Fig. 1). Hence, humoral and cellular immune responses are induced at points of HIV entry, principally rectal/genital mucosa, and also at sites of HIV replication including blood, lymph nodes, and intestinal epithelium. Other practical features of Ad vectors include their growth to very high titers, typically $1x10^{11}$ pfu/ml, making them suitable for mass vaccine production. Because Ad virions are non-enveloped, they are physically stable, can withstand high pressure and can thus be lyophilized and stored for weeks or months in various formulations without losing their shell integrity[8]. An added advantage is that Ad-based HIV vaccines can be delivered orally and intranasally[9,10], with no invasive procedures requiring needles. Further, because there are several non-oncogenic and highly-characterized serotypes (including Ad1, Ad4, Ad5 and Ad7), different serotype backbones can be used to circumvent potentially unfavorable prior immunity to a particular Ad serotype in endemic areas. A recently-developed chimpanzee Ad vector[11] may also prove to be very useful in this regard. The replication of human Ad is restricted to humans and chimpanzees. However, the availability of an Ad5 host range mutant[12] and development of an Ad5 host range mutant (Ad5hr) vector able to replicate in monkey cells[13] has made it possible to test the immunogenicity, safety and efficacy of a variety of Ad-HIV and Ad-SIV vaccines in appropriate animal models, including rhesus macaques.

Several Ad-HIV and Ad-SIV vaccines have been developed and are currently under investigation in preclinical and clinical trials (Table 1). These vaccines fall into two general categories, replication-defective or replication-competent, based on the nature of early gene deletions described below. These replicative properties also lead to Ad-based vaccines with distinct characteristics and clear advantages and disadvantages, as we will also discuss.

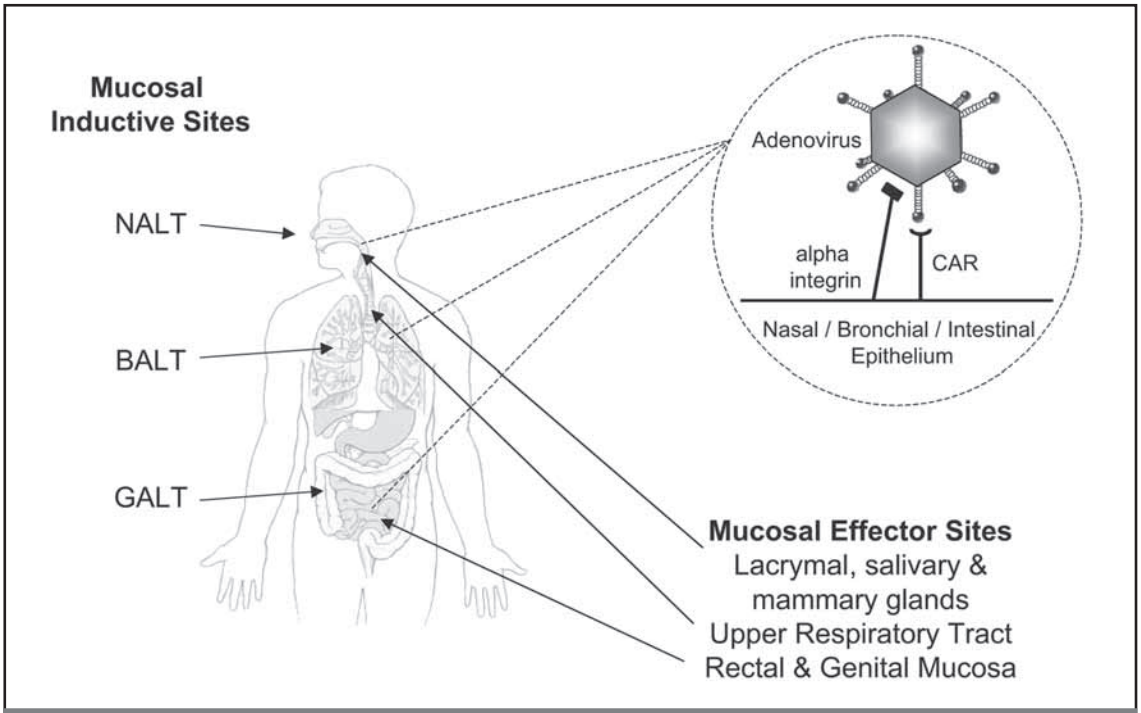

Figure 1. Ads naturally target mucosal inductive sites making them suitable vectors for AIDS vaccines. *Adenoviruses can be delivered intranasally, intratracheally and orally, and target mucosal inductive sites (i.e. nasal, bronchial and gastrointestinal-associated lymphoid tissue: NALT, BALT and GALT). Immune responses against HIV inserts are elicited at mucosal effector sites, including the rectal and vaginal mucosa, principal areas of HIV infection.*

Table 1. *Progress of pre-clinical and clinical Ad-recombinant AIDS vaccine development*

Pre-clinical studies

Replicating vectors	Inserted gene	Test species	References
Ad4-ΔE3	HIV_{MN}env; HIV_{IIIB}gag	Cotton rat	14
Ad4-, Ad5-, and/or Ad7-ΔE3	HIV_{IIIB}env/rev	Dog	15
Ad5-ΔE3	HIV_{BH10}gag	Rhesus macaque	16
Ad4-, Ad5-, and/or Ad7-ΔE3	HIV_{IIIB}env/rev; HIV_{IIIB}gag/rre/rev	Chimpanzee	17,18
Ad4-, Ad5-, and/or Ad7-ΔE3	HIV_{MN}env/rev	Chimpanzee	10,19,20
Ad5-ΔE3	SIV_{mac251}gag	Mice	21
Ad5hr-ΔE3	SIV_{smH4}env/rev	Rhesus macaque	22,23
Ad5hr-ΔE3	SIV_{smH4}env/rev; SIV_{mac239} gag	Rhesus macaque	24,25
Ad5hr-ΔE3	SIV_{smH4}env/rev; SIV_{mac239}gag; SIV_{mac239}nef	Rhesus macaque	26,27
Non-replicating Vectors			
Ad5-ΔE1	HIV_{IIIB}env/rev	Mice	28
Ad5-ΔE1	HIV_{BAL}env	Mice	29
Ad5-ΔE1	HIV_{CAM1}gag	Rhesus macaque	30
Ad5-ΔE1, ΔE3	HIV_{BX08}env	Rhesus macaque	31
Ad5-ΔE1, ΔE3	SIV_{mac142}env; HIV_{LAI}tat; HIV_{LAI}rev	Mice	32
Ad5-ΔE1, ΔE3	SIV_{mac239}gag	Rhesus macaque	33
AdC68-ΔE1	HIV_{HXB2}gag	Mice	34

Clinical studies

Replicating vectors	Inserted gene	Status	Sponsor
Ad4-ΔE3	HIVenv (clade B); HIVgag (clade B)	Planned Phase I	NCI, NIH[a]
Non-replicating vectors			
Ad5-ΔE1	HIVgag (clade B)	Planned Phase II	Merck[b]
Ad5-ΔE1, ΔE3	HIVgag/pol/nef (clade B); HIVenv (clade A,B,C)	Planned Phase I	VRC, NIH[b]

[a]MRG, unpublished. [b]The Pipeline Project, Vaccines in Development, UCSF Center for HIV Information and the HIV Vaccine Trials Network, 2003.

Genomic structure and production of replication-incompetent and replication-competent Ad recombinants

Traditionally, Ad genes are subdivided into early (E) and late (L) genes, which are expressed before and after Ad-DNA replication, respectively. A detailed description of the Ad replication cycle and the functions of the various viral genes is provided by Shenk[35]. Here we will restrict discussion to the E1 and E3 genes, most commonly deleted in Ad vectors used in vaccine development. The E1-region genes are essential for the regulation of adenovirus transcription. Thus, genetically-engineered E1-deleted adenoviruses, with insertions of heterologous promoters and foreign genes, are able to infect cells, but are unable to replicate further. Expression of the foreign gene is limited to a single occurrence following initial infection. In contrast, the E3 region is not essential for viral replication and, although it encodes several proteins that allow adenoviruses to evade or modulate the host immune response[36], E3-deleted adenoviruses are otherwise functional and replication-competent. Following infection, expression of foreign gene inserts is mediated by endogenous adenovirus promoters, and occurs with each round of Ad replication. Thus, E1- and E1/E3- deleted Ad vector cassettes are useful for cloning replication-incompetent Ad vaccines, while E3-deleted vectors are suitable for replication-competent vaccine construction. E3-deleted vectors can accommodate up to 4 kb of HIV or SIV DNA. The first generation E1/E3-deleted Ad vector cassettes have a greater insert cloning capacity of up to 7 kb.

Several additional replication-incompetent second generation Ad vectors have been described with more extensive deletions of early region genes. Completely "gutless" Ad vectors, possessing only the viral inverted terminal repeats and packaging signal[37], have also been developed. In spite of their greater cloning capacity and their reduced propensity for inducing an immune response to the vector itself, neither the second generation nor the "gutless" vectors have yet been used in AIDS vaccine design.

Replication-defective Ad-HIV/SIV recombinants require selection and production on E1-expressing cell lines, which compensate for the missing E1-regions genes by providing their functions *in trans*. The 293 cell line[38] has been used extensively. However, prophylactic AIDS vaccines intended for use in healthy people must meet more rigorous safety criteria than recombinants intended for therapeutic use in patients who will directly benefit from such gene therapy. Thus, to ensure that E1-deleted Ad recombinants are fully replication defective, cell substrates must be developed containing E1-region inserts matched precisely to each E1-deleted vector in order to avoid recombination resulting from overlapping nucleotide sequences[37]. Such design features prevent unintended contamination of Ad-recombinant vaccine lots with replication-competent Ad (RCA) arising from recombination events. In con-

trast, production of replication-competent Ad recombinants does not require complementing cells, and RCA is not a safety concern. E3-deleted Ad recombinants can be grown on a variety of primary human epithelial cells or cell lines. Here, as is also the case with replication-defective Ad recombinants, cell substrate selection must take into account issues of possible tumorigenicity and adventitious agents[39].

Replication-incompetent Ad-HIV/SIV vaccines

Both classes of Ad recombinants (replication competent and replication incompetent) are being developed as vaccines against HIV. We will summarize progress to date on each approach, followed by a general discussion of the strengths and areas of concern for each vaccine type. Recent pre-clinical studies have established replication-incompetent Ad recombinants as prime vaccine candidates. Studies in Mamu-A*01 rhesus macaques showed that three intramuscular immunizations with $1x10^{11}$ replication-incompetent Ad5-SIV*gag* viral particles generated sufficient CD8-mediated cellular immunity to lessen acute-phase viremia and control set-point viral burdens at low levels in comparison to control monkeys, and to protect against the sudden, dramatic loss of CD4+ T-cells induced by an intravenous challenge with the genetically-engineered, pathogenic HIV/SIV chimeric virus, SHIV89.6P[33]. In addition, monkeys primed with three 5 mg doses of SIV*gag* DNA, administered with the proprietary adjuvant CRL 1005 and boosted with a single dose of $1x10^{11}$ Ad5-SIV*gag* viral particles, were protected from this intravenous challenge to the same extent. Although these results are very encouraging, their relevance to a sexually-acquired human AIDS virus infection remains to be established. In fact, the same degree of protection using this vaccine approach was not achieved when similarly-immunized macaques were challenged intrarectally with the pathogenic SIVmac239 strain[40], a challenge model that resembles mucosal transmission of an AIDS virus infection, and one of several virulent SIV strains that produces an AIDS-like disease in macaques that more closely mimics HIV-induced AIDS in people[41]. Macaque studies of an Ad5-HIV*gag* recombinant have confirmed the potent cellular immune responses elicited by the adjuvanted DNA prime/Ad-recombinant boost protocol. In fact, a low-dose (10^7 viral particles) Ad-HIV*gag* recombinant boost elicited comparable immune responses to a high-dose boost (10^{11} viral particles)[30].

The promise of this replication-incompetent Ad vaccine approach has led to human trials (Table 1). However, in spite of the strong cellular immunity elicited in response to immunization with the Ad5-HIV*gag*, it is likely that humoral immune responses will also be necessary for an effective vaccine against HIV. In the absence of functional envelope antibodies which mediate neutralization of viral infectivity or other antibody responses able to blunt the initial infecting dose of virus, vaccines are likely to fail, due to the inability of even potent cellular immunity to control a high virus exposure and subsequent cell-to-cell spread of replicating virus. Neutralizing antibodies and cellular immunity can independently provide selective pressure leading to escape of non-recognized viral strains[42-45]. A dual approach, however, targeting conserved functional domains and making use of both humoral and cellular arms of the immune system, may better retard this phenomenon. Replication-incompetent Ad-recombinant vaccines with HIV envelope inserts are being developed by other research groups[28,31]. In one case, high titers of envelope-specific antibodies were elicited in mice immunized with an Ad5-HIV*env* recombinant encoding the envelope from the T-cell line adapted HIV-1 strain IIIB[28]. However, it was not established whether such antibodies had a neutralizing capacity *in vitro*. In a more recent study, Mamu-A*01 rhesus macaques were primed with DNA encoding the envelope gene from the primary HIV isolate, Bx08, and boosted with a single intramuscular injection containing $4.4x10^{11}$ pfu of replication-incompetent Ad5-HIV*env* recombinant encoding HIV_{Bx08} gp120[31]. Very high levels of anti-envelope antibodies were elicited in sera, and neutralizing antibody activity against the homologous primary HIV strain was detected in most of the immunized macaques. The neutralizing activity was of low titer and transient, however, and lacked cross-reactivity with other CCR5-tropic HIV isolates. Nevertheless, the induction of neutralizing antibodies against a primary isolate is an encouraging advance. The fact that it was achieved in the absence of a protein subunit booster immunization is significant.

Replication-competent Ad-HIV/SIV vaccines

The use of replication-competent Ad recombinants in AIDS vaccine design has been pursued by a few groups, but principally our own (Table 1). Using a vaccine strategy incorporating Ad-recombinant priming and boosting with subunit proteins, a complete spectrum of immune responses has been achieved; both systemic and mucosal cellular and humoral immunity to HIV or SIV in non-human primate models[10,17-20,22-27]. In initial studies, chimpanzees primed intranasally with $1x10^7$ pfu of a replication-competent Ad5-HIV*env* recombinant, and boosted with a native gp120 protein subunit, were protected from multiple intravenous HIV-1 challenges[10,19]. Key findings in these studies were the duration of protection[10] and the development of both CTL and antibodies able to neutralize primary isolates[20], achieved with only three immunizations.

To assess the ability of this vaccine approach to protect against a pathogenic virus, we turned to the SIV rhesus macaque model. Initially, we showed that mucosal priming with an Ad recombinant encoding SIV*env/rev* based in an Ad5 host range mutant vector, followed by boosting with gp120, elicited humoral, cellular, and mucosal immune responses and resulted in reduced viral burden during the acute phase of infection following a vaginal challenge with SIV_{mac251}[22]. Subsequently, we have systematically investigated

the impact of priming immunizations with recombinants encoding additional viral genes. Oral and intranasal immunization with Ad5hr-SIV*env/rev* and/or Ad5hr-SIV*gag*, followed by boosting with a SIVgp120 subunit protein, elicited immune responses to all three viral gene products, and resulted in reduced acute phase and set point viral burdens following an intrarectal challenge with SIV_{mac251}[25]. Oral, intranasal and intratracheal priming with these Ad5hr-SIV recombinants, in addition to an Ad5hr-SIV $nef_{\Delta 1\text{-}13}$ component, elicited potent cellular immunity to all four encoded SIV genes (*env*, *rev, gag*, and *nef*) and extended to both dominant and sub-dominant CTL epitopes[26,27]. The immunity was persistent, extending to 30 weeks after the last Ad immunization. Following an intrarectal SIV challenge, the vaccine efficacy was impressive, as significant protection was achieved in macaques primed with three or more SIV genes. A subset of animals (39%) exhibited exceptionally strong protection, being completely aviremic or clearing or controlling viremia at the threshold of detection (Patterson, et al. [submitted for publication]). Overall protection during the acute phase of infection was associated with anti-gp120 antibodies, while CTL responses were associated with reduced viral burdens at set-point. This solid, sustained protection from a pathogenic SIV_{mac251} challenge persisted over a year after the first challenge. The protection elicited is remarkably durable, as shown by continued protection from a second intrarectal SIV challenge administered with no intervening immunization in 8 of 11 macaques. These macaques continue to be aviremic at 24 weeks after the second challenge, with no signs of immunosuppression (Malkevitch N, Patterson LJ, et al. [unpublished observations]).

Choice of replication-competent versus incompetent Ad-HIV-recombinant vaccines

Clearly, both types of Ad-recombinant vaccines are highly promising vaccine candidates. Protection against the pathogenic SHIV89.6P challenge in rhesus macaques using a non-replicating Ad-SIV recombinant[33] was as good or better than that seen using other viral vector recombinant vaccines, including MVA[46] and VSV[47]. At the same time, the solid protection obtained against pathogenic SIV_{mac251}using replicating Ad-SIV recombinants for priming (Patterson, et al. [manuscript submitted]) has exceeded that seen against virulent SIV strains with other vectored approaches, including the attenuated vaccinia vectors ALVAC[48], NYVAC[49], and MVA[50,51], Venezuelan Equine Encephalitis virus replicons[52], herpesvirus[53], and salmonella[54]. What factors must be considered, then, in moving forward with either vaccine approach? Several issues for consideration are discussed below.

Practical considerations, in addition to the choice of cell substrate discussed earlier, include how cloning capacity may impact on development of broadly-effective vaccines. To date, HIV clade B gene inserts have been most extensively used in vaccine materials. For global use, however, genes representative of HIV strains prevalent worldwide, including circulating recombinant forms, will be needed. A key manufacturing issue will be whether these will be produced separately or incorporated as multi-gene, multi-epitope inserts in Ad recombinants. In this regard, the replication-incompetent Ad vector has an advantage in being able to accommodate approximately twice as much foreign genetic material.

The choice of Ad serotype for use as the vector backbone, as it relates to the prevalence of Ad subtypes worldwide, is important for both approaches. Prior immunity resulting from previous Ad infection could result in lessened effectiveness of the Ad-vectored immunogen, as suggested by gene therapy studies in which repeated administrations of the same Ad-recombinant vector led to anti-vector immunity and decreased expression of the foreign gene insert[55]. We, and others, have shown that limited sequential administrations of the same Ad vector can still boost immune responses to inserted gene products[24,56], suggesting prior immunity may not be so deleterious if vaccine regimens require few immunizations. Alternatively, Ad vectors of different serotype may be used for sequential administrations[10], rare serotypes may be selected for vector development[11,57], or other vectored vaccines such as naked DNA may be used for initial priming followed by Ad vector boosting[33,58]. With regard to alternative serotypes, the need for development of complementary cells for replication-defective Ad vaccine production may be problematic.

Safety issues are critical in vaccine development. Although replication-incompetent Ad recombinants are theoretically safer, it remains to be determined if the high doses necessary (10^9-10^{11} plaque forming units [pfu]) to elicit potent immune responses with these recombinants are tolerable, due to local inflammatory responses. Replicating Ad recombinants can be administered at significantly lower doses (10^5 -10^7 pfu), thereby greatly lessening any initial toxicity. From an immunological standpoint, the ability of an E3-deleted Ad-HIV vaccine to replicate in respiratory and intestinal epithelium may provide its greatest advantage in inducing the mucosal immunity so needed for prevention of HIV transmission. Conversely, this ability to replicate, together with the potential for causing disease, leads to greater safety concerns. Nevertheless, replicating Ad wild-type vaccines have an impressive history of safe and effective use in preventing acute respiratory disease (ARD) in military personnel. Ten million US army recruits were immunized orally with replication-competent Ad type-4 and Ad type-7 vaccines from 1971 to 1996, resulting in unprecedented levels of protection from ARD[59]. At the same time, no safety issues or clinically significant events arose. The effectiveness of this vaccination program is emphasized by recent ARD outbreaks[60] and two fatal cases of adenovirus-related illness in military personnel[61] attributed to suspension of the replication-competent, wild-type Ad4 and Ad7 oral vaccination program. Hence, safety concerns about oral vaccination with replication-competent adenovirus would seem rather paradoxical.

Intranasal administration of Ad-recombinant vaccines may be more effective than the oral route in eliciting the desired mucosal immunity, yet dose tolerance may be more limited. In gene therapy studies, repetitive intranasal doses of 10^9 pfu of non-replicating Ad recombinants were safely tolerated[62,63] while 10^{10} pfu resulted in local inflammation. The wild-type Ad4 vaccine has been safely administered intranasally to Ad-seropositive people at a dose of $2x10^5$ pfu[64] while an intranasal dose of $4x10^4$ pfu was well tolerated in Ad seronegative individuals, although 1 of 4 people developed minor clinical symptoms[64]. As summarized above, although not the natural hosts of human Ad, both chimpanzees and rhesus macaques have developed impressive immunity to HIV and SIV gene products following intranasal administration of replicating Ad recombinants, while exhibiting no clinical symptoms attributable to Ad immunization. Clearly, human trials will need to determine the safe intranasal dose and whether it is sufficient to elicit the desired immune responses.

Vaccine programs using replicating Ad recombinants introduce additional safety concerns with regard to the possible spread of the live vaccine to non-vaccinated individuals. Yet transmission to jointly-housed individuals in close contact with subjects who received oral Ad4 and Ad7 vaccines was not observed[65]. Intimate contact was required for such transmission among adults[66], mother-to-child transmission was rare, and transmission from vaccinated children to parents or siblings occurred at a low frequency of 10 to 20 %[67]. Similarly, transmission following intranasal vaccination has not occurred among jointly-housed vaccinees and control subjects[64]. These findings suggest that with appropriate counseling, transmissibility issues should not preclude the use of replicating Ad recombinants.

Finally, the inherent safety of the replicating Ad backbone containing the E1 genes, but lacking the E3 region, should be addressed. Although Ad-E1 genes were initially reported as possessing transforming activity, and some human Ads are able to induce tumors in rodent cells, importantly, extensive studies have not shown any link of Ads with human cancer[68]. E1a, in fact, has been shown to reverse the transformed phenotype of human tumor cells, suggesting that the context of E1 interaction with host-cell proteins is critical in directing cells towards the transformed or normal state[69]. With regard to E3, previous reports indicated that deletion of the E3 region increased the pathogenicity of Ad5 in the cotton rats, suggesting that use of such recombinants in people might be problematic. Using Ad4-HIV*env* and Ad4-HIV*gag* recombinants planned for use in phase I human trials, we have shown, on the contrary, that insertion of foreign DNA in the E3 region abrogated any increased pathogenicity[14], substantiating the safety of these replicating vectors.

While replicating Ad recombinants provoke more safety concerns than the non-replicating vectors, they may be more immunogenic. Overall they can provide a greater antigen dose by replicating to levels that exceed the total dose of replication-incompetent vector. Their replication also provides prolonged expression of inserted genes and perhaps, therefore, a more persistent immune response. Replicating vectors induce pro-inflammatory cytokines and co-stimulatory molecules, thus providing their own adjuvant effect. Replicating Ad recombinants may also be better able to overcome pre-existing Ad immunity, as indicated by the ability to re-infect Ad-seropositive people. Most importantly, however, replicating Ad recombinants may elicit more potent mucosal immune responses due to their prolonged replication in epithelial cells of the upper respiratory tract and gut. In contrast, it is problematic whether replication-incompetent, E1/E3-deleted Ad recombinant can be safely delivered at high enough doses to mucosal inductive sites to generate desired mucosal immune responses. To our knowledge there is, as yet, no evidence that replication-incompetent Ad-vectors can generate substantial long-lasting mucosal immunity against HIV or SIV in the gut or vagina.

Future directions for Ad-HIV research

Clearly, Ad recombinants are highly immunogenic and suitable as vectors for delivery of HIV vaccines. Replication-incompetent Ad-SIV*gag* and replication-competent Ad-SIV*env/rev/gag/nef* have protected non-human primates from intravenous/SHIV89.6P and intrarectal/SIV_{mac251} challenges, respectively. A systematic comparison of the immunogenicity and efficacy of replication-incompetent and replication-competent adenovirus-SIV/HIV recombinants encoding the same genes, however, has not been performed in non-human primates. This would be extremely useful in determining the future design of Ad-HIV recombinant vaccines. Additional studies that might lead to highly-efficacious vaccines and strong mucosal immunity include combination regimens in which replication-competent and -incompetent Ad recombinants are used in prime-boost strategies. It has been shown, for example, that priming with live replicating poliovirus vaccine is necessary for induction of strong mucosal immune responses following boosting with inactivated poliovirus vaccine[70]. An attractive strategy, therefore, also avoiding vector-induced immunity, might be priming with replicating Ad4-HIV recombinants and boosting with non-replicating Ad5-HIV recombinants.

Improved administration of vaccines to mucosal sites is also needed. Mucosal protection in our most recent macaque study was achieved using a combined oral/intranasal and intratracheal Ad-SIV immunization approach (Patterson [submitted]), one which would be difficult to implement in a mass-vaccination scenario in humans. Oral delivery of Ad-recombinant vaccines to the intestine, used successfully in the military Ad4 and Ad7 vaccine program, is therefore under study in the macaque model. New targeting approaches, including modifying Ad pentons and fibers to increase Ad affinity for specific receptors, are being developed for Ad gene therapy and Ad vaccine applications[71-73] and might also be used to improve mucosal immune responses.

Finally, since Ad-HIV vaccines would ultimately be used for mass-vaccination purposes, an equally important question is whether they would be suitable for use in the general population, including immunocompromised individuals. It is clear that immuno-suppressed individuals, such as transplant recipients or those with frank AIDS, have a greater likelihood of exacerbated Ad-induced disease[74]. It is highly unlikely that these individuals would be vaccine recipients. However, it will be important to determine the relationship of Ad-induced disease to the extent of CD4 depletion, a question that may be initially addressed in non-human primate models. Human trials of replicating Ad-HIV recombinants will soon be implemented. Ultimately, the safety of Ad-recombinant immunization in HIV-infected healthy individuals will also need to be explored.

In conclusion, the safety, immunogenicity and efficacy observed so far in non-human primates, coupled with the necessity for an effective mucosal vaccine against HIV, makes the testing of additional replication-competent and replication-incompetent Ad-HIV vaccine candidates in humans a scientific matter of simple logic and urgency.

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