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Replicating adenovirus vector prime/protein boost strategies for HIV vaccine development

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Abstract

Background—In the last few years the HIV vaccine field has moved forward a number of promising vaccine candidates into human clinical trials.

Objective—In this review we briefly discuss the advances made in vaccine development and HIV pathogenesis and give an overview of the body of work our lab has generated in multiple animal models on replication-competent Ad recombinant vaccines.

Methods—Emphasis is placed on comparative examination of vaccine components, routes of immunization and challenge models using replicating Ad vectors.

Results/conclusion—The overall findings make the case that replicating Ad vectors are superior in priming multiple arms of the immune system, and in conjunction with protein boosting, have resulted in dramatic protective efficacy leading to their advancement to phase 1 trials. Implications of the recent halting of the Merck Ad5-HIV phase 2b clinical trial for our vaccine approach and other vectored vaccines are discussed.

Keywords

HIV vaccine; replication competent adenovirus vectors; mucosal immunity; protein boost; multigenic priming; animal models

1. Introduction

Ever since HIV was discovered to be the causative agent of AIDS almost 25 years ago, researchers have been trying to develop an effective strategy to oppose it. Initial vaccine candidates were based on approaches that had been successful in preventing other viral infections, such as inactivated virus and viral subunits. Yet the first HIV vaccine candidate advanced to phase 3 efficacy trials, a monomeric HIV envelope subunit, recombinant gp120, failed to elicit effective immunogenicity let alone protection in humans [1]. In retrospect, this early failure was due to lack of basic knowledge of the dichotomous interaction between the virus and host and the cryptic nature of neutralizing epitopes critical for protection [see 2 for review]. Unlike viruses for which vaccines are currently available, HIV is special in its ability to simultaneously attack a key element of the immune system, CD4⁺ T-cells, and elude host immune responses by a variety of mechanisms such as immune selection and down regulation of MHC class I molecules. Moreover, HIV integrates into the host genome as a crucial step in its life cycle, leading to life-long infection. Because it is an RNA virus with an error prone

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polymerase, it is further able to tolerate high levels of sequence variation. For vaccinologists, the goal is to design a vaccine that will stimulate lasting protective immunity effective against a broad spectrum of HIV variants and be able to withstand viral immune escape. Given the difficulty to date in designing a vaccine able to prevent transmission of HIV, current strategies have evolved to managing the course of disease by lowering viral load, with the aim of keeping the immune system intact and tipping the balance in favor of the host, while also decreasing potential transmissions. In an Ugandan cohort [3] viremia levels were shown to directly correlate with heterosexual transmission rates, and individuals with less than 1500 HIV-1 RNA copies in their blood rarely transmitted HIV to their partners. Overall, developing a vaccine that would succeed in providing long term protective immunity able to blunt or even prevent viremia along with implementing other preventive strategies are vital to solving the AIDS pandemic.

A major obstacle to HIV vaccine development is that no clear immune correlate of protection has been identified. “Elite controllers”, a highly protected subset of long-term nonprogressors who maintain normal CD4+ counts ≥ 10 years are further defined as naturally resistant to infection or able to control viremia to undetectable levels (< 50 copies RNA/ml). One percent of HIV infected individuals have the ability to control viremia at this level, apparently unassociated with route of exposure, race, gender or sociologic factors [4]. Elite controllers are currently being studied extensively for clues which will inform targeted vaccine design. A recent review points to multiple factors associated with viral control, with no common factor identified [4]. Adaptive immunological responses, such as elevated levels of HIV specific CD4 + T-cells expressing potent IL-2 and IFN- γ cytokines as well as CD8+ T-cells which are polyfunctional in their ability to produce multiple cytokines and rapidly divide upon antigen stimulation, are measurably higher in elite controllers. CD4+ T-cells from elite controllers also have been shown to lack the immunosuppressive molecule CTLA-4 which is often up-regulated on cells of HIV infected patients. Innate immune mechanisms are the first line of defense from viral infection. It has been reported that both natural killer and plasmacytoid dendritic cells are higher in number in elite controllers, with some expressing certain regulatory receptors that are associated with control of viremia. Finally, certain HLA alleles are enriched among elite controllers, highlighting the role host genetic factors can play in impacting the level of virus-specific immunity that the host can mount. Individuals possessing deletions or polymorphisms within the coreceptor CCR5 required for HIV entry can also influence the ability of the virus to infect.

Other models are also potentially useful in determining immune correlates of protection. For example, some non-human primate hosts naturally infected with SIV, including sooty mangabeys, exhibit high viral loads with resulting profound depletion of mucosal target CD4 + T- cells, however with no disease progression [5,6]. Underlying mechanisms associated with immune activation may provide clues to the apathogenic nature of the disease course in these animals.

In the absence of a defined protective correlate, cellular immunity is believed to be important based on several lines of evidence. In natural infection, the role of T-cells in controlling viremia is evident when after an initial peak of viremia at week 3, HIV is brought under control by the emergence of CD8+ and CD4+ T-cells capable of lysing infected cells. Although the virus is not cleared to undetectable levels in most people, a set point is reached a few months after infection which is 1–2 logs lower than the peak, and is maintained for years until total immune exhaustion leads to AIDS and death [7]. T-cells are also important for control of SIV in the macaque model of AIDS. CD8+ T-cells elicited by vaccines are responsible for reducing viral load [2,8–11]. Conversely, *in vivo* depletion of CD8+ T-cells prior to challenge with SIV results in uncontrolled viral replication [12].

Antibodies able to neutralize HIV infection will also be important components of an effective vaccine. Passively administered neutralizing antibodies have resulted in sterilizing protection of rhesus macaques, completely blocking both systemic and mucosal transmission of SIV. However, very high antibody levels were required [7,13–16]. These crucial experiments stand as the only proof-of-concept that HIV infection can be thwarted at the level of transmission. However, a seemingly insurmountable difficulty is in designing an immunogen which mimics the envelope's native trimer structure on the virion as well as exposing highly conserved epitopes, hidden as a result of native conformation or surface glycans. Few antibodies capable of recognizing these sites exist even in infected patients. In this regard, the CD4+ binding site of gp120 is a clear target for vaccine design [17] as well as the membrane proximal region of gp41 and the conformational co-receptor binding site on gp120.

Without broad, potent neutralizing antibodies able to prevent HIV infection altogether, a vaccine strategy aimed at controlling viral infection at a low level must induce sufficient immunity to tip the balance in favor of the host. Studies conducted in the rhesus macaque model have shown that within days of initial infection, CCR5-expressing effector memory CD4+ T-cells, the majority of which reside in the gut, are targeted by the virus and eliminated [18–20]. These same cells are the ones that mount an effective immune response to the virus. A vaccine designed to simply control infection must elicit sufficient HIV-specific central memory cells in the T-cell pool able to give rise to effector cells capable of controlling virus over time. It is now thought that the deciding blow to the immune system in favor of the virus is a generalized immune activation characterized by activation of all T-cells, inflammatory cytokine secretion and eventually immune exhaustion [21]. Once this process is initiated, vaccine-elicited responses might be unable to reverse the effect. Recent studies of the pathogenic mechanisms of HIV infection in the gut and mucosa and association of immune activation with microbial translocation and metabolic dysfunction may suggest approaches for restoring immune function in people with HIV [22]. The extent to which vaccine-elicited immune responses might contribute to such restoration needs to be explored.

It has become apparent since the first attempts at vaccine development that all components of the immune system will need to be engaged in fighting HIV, including humoral and cellular adaptive and innate immunity, and that immune responses will need to be elicited in multiple compartments, both systemic and mucosal. Use of live attenuated SIV vaccines showed great promise in preventing infection in the monkey model [23–26], perhaps due to their mimicking of natural infection and engagement of the total immune system. But use of such vaccines is precluded due to safety concerns, since persistent replication of the vaccine virus eventually results in reversion to virulence [27]. Therefore, researchers have looked to numerous alternative approaches to deliver HIV genes to the immune system with a number of them already in clinical trials [see 2,7 for reviews]. These include replication competent and incompetent viral vectors in addition to DNA. As discussed below, one alternative to a live attenuated vaccine is provided by a replicating vector. Here we briefly review a number of candidate replication-competent viral vectors under development for application in HIV vaccine approaches and then focus extensively on replicating Ad vectors which have been shown to be highly effective in priming multiple arms of the immune system. Together with protein boosting in rhesus macaques, replicating Ad vectors have elicited some of the most potent and persistent protective efficacy against virulent SIV mucosal challenges, providing the basis for moving the replicating Ad-recombinant approach into clinical trials. However, the recent failure of the Merck replication-defective Ad5-HIV vaccine in the STEP trial, and the apparent correlation of high pre-existing Ad5 antibody titers with a greater number of HIV infections in vaccinees necessitates consideration of possible mechanisms contributing to this outcome and careful clinical trial design until the trial results are completely understood. In the final analysis, the STEP trial may have important implications not only for Ad-based vaccines, but perhaps also for other vectored vaccines and all vaccine approaches that aim to

induce cellular immune responses. At the end of this chapter we discuss the trial results as they are currently known with regard to specific vectors including Ad vectors, vaccine design, and translation into clinical trials.

2. Replicating vectors as vaccine candidates

HIV is primarily transmitted via the mucosal route, similar to poliovirus, *Salmonella typhi*, influenza and rotavirus. It is no coincidence that vaccines for these latter infectious agents all induce mucosal immunity, are live attenuated, and are administered mucosally, primarily orally [see 28 for review]. While there are lingering safety concerns related to their use and some difficulties in manufacturing, they have exhibited potent immunity and protective efficacy in clinical trials and are approved for human use.

Since use of a live attenuated HIV vaccine has been ruled out due to the implications of reversion to wild type and subsequent disease induction [29,30], a more practical approach is utilizing replication competent mucosal vectors expressing foreign genes of interest, thereby eliminating the safety concerns associated with live attenuated viruses. Live vaccine vectors delivered in such a way as to closely mimic natural infection by a virus or bacteria, should prove optimally effective. They often target the same cell types, present antigens in native conformations, and additionally persist for more than a single replication cycle, enabling prolonged antigen presentation to the immune system.

A few of the most promising replicating vectors will be detailed below, although it is worth mentioning that additional vectors such as an attenuated Sendai virus expressing SIV-gag and polioviruses expressing the entire SIV genome in overlapping fragments have been developed and tested, resulting in induction of virus-specific immunity and protection in the macaque model [31,32]. Furthermore, a phase I vaccine trial in China has recently begun using an attenuated, replicating Tiantan vaccinia vector expressing multiple HIV genes [33].

2.1 Measles Virus

A close examination of licensed vaccines against infectious diseases reveals that some of the most potent, efficacious constructs are replicating [34]. In addition to the well described vaccines for polio, typhoid fever, and rotavirus mentioned earlier, the measles virus vaccine is also live attenuated and has been given to millions of children with no major adverse side effects [35,36]. One factor contributing to this safety is that the vector is a single-strand negative RNA virus which replicates, like polio, yellow fever and vesicular stomatitis virus (VSV), solely in the cytoplasm without a DNA step. Thus, integration is not a concern. The measles vaccine elicits durable, broad immunity (both humoral and cellular) and conveys life long protection. The vector can accommodate large inserts up to 5Kb with no detectable genetic instability and has been used as a backbone for insertion of a number of foreign genes, including SIV, HIV, hepatitis B, mumps, and West Nile virus [37–40]. There is no apparent restriction due to anti-vector immunity, suggesting that the measles vector could be used in additional vaccine applications in spite of previous measles vaccination. In a study published by Lorin, *et al.* [38], a live attenuated measles virus expressing an HIV_{89.6P} gp160 envelope induced high titer antibodies in both mice and macaques and CD4+ and CD8+ T-cell responses in mice – all in the face of high pre-existing immune titers to measles virus. This vaccine has been given safely in aerosol form and was successful in inducing mucosal immunity [41,42]. It needs to be explored further in the rhesus macaque model, preferably allowing for assessment of protection following a virulent SIV challenge.

2.2 Vesicular Stomatitis Virus

The utility of VSV as a vaccine vector has also been explored. First developed for use in domestic livestock as a vaccine to prevent lethal foot-and-mouth disease, its lack of seroprevalence in the human population together with its ability to infect mucosal tissues, accommodate large inserts and replicate to high titers in tissue culture make it a highly attractive vaccine vehicle [43–45]. After success in inducing immunity and protection against influenza in mice by the intramuscular and intranasal routes [45,46] was achieved, attention turned to use of VSV as an HIV vaccine vector. Studies in rhesus macaques showing that live recombinant VSV expressing HIV envelope and SIV Gag stimulated both CD8⁺ T-cell responses and neutralizing antibodies and led to protection from a SHIV_{89.6P} challenge [47–49], brought this strategy to the forefront of vaccine development. Nevertheless, a key safety concern is the vector's neurotropism. Mice inoculated intranasally with the vector developed encephalitis [50]. Neuropathology was confirmed later in rhesus macaques, but only after direct intrathalamic inoculation [51]. The vector has been further modified by deleting the entire G gene, attenuating neuropathogenicity. With this deletion, however, comes the additional problem of requiring specialized cell substrates able to complement this protein for large scale manufacture of virus stocks [52]. Nevertheless, when attenuated, non-replicating VSV vectors were used as a boosting immunization following priming with DNA plasmids expressing SIV Gag and IL-12, the combination strategy elicited better immunity and resulting protection from a SHIV_{89.6P} challenge than VSV alone [53]. Although still to be tested using the more virulent SIV challenge model, continued optimization of these DNA-VSV combination strategies is ongoing.

2.3 Poxviruses

Vaccinia virus is one of the most successful vaccines ever developed, with worldwide use resulting in eradication of smallpox. As recombinant vaccinia vectors can induce both long-lasting antibody and cellular immune responses, the vector was a natural choice for development of an HIV vaccine and was one of the earliest strategies undertaken. A number of clinical studies in which a vaccinia-HIV_{env} recombinant prime was followed by an envelope protein boost were shown to be safe in the controlled trial setting [54]. However, the vaccine unfortunately proved less immunogenic in people [55] than in earlier nonhuman primate studies [56], presumably due in part to high levels of preexisting immunity to the vector as a result of the smallpox eradication program. Moreover, continued safety concerns regarding the potential for spread of vaccinia to unvaccinated contacts [57] with the possibility of eye infection, myopericarditis and unintended seroconversion against the inserted HIV gene product dampened enthusiasm for this approach. Accidental spread to immune suppressed individuals can have severe consequences [58]. Nevertheless, the success of the vaccinia vaccine program prompted development of nonreplicating poxvirus vaccine vectors, including the multiply passaged modified vaccinia Ankara (MVA) and the genetically modified NYVAC vector, both of which no longer replicate in human cells, and fowlpox and canarypox (ALVAC) vectors which are restricted to replication in avian species. These non-replicating poxvirus recombinant vaccines have entered clinical trials, and have shown promising results. Although the focus of this review is on replication-competent Ad-recombinant vaccines, promising, non-replicating poxvirus vaccine candidates will be briefly summarized below.

2.4 Adenoviruses

Replicating adenoviruses (Ad) are particularly attractive vehicles for mucosal delivery due to the fact that they target mucosal inductive sites, including the gut and upper respiratory tracts [see 59,60 for reviews]. They can infect dividing and non-dividing cells, with no integration into the host genome. Given mucosally, after infecting the epithelial cells lining the mucosa, they can infect localized dendritic cells which migrate to draining lymph nodes and efficiently

present antigens to T-cells, a vital step in generation of protective immunity. In addition, adenoviruses induce local innate immune responses upon inoculation such as cytokines and chemokines and upregulate co-stimulatory molecules which additively result in elicitation of potent immune responses [61]. Replication of the vector ensures ongoing antigen expression and presentation to the immune system, allowing administration of these vaccines at low, safe doses. The latter “dose sparing” feature represents a practical manufacturing advantage. Moreover, Ad stably express inserted genes with high levels of expression, and can be grown to high titers *in vitro*, further addressing large scale manufacturing concerns.

As with other replicating vectors, safety is a prime concern for a vaccine that will be administered to healthy individuals and some people perhaps already unknowingly infected with HIV and immune suppressed. In natural infection, Ad has been routinely isolated from adenoids, tonsils and lungs of human patients [62,63]. However virus has not been isolated from recipients of recombinant Ad vectors or non-attenuated Ad vaccines. Non-attenuated live Ad4 and Ad7 vaccines have been administered to more than 10 million military recruits in the past in order to protect them from acute respiratory syndrome (ARD) [64,65]. Given orally in the form of enteric-coated tablets, they replicated in the gut without causing disease and could be detected in stool for up to 3 weeks [66–68]. Only after intimate contact within the same household was transmission shown to occur, but with no resulting clinical symptoms [69]. These vaccines proved highly effective in reducing cases of ARD. While vaccination was suspended the last few years due to lack of a manufacturer, the benefit of the oral Ad4 and Ad7 vaccine program became apparent and the military is once again producing the vaccines for routine immunization of recruits.

Safety of replication competent adenoviruses has also been demonstrated in the use of non-attenuated Ad in the treatment of cancer [see 70 for review]. At fairly high doses of 10^7 to 10^{13} particles in some cases, no serious side effects were observed. Replication-competent Ad4, 5 and 7 recombinant vaccines have also been developed for prevention of hepatitis B infection [71–74]. In both chimpanzees and in humans, recombinants encoding the surface antigen of hepatitis B given orally were well tolerated. In a phase 1 human trial, although virus shedding was detected, no transmission of an Ad7-hepatitis B vaccine to similarly housed individuals occurred.

3. Prime/boost approaches using Ad replicating vectors

The earliest studies using replicating Ad encoding HIV-1 *env* and *gag* were performed in mice and rhesus macaques, both non-permissive for human Ad replication. Nevertheless, in both models, cellular and humoral immunity were elicited [75]. Dogs proved to be a better model, allowing abortive replication. Intratracheal immunization of dogs with recombinant Ad4, 5 and 7 expressing HIV *env* resulted in enhanced, functional anti-Env antibodies with neutralizing activity [76]. Later the intranasal route compared to oral was found to give the highest level of vector replication in chimpanzees [77], paving the way for vaccine studies in this animal model.

Subsequently, intranasal priming with replicating Ad-HIV_{MN} *env* recombinants and boosting with HIV-1_{SF2} gp120 envelope protein [78] were shown to protect 4 of 4 chimpanzees against a low-dose HIV-1 challenge, and 3 of 4 against a high-dose challenge administered one year later with no intervening immunization. The study importantly showed that only three immunizations were sufficient to achieve protection: one Ad prime followed by two protein boosts or two Ad priming immunizations followed by a single protein boost. Notably, the sequential priming immunizations made use of different Ad serotypes (Ad 5, 7 and 4), establishing a useful strategy for eventual translation into humans. High titer neutralizing antibodies to both HIV_{MN} and HIV_{SF2} primary and laboratory-adapted isolates were elicited

which correlated with protection. A third challenge 3.5 years later with a non-syncytium inducing, minimally passaged HIV-1₅₀₁₆ stock followed re-priming with Ad5, 7 and 4 recombinants, each expressing HIV-1_{MN} gp160, and a boost with HIV-1_{SF2} gp120 protein. One of three of the previously protected chimps was completely protected. Again, broad neutralizing antibody including inhibition of the challenge virus correlated with protection [79,80].

The chimpanzee studies demonstrated the potential of the replicating Ad-recombinant vaccine approach, and implied that even better efficacy might be achieved in humans, the natural host of Ad4, Ad5, and Ad7. However, the protective efficacy achieved was discounted by the field, due to the apathogenic nature of the challenge viruses in chimpanzees. Use of virulent viruses was not possible in the endangered animals. Therefore, the SIV-rhesus macaque model was developed and used extensively to explore the replicating Ad vaccine approach in a series of studies (Table 1). These were made possible after the publication by Cheng, *et al.* [81] reporting that a host range mutant of Ad5 (Ad5hr) was able to replicate in monkey cells. We confirmed that an Ad5hr-SIV_{smH4env/rev} recombinant replicated *in vivo* in rhesus macaques [82], although replication was attenuated compared to levels observed in chimps. Priming first orally and intranasally with Ad5hr-SIV_{SMenv/rev}, then intratracheally twelve weeks later generated low level T-cell proliferative responses to envelope, and strong anti-SIV Env-specific binding and neutralizing antibodies in serum. Env-specific IgG and IgA binding antibodies were also observed in mucosal secretions, and both systemic and mucosal antibodies were continually boosted after gp120 protein administration. The Env-only vaccine approach was shown to induce acute-phase protection following intravaginal challenge with SIV_{mac251} [82] (Table 1A). Partial protection achieved after re-boosting and rechallenge of the transiently viremic macaques was associated with both cellular and humoral immune responses [83].

During this same time period, others using primarily Gag-based vaccines to induce cellular immunity were achieving dramatic reduction of SHIV_{89.6P} viral loads following challenge of rhesus macaques [10,11,84]. Due to the high variation among HIV-1 *env* sequences, the isolate-specific env-only vaccine might have been too narrow in scope. Immune escape from vaccine-induced responses had already been shown [85]. Therefore, in order to expand the breadth of immune responses we incorporated an Ad5hr recombinant expressing SIV Gag, optimized as described for HIV-1 *gag* [86,87] into the immunization regimen [88]. Using both Ad5hr-SIV_{env/rev} and -SIV_{gag} we showed that priming a second time with identical Ad recombinants did not dampen cellular immune responses - in fact responses to both antigens were boosted as well as anti-SIV Env binding antibodies. Subsequent SIV gp120 protein boosts concomitantly elevated cellular, mucosal and humoral immune responses, with detection of anti-SIV gp120 IgA and IgG antibodies in serum, nasal and vaginal secretions, and saliva. Following intrarectal challenge with SIV_{mac251}, protection was maintained into the chronic phase of infection [89]. Cellular immunity to SIV Gag and Env post and pre-challenge, respectively, as well as Env-specific IgG binding antibodies in nasal and vaginal secretions observed at the time of challenge correlated with significant reduction of acute phase viremia by 1 log, and of set point viremia by 1–1.5 logs among *Mamu A*01⁽⁻⁾* immunized and control macaques. Clearly the additional SIV_{gag} recombinant led to improved protection (Table 1B).

The conclusion that broad cellular immune responses plus mucosal antibodies contributed to protection led us to design a larger study in rhesus macaques. We had already shown that Ad5hr recombinants containing SIV_{239nef} genes were immunogenic in rhesus macaques [90]. Therefore we addressed the relative contribution of multigenic Ad recombinants in combination with Env protein boosting to immunogenicity and protection from a pathogenic mucosal SIV challenge. In addition to a control group, five immunization groups included the Ad5hr-SIV_{smH4env/rev} recombinant based on prior observations that it stimulated both cellular and humoral immune responses. As summarized in Table 1C, priming immunizations were

administered orally + intranasally and then intratracheally, using Ad5hr-SIV_{env/rev} alone or in combination with Ad5hr-SIV_{239gag}, -SIV_{239nef} or all three recombinants. Four groups were boosted twice with SIV₂₅₁ gp120 envelope, while the fifth was primed with Ad5hr-SIV_{env/rev} but boosted with a novel CD4 binding site multimeric peptide, termed “peptomer”, given in the absence of adjuvant so as not to destroy conformational structure [91,92].

Significant Env, Rev, Gag and Nef specific cellular immune responses were elicited according to immunization groups throughout the priming and boosting phases of the vaccine regimen [93]. Interestingly, the addition of Ad5hr-SIV_{gag} to the Ad5hr-HIV_{env} immunization regimen enhanced Env-specific responses, whereas Nef-specific responses were transiently down-modulated in the priming phase when all three recombinants were given together. No significant differences in Ad replication as assessed by viral shedding in nasal secretions were detected however, which might have accounted for the immune modulation. As shown by tetramer staining of T-cells of the *Mamu-A*01⁽⁺⁾* macaques that were included in these groups, and IFN- γ ELISpot assays, the replicating Ad vectors were able to prime for persistent, potent CD8⁺ T-cell responses to both dominant and subdominant Gag and Env epitopes [94]. High titer SIV Env-specific binding antibodies and antibodies able to neutralize T-cell line adapted but not primary SIV_{mac251} were elicited for all groups except the peptomer boosted group and the controls.

Upon challenge with SIV_{mac251}, all groups receiving two or more Ad recombinant immunizations showed statistically significant reductions in both acute phase and set point viremia, the later recently determined after reassessing samples using a more sensitive NASBA assay (<50 copies/ml plasma) [95 and unpublished observations]. Notably, 12 of 31 macaques (39%) in these immunization groups had no detectable viral load or displayed strong control of chronic phase viremia with geometric mean viral loads ≥ 3 logs below control levels. Overall protection at set point was correlated with Env- and Rev-specific cellular immunity at the time of challenge, while anti-Env binding antibody titers correlated with reduction in acute phase viremia. A later study characterized these non-neutralizing binding antibodies that contributed to protection as mediating antibody-dependent cellular cytotoxicity (ADCC) [96]. This finding focused the field on the importance of evaluating antibodies for additional functions which might contribute to protective efficacy in addition to neutralization. In fact a recent paper reported that the ability of the broadly reactive b12 monoclonal antibody to passively protect against viral challenge was partly attributable to a function mediated by its Fc receptor and not solely neutralizing activity [97].

The protection achieved in the above study was durable. Following intrarectal challenge one year later with the same SIV_{mac251}, eight of eleven of the strongly protected, immunized macaques resisted re-challenge, exhibiting undetectable or low level viremia, with an overall reduction in geometric mean chronic viral load of 4 to 5 logs compared to controls [98]. The three remaining macaques exhibited higher viral loads, which nevertheless remained 1 to 2 logs below control levels. Overall, the durable protection was CD8⁺ mediated as shown by *in vivo* CD8⁺ T-cell depletion experiments and the presence of SIV-specific memory T-cells. Of special interest, 3 *Mamu A*01⁽⁻⁾* macaques originally primed with Ad5hr-SIV_{env/rev} and boosted with SIV peptomer emerged as “elite controllers”. Two consistently displayed no detectable viral load, while the third exhibited transient low-level viremia only after the initial SIV_{mac251} challenge. The basis for the strong protection exhibited by these macaques is unknown, but we continue to follow them and explore potential correlates of immune protection (Table 1C).

The study summarized above suggested that intratracheal immunization was superior to the intranasal/oral route in the macaque model. However, intratracheal immunization would not be used in human trials. Further, oral administration of replicating Ad is inherently safer than

the intranasal route in people. Therefore we optimized the oral delivery of our vaccine for testing in rhesus macaques. Previously, following neutralization of stomach acid with sodium bicarbonate, Ad5hr-recombinants were administered in PBS via stomach tube. Resulting Ad replication occurred in about one-third of the macaques and was of short duration. Subsequently, Ad5hr-SIV recombinants were formulated in enteric-coated tablets, insoluble in acid, to allow the virus to bypass the stomach and reach the intestine where the tablets dissolve at neutral pH. Using such tablets, a recent study compared sequential oral/oral priming versus intranasal/oral priming with Ad5hr-SIV_{env/rev, gag} and *nef* recombinants followed by two boosts with SIV gp120 (Table 1D). The tablets successfully delivered the oral vaccine, as 100% of the immunized macaques shed virus in stool samples, although again duration of shedding was limited. Both the oral/oral and intranasal/oral regimens resulted in viremia reductions of approximately 1.5 logs at set point after an SIV_{mac251} rectal challenge [99]. The equivalent protection was unexpected since cellular immune responses in peripheral blood were low for the oral/oral group. However, both groups displayed mucosal cellular and humoral SIV-specific immunity which provided an explanation for the challenge outcomes. The results reflect the inability of responses measured in peripheral blood to predict immunity at mucosal sites, and highlight the need for development of sensitive, validated assays for monitoring future clinical trials that will assess mucosally delivered vectored vaccines.

Continued investigation of the best combination of gene inserts to include in the replicating Ad vector led us to examine HIV Tat. Native Tat protein or DNA encoding *tat* has shown promise in protecting cynomolgus macaques from a SHIV_{89.6P} challenge [100,101]. Recently, we compared replicating Ad5hr-HIV-1*tat* alone, Ad5hr-HIV-1*tat* in combination with Ad5hr-HIV-1*env* gp140 or a multigenic group including not only the *tat* and *env* recombinants but also Ad5hr-SIV_{gag} and Ad5hr-SIV_{nef} as priming immunogens followed by boosting with Tat, and/or Env or Nef proteins as appropriate (Table 1E) [102]. All immunization groups exhibited comparable HIV and/or SIV specific cellular and humoral immune responses to individual antigens. The *tat* only group was not protected from an intravenous challenge with SHIV_{89.6P}, but the multigenic and *tat-env* groups exhibited strong protection, reducing set point viremia 3 and 4 logs, respectively compared to controls. The greater reduction in set point viremia seen in the *tat-env* group compared to the multigenic group was statistically significant and associated with higher levels of binding antibodies to Env and Tat. The exact mechanisms accounting for this enhancement of antibody induction and protective efficacy are currently being investigated. Tat is known to act as an adjuvant, but generally with regard to cellular immune responses. Co-immunization of mice with Ad5hr-SIV_{gag} and Ad5hr-HIV-1*tat* induced significant enhancement of SIV Gag-specific T-cell responses [102]. Tat is also known to facilitate MHC class I presentation of antigens [104,105]. With regard to the observed antibody association, a potential synergy between the *tat* and *env* immunogens is under investigation.

The availability of SHIV models has made it possible to evaluate HIV envelope vaccines, more relevant to people than the SIV envelope. The contribution of a protein boost to the replicating Ad-recombinant vaccine approach should not be overlooked. Novel boosting immunogens will undoubtedly be needed to elicit broadly neutralizing antibodies. Further, as reported by others [106,107], boosting with protein can lead to increased and broader cellular and humoral immune responses. The studies reviewed to this point all were conducted with two replicating Ad-recombinant priming immunizations followed by two protein boosts. Recently, we examined the role of the protein boost, using Ad5hr-recombinants encoding HIV_{89.6Penv}, SIV_{gag} and SIV_{nef} as priming immunogens. Immunization groups were either not boosted or received two HIV gp140 protein boosts or two boosts with an HIV peptomer, representing the CD4⁺ binding site of the HIV envelope. Significant protection was not achieved in the peptomer group. However, control of chronic viremia 3 logs below control levels, was exhibited by Mamu-A*01⁽⁻⁾ macaques immunized with and without gp140 boost regimens

following intravenous challenge with SHIV_{89.6P}. The benefit of the protein boost was observed during the acute phase of infection where only the protein boosted group showed significant reduction of acute phase viremia of 1.5 logs [108].

More recently, the value of boosting with components designed to elicit neutralizing antibodies was also demonstrated using a second SHIV model. Two mucosal priming immunizations with replicating Ad5hr-HIV_{89.6Penv} followed by boosting with either HIVgp140SF162 Env protein or VEE/SIN chimeric alphavirus replicon particles expressing HIV V2gp140SF162 protein elicited not only cellular immune responses, but also high titer neutralizing antibodies. Following intrarectal challenge with SHIV_{SF162P4}, 5 of 8 immunized macaques were protected. The protection was correlated with systemic neutralizing antibody titers [109].

4. Replication Defective Vectors

While this review has focused on replication-competent Ad-HIV recombinant vaccines, we would be remiss in not mentioning several non-replicating vectors that are being developed or are already in clinical trials [7], including replication-defective Ad, poxviruses, adeno-associated virus (AAV), Venezuelan equine encephalitis virus (VEE), and herpes virus as well as DNA alone or in combination with other vectors. Due to the scope of this review, they will not be covered in detail, however, as poxvirus and non-replicating Ad approaches represent the most advanced strategies to date, they will be briefly mentioned here.

Non-replicating poxviruses were among the earliest vectors developed for HIV vaccines, primarily due to safety concerns associated with the highly successful vaccinia vector as discussed above. In general, non-replicating poxvirus-recombinants have proven to be less immunogenic in people than predicted by non-human primate studies. Moreover, modifications including multigenic inserts and alternative promoters have not resulted in increased immunogenicity in people [2]. Results from more recent trials are not yet available, however. In a phase 2 study, a multigenic ALVAC-HIV recombinant vaccine followed by boosting with gp120 was not advanced to a proof-of-concept trial as it failed to elicit a CD8+ CTL frequency of 30% in healthy volunteers [110]. However, results of a phase III efficacy trial in Thailand of an ALVAC-HIV_{env}(B/E), *gag*, *pol* prime and gp120(B/E) boost are pending [111].

MVA recombinants in combination with DNA priming have also been disappointing in Phase I and II clinical trials, inducing cellular immune responses in a low percentage of vaccinees [112]. However, heterologous prime/boost combinations using MVA and fowlpox virus (FPV) first evaluated in rhesus macaques [113], then in humans [2] have shown promise. In the rhesus macaque model, higher cellular immunity was seen in both heterologous prime/boost groups (MVA/FPV prime/boost in either order) compared to the homologous MVA/MVA immunization group, however the level of subsequent protection was equivalent for all groups after challenge with SHIV_{89.6P}.

The most promising non-replicating poxvirus approach to date has made use of the NYVAC vector. A recent phase I trial evaluating a DNA prime/NYVAC recombinant boost regimen (both expressing Env, Gag, Pol and Nef) showed the approach was highly immunogenic, eliciting potent, broad, polyfunctional, and durable T-cell responses in 90% of vaccinees [114]. Clearly this approach merits further development.

Replication-defective Ad (by virtue of an E1 deletion) offer some of the same attractive features as replicating Ad vectors, including infection of dendritic cells and cells lining mucosal inductive sites [11,59,115]. Likewise, they do not integrate into the host DNA, can be easily scaled up, and stably express gene inserts. We have shown in the chimpanzee model that replicating Ad recombinants are more immunogenic than non-replicating Ad recombinants

[116]. Priming with replicating Ad5-HIV_{MNenv/rev}, followed by Ad7-HIV_{MNenv/rev}, resulted in higher cellular and humoral immune responses that were further boosted with HIV-1_{SF162} gp140 V2 protein. Importantly, the humoral immunity elicited included binding, neutralizing, and ADCC mediating antibodies [117]. However, non-replicating Ad vectors are often deleted in both E1 and E3 genes allowing for larger gene inserts. And although higher doses must be used for immunization, non-replicating Ad are safer than replicating vectors since they can't cause disease. A variety of non-replicating rare-subtype Ad, Ad of non-human species, and chimeric Ad vectors in which neutralizing epitopes on the virion surface have been engineered to contain sequences of rare serotypes are under development, which may circumvent pre-existing immunity in the population [65,118,119]. Blunted immunogenicity of inserted genes can result from high prior levels of anti-vector immunity. A concerted effort has been put forth to develop these vectors for HIV/AIDS vaccines due to a number of promising studies performed in rhesus macaques. Studies in non-human primates have evaluated recombinants based in Ad of subgroup B (Ad11, Ad35 and Ad50 and closely related chimp AdC1), subgroup C (Ad5), subgroup D (Ad26, Ad48, Ad49), and a second chimp AdC68 which shares homology with Ad4 from human subgroup E. Some level of anti-vector cross reactivity has been observed among all human and chimpanzee adenovirus serotypes. Further, the most potent immune responses to HIV or SIV inserted genes continue to be seen using the Ad5 vector. Unfortunately, Ad5 also elicits the highest cross-reactive vector immunity, rendering a subsequent boosting immunogen with this same vector ineffective. This result is especially apt, considering that 60–90% of the population has already acquired immunity to Ad5 by adulthood [120]. Overall, the most promising rare serotype used to date is Ad26, which has proven to be highly immunogenic. When used as a priming immunization followed by a heterologous Ad5 boost, it results in high antibody titers, and durable immunity in rhesus macaques [118].

Replication-defective Ad have also proven effective in inducing immune responses as a boost in combination with DNA priming [11,121,122]. One of the most immunogenic vaccines to date in Phase 1 clinical trials is the Ad5 multiclade combination (clade B *gag-pol*, and clade A, B and C *env*) which has elicited T cell responses in up to 70% of vaccine recipients as measured by IFN- γ ELISpot [123] in addition to Env-specific antibody responses. The future plan has been to administer this vaccine following priming with matched multiclade HIV DNA vaccines developed by the Vaccine Research Center (VRC), NIH. These were also shown to be safe, however less immunogenic, in phase 1 trials [124]. Whether this trial (termed PAVE100) will go forward with a modified trial design to exclude Ad5-seropositive individuals is currently under review as a result of the recent halting of the phase 2b STEP and Phambili trials of Merck's non-replicating Ad5-HIV*gag-pol-nef* vaccine in the Americas, the Caribbean, and Australia, and South Africa, respectively. The STEP trial was undertaken in December, 2004, to address two hypotheses: that the trivalent vaccine would prevent HIV infection, and that it would lower viral loads in individuals that became infected. An interim analysis showed conclusively that neither endpoint was achieved, and the trial was halted September 21, 2007 [125]. However, an unexpected trend of greater HIV infections in the trial's vaccinees compared to placebo recipients with high Ad5 antibody titers prior to immunization was reported November 7, 2007 [<http://www.hvti.org/science/1107.html>], and statistical and laboratory analyses are continuing to explore possible mechanisms behind the surprising finding. Results of multivariate analyses reported at the recent 15th Conference on Retroviruses and Opportunistic Infections in February, 2008, revealed that both high pre-existing Ad5 antibody titers and lack of circumcision increased the risk of acquiring HIV in the vaccinated individuals, whereas no increased risk was associated with low pre-existing Ad5 antibody titers and circumcision [126]. Other potential factors remain to be investigated, such as Herpes simplex virus status and HLA type. Until further information is obtained, it is likely that Ad-based vaccine trials will proceed in volunteers at low risk for HIV acquisition who

also lack prior humoral immunity to the vector. Further implications of the STEP trial outcomes are discussed at the end of this chapter.

Conclusions

Based on the strong safety record of wild-type Ad4 and Ad7 vaccines in people and the proven immunogenicity and protective efficacy in non-human primates of replicating Ad vectors containing SIV/HIV inserts, a human clinical trial in collaboration with the Division of AIDS and the NIAID is planned. A phase 1 dose escalation study of an Ad4-HIV_{env} vaccine, formulated to be given as an oral tablet, will be performed. Once a safe dose is established, further testing of the overall strategy, including boosting with an Ad7-HIV_{env} vaccine to overcome possible anti-vector immunity is contemplated. As a vital component of any vaccine should include induction of humoral immunity, we have also proposed boosting with HIV envelope protein, matched to the insert included in the Ad vectors. Based on the experience with oral Ad vaccines in the U.S. military, we anticipate establishing safety and expanding testing of the vaccine in additional volunteers in order to obtain extensive immunogenicity data. In view of the STEP trial results and until a better understanding of the findings is obtained, testing of the replicating oral Ad4- and Ad7-HIV vaccines is planned to proceed in Ad4 and Ad7 seronegative people.

Expert Opinion

The potential for elicitation of superior immune responses and protective efficacy resulting from replication-competent Ad-HIV recombinant vaccination is good. Results obtained in non-human primate models have been very promising, yet the models are not optimal for evaluating this vaccine approach. Human Ads have a very restricted host range. The most permissive animal species for Ad replication is the chimpanzee. However, in this model dosages of replicating Ad administered intranasally have been 3 to 4 logs higher than the permissible dose in people, suggesting impaired replication. For example, chimpanzees have been administered 10^7 to 10^8 plaque forming units (pfu) intranasally [77,116,127] while a safe dose in humans is no more than 10^4 [128]. Similarly, using the Ad5hr vector in macaques, doses of 10^9 have been administered intranasally and intratracheally with no clinical consequences [88,93,102]. Duration of shedding of Ad vector in stool samples is also used to estimate Ad replicability *in vivo*. Whereas humans shed Ad in stool samples after oral administration on average for 3 weeks [66,67,69,129,130], following oral administration to rhesus macaques of enteric coated tablets containing Ad5hr vector or SIV recombinants, shedding in stool samples was observed only for 2 to 4 days [99,131]. Overall the data suggest relatively poor replication of the Ad vector in non-human primates, implying that improved replication in humans will lead to more potent immune responses.

The recent failure of the Merck Ad5-based vaccine may have implications for all T-cell vaccine approaches, not only those using Ad5. The clearest outcome of the phase 2b trial was lack of efficacy of the Merck Ad5-HIV_{gag-pol-nef} vaccine. The fact that not only were infections not prevented but also that viral loads were not reduced suggest that T-cell responses alone will not be sufficient for effective protective efficacy. That new infections were not prevented is perhaps not surprising, but the lack of viremia reduction was disappointing. This latter outcome was previewed, however, by results of earlier preclinical studies in macaques in which three sequential immunizations with replication-defective Ad5-SIV_{gag} failed to reduce viremia in comparison to controls following challenge with SIV_{mac239} [122]. With this in mind, better attention to non-human primate studies in the future might avoid lengthy and costly human trials. Overall, the response rate to the Merck vaccine as measured by IFN- γ ELISpot was excellent [132]. While response levels appeared lower in individuals with higher anti-Ad5 antibody titers, this did not influence the trial outcome as the CD8+ and CD4+ responses were

polyfunctional and comparable between cases and non-cases in the various Ad5 antibody groups [133]. However, it is not known to what extent the cellular immunity elicited was present at mucosal sites. As similar set point viremia levels were observed in both vaccinees and controls, the trial has established a lower bar for cellular immunity which future T-cell vaccines will need to exceed in order to potentially diminish viremia if protection against infection is not achieved.

Identification of the underlying basis for the trend of increased HIV infections in individuals with high pre-existing Ad5 antibody titers is currently a subject of intense investigation, as it may broadly impact not only Ad5-based vaccines, but possibly all vectored vaccines and T-cell vaccines in general. One prime hypothesis considers induction of activated CD4+ T-cells at the site of HIV transmission, providing more targets for HIV infection. This might occur in individuals with pre-existing Ad5 immunity upon re-exposure to the vector, and might also arise in response to other vectored vaccines in which vector-immunity is pre-existing or elicited by repeated sequential administrations. Activation may also pose a problem for any T-cell vaccine which induces CD4+ T-cells together with an inadequate CD8+ T cell response able to control a potential increase in HIV transmission. In the STEP trial, however, T-cell activation as a causative factor for the increased trend in HIV infection does not seem likely. While the percentage of circulating activated CD4+ CCR5+ cells was higher in individuals with high Ad5 titers, activation levels were no different between vaccine and placebo recipients with similar anti-Ad5 titers [133].

An unusual outcome of the STEP trial was a decline in the risk for acquiring HIV infection in placebo recipients as Ad5 antibody titers increased, while the risk was similar across all Ad5 antibody levels in vaccinated individuals (<http://www.hvti.org/science/1107.html>). This pattern suggests that other confounding variables may have contributed to the trial results. Circumcision has already been identified as a factor associated with the increased HIV infections, and greater acquisitions were also seen in North America [126]. Results of additional investigations on STD status, HLA type, behavioral aspects, etc. will help elucidate the mechanisms underlying the increased HIV acquisitions in vaccine recipients. Until the results are fully understood, testing of Ad recombinant vaccines and other vectored vaccines to which pre-existing immunity in the population exists or might be elicited by sequential immunizations should proceed cautiously.

Current vectored vaccine approaches based in both replicating and non-replicating Ad-recombinants differ in significant ways from the Merck Ad5 vaccine strategy. The latter relied on repeated immunizations of the same Ad5 vector, thus presumably eliciting increasing levels of Ad-specific immunity and re-exposing vaccinated individuals to the antigenic stimulus. The planned VRC Ad5 multiclade PAVE100 vaccine trial discussed above is designed with initial DNA immunizations followed by a single Ad5 recombinant boost. Similarly, the strategy for our replicating Ad4 vaccine approach currently moving toward human trials is to use sequential vaccination, in which a second booster immunization will be based in an Ad7 vector, followed by a boost with envelope protein. Thus both approaches should minimize and circumvent anti-vector humoral immunity, although the possibility of activation among CD4+ T-cells still exists and anti-vector cellular immunity should be monitored closely throughout the trial. With regard to the replicating Ad4 and Ad7 vaccines, a recent survey reported that 66% and 73% of U.S. military recruits lacked protective antibodies to Ad4 and Ad7 respectively [134], and 88% lacked protective immunity to both vectors. A priority for future research on the Ad4/Ad7 approach will be to develop a highly sensitive assay to appropriately survey Ad4 and Ad7 serostatus at future vaccine sites outside the United States.

One immediate outcome of the STEP trial failure will be renewed efforts to develop anti-envelope vaccines able to elicit neutralizing antibodies. This vaccine approach should be able

to prevent HIV entry, therefore circumventing potential issues of vaccine-induced T-cell activation and enhancement of infection. Such vaccines will have to elicit potent and broad neutralizing antibodies, however, in order to protect across HIV clades and also avoid a related potential problem of antibody dependent enhancement of infection [135]. Design of the immunogen needed to elicit such immunity hopefully will come from on-going structural biology studies of the viral envelope. Yet eventual immunizations will likely require use of adjuvants and/or vector priming, again requiring care in vector selection and design of the vaccine regimen.

It is our belief that an effective vaccine will ultimately depend on engaging all elements of the immune system, both innate and adaptive immunity elicited systemically and at mucosal sites. Knowledge of immune correlates of protection gained from natural HIV/SIV infection will be critical for suggesting additional vaccine components to augment existing vaccine strategies. Immune responses generated during natural infection are generally insufficient to control the virus, and only individuals with a genetic predisposition for blocking viral entry or a select few “elite controllers” have been resistant to infection. Clues for vaccine design may come from investigations of immune mechanisms responsible for elite control in people, and in animal models from the few vaccinees highly protected from challenge exposure. Technological advances such as multiparameter flow cytometry and microarray analysis of peripheral blood and tissues may shed light on pathogenesis and resulting immune control mechanisms. Immune responses at mucosal sites also need to be elucidated and evaluated as protective correlates. Alternatively, sensitive surrogate assays for mucosal responses that can be performed readily and repeatedly on peripheral blood need to be developed. Important information can also be gained from animal models where SIV infection does not cause disease, and where innate immune mechanisms seem to be playing a role, including sooty mangabeys, African green monkeys, and the recently described Caribbean African green monkey [136]. Only through these collective efforts can we hope to design an efficacious vaccine and conquer what has proven to be one of the greatest pandemic diseases in our lifetime.

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Table 1
Effect of immunization route and number and identity of priming immunogens on vaccine elicited protection in the rhesus macaque model.

Study	Prime 1	Prime 2	Boost	Challenge	Control of acute viremia	Control of set pt. viremia	Immune Response at challenge	Comment; Implications	Ref.
A	O + IN	IT	IM	IVag					
Group 1	Ad5hr-SIV _{SM} env/rev gp160	Ad5hr-SIV _{SM} env/rev gp160	SIV gp120, 2X, synex adj.	SIV _{mac251}	yes	transient (not past 20 weeks)	Nab. anti-gp120 IgG and IgA in nasal, rectal, vaginal secretions	Env-only vaccine contributes to acute phase protection	82,83
B	O + IN	O + IN	IM	IR					
Group 1	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag}	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag}	SIV gp120, 2X, QS21 adj.	SIV _{mac251}	yes	yes persistent control in non- <i>Mamu A*01</i>	anti-gp120 IgG; env + gag cellular immunity	Additional Gag vaccine component increases chronic phase control	88,89
C	O + IN	IT	IM	IR					
Group 1	Ad5hr-SIV _{SM} env/rev gp160	Ad5hr-SIV _{SM} env/rev gp160	SIV gp120, 2X, MPL-SE adj.		no	no			93–96,98
Group 2	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag}	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag}	SIV gp120, 2X, MPL-SE adj.		yes	yes	Env-specific binding antibody, ADCC correlated with acute phase protection	IT route very effective in macaque. 73% (8 of 11) highly protected macaques durably protected 1 yr later against 2nd SIVmac251 IR challenge.	
Group 3	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 nef}	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 nef}	SIV gp120, 2X, MPL-SE adj.	SIV _{mac251}	yes	yes			
Group 4	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag} + Ad5hr-SIV _{239 nef}	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag} + Ad5hr-SIV _{239 nef}	SIV gp120, 2X, MPL-SE adj.		yes	yes	Env-specific cellular immunity correlated with chronic phase protection	3 "elite controllers" under further study	
Group 5	Ad5hr-SIV _{SM} env/rev gp160	Ad5hr-SIV _{SM} env/rev gp160	SIV peptomer, 2X, in PBS		no [#]	no [#]			
D	O	O	IM	IR					
Group 1	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag} + Ad5hr-SIV _{239 nef}	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag} + Ad5hr-SIV _{239 nef}	SIV gp120, 2X, MPL-SE adj.		yes	yes	Broad cellular mucosal immunity; systemic and secretory anti-SIV	Systemic cellular immune responses do not predict protective efficacy against mucosal challenge.	99 Hidajat in prep.
Group 2	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag} + Ad5hr-SIV _{239 nef}	Ad5hr-SIV _{SM} env/rev gp160 + Ad5hr-SIV _{239 gag} + Ad5hr-SIV _{239 nef}	SIV gp120, 2X, MPL-SE adj.	SIV _{mac251}	yes and lower than O/O group at week 2	yes	Env IgG and IgA		
E	O + IN	IT	IM	IV					
Group 1	Ad5hr-HIV _{INB tat}	Ad5hr-HIV _{INB tat}	HIV tat, 2X, alum		no	no	Low level Tat. Env, Gag and/or Nef	Tat/env significantly better than multigenic.	
Group 2	Ad5hr-HIV _{INB tat} + Ad5hr-HIV _{89,6P env} gp140	Ad5hr-HIV _{INB tat} + Ad5hr-HIV _{89,6P env} gp140	HIV tat, 2X, alum HIV _{89,6P} Env, 2X, in MPL	SHIV89.6P	no	yes	cellular immunity according to group. Binding Ab to Env and Tat higher in Tat/Env group	The tat/env vaccine combination should be further explored	102

Study	Prime 1	Prime 2	Boost	Challenge	Control of acute viremia	Control of set pt. viremia	Immune Response at challenge	Comment; Implications	Ref.
A	O + IN	IT	IM	IVag					
Group 3	Ad5hr-HIV _{11B} <i>tat</i> + Ad5hr-HIV _{89,6P} <i>env</i> gp140 + Ad5hr-SIV ₂₃₉ <i>gag</i> + Ad5hr-SIV ₂₃₉ <i>nef</i>	Ad5hr-HIV _{11B} <i>tat</i> + Ad5hr-HIV _{89,6P} <i>env</i> gp140 + Ad5hr-SIV ₂₃₉ <i>gag</i> + Ad5hr-SIV ₂₃₉ <i>nef</i>	HIV <i>tat</i> , 2X, alum HIV _{89,6P} <i>Env</i> , 2X, SIV <i>Nef</i> , 2X, both in MPL		yes	yes			

#. includes a subset of 3, non-*Mamu A*01*⁺ elite controllers exhibiting either undetectable or transient low level viremia; ongoing investigation of immune correlates
IN = intranasal; IM = intramuscular; IV = intravenous; O = oral; IT = intratracheal; Ivag = intravaginal; IR = intrarectal