Adenovirus vectors as HIV-1 vaccines: where are we? What next?

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Introduction

Although antibody studies have moved to the forefront of HIV-1 vaccine research since the RV144 trial [1,2], it seems likely that complementary $CD8^+$ T-cell immunity will be necessary. This is the best defined protective factor in established HIV-1 infection, based on numerous lines of evidence, including reduced simian immunodeficiency virus (SIV) containment after depletion of $CD8^+$ cells in infected macaques [3–5], HIV-1 sequence evolution predominately in $CD8^+$ T-cell epitopes [6], class I human leukocyte antigen (HLA-I) locus being the greatest genetic determinant of immune control [7] and temporal correlation of the $CD8^+$ T-cell response to drop of viremia during acute infection [8,9]. Although less certain, this arm of immunity may contribute to preventing HIV-1 infection as well. HIV-1-specific $CD8^+$ T cells can kill infected cells before virion production and sterilize viral cultures in *vitro* [10,11], and have been observed in some highly exposed yet uninfected persons such as a cohort of commercial sex workers in Nairobi [12].

Generating HIV-1-specific $CD8^+$ T-cell responses by vaccination has been challenging. Exogenous proteins have poor access to the HLA-I pathway, thus numerous vectored approaches have been tested [1]. The most potent in humans has been recombinant adenovirus serotype 5 (rAd5), in versions from Merck Research Laboratories (MRK) and the NIH Vaccine Research Center (VRC). Unfortunately, efficacy trials have been disappointing and raised questions about the safety of rAd5 vectors.

Failure of two recombinant adenovirus serotype 5 HIV-1 vaccines in human efficacy trials and a question of increased susceptibility to infection caused by recombinant adenovirus serotype 5

HVTN 502 (Step) [13] administered three MRK rAd5 doses to MSM and at-risk women in the USA and Australia (Table 1), and was halted for futility at the midpoint. Infection rates between vaccine (24/741) and placebo (21/762) groups were not statistically different, but subgroup analyses suggested an increased incidence of infection in vaccinated men who were either Ad5-seropositive prevaccination or uncircumcised [14]. The South African Phambili study [15] of the same regimen was discontinued and unblinded early (after one or two vaccinations in most participants) due to the Step results. In 42 months of unblinded follow-up, there was a higher rate of HIV-1 infection (largely in men) in the vaccine group (63/400 vaccines versus 37/400 placebo recipients), although unlike Step this was unrelated to Ad5 serostatus or circumcision [16].

HVTN 505 tested VRCrAd5 (containing additional genomic deletions) as a single boost after three DNA

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Table 1. Human efficacy studies of recombinant adenovirus serotype 5 HIV-1 vaccines.

Study	rAd5	HIV-1 inserts	DNA prime (months)	rAd5 vaccination schedule (months)	Control vaccine	Location (Dominant clade)	Individuals			Hazard ratio	
							Male Baseline			Women	
							MSM	Circumcision	Ad5	<18 months	>18 months
Step (HVTN 502)	MRL	Clade B *gag/pol/nef*	N	0, 1, 6	Placebo	America and Australia (B)	Y	+/–	+/–	Y	1.22	1.47
Phambili	MRL	Clade B *gag/pol/nef*	N	0, 1, 6	Placebo	RSA (C)	N	+/–	+/–	Y		
HVTN 505	VRC	Clades A+B+C *env*, Clade B *gag/pol*	Y (0, 1, 2)	6	Placebo	USA (B)	Y	+	–	(TG)		

MRL, Merck Research Laboratories; VRC, NIH Vaccine Research Center.

priming vaccinations [17]. Only Ad5-seronegative circumcised men and transgender women were enrolled, based on the Step results. This study was also halted for futility, with HIV-1 infections in 41 out of 1251 vaccines and 31 out of 1251 placebo recipients, of which 27 and 21, respectively, occurred at least 4 weeks after completing all vaccinations. In contrast to Step, HVTN 505, and preceding studies of VRC rAd5 vaccination, did not reveal increased infections [18], although it is unclear whether this is due to a biological difference, the different participant population or insufficient statistical power. VRC and MRK rAd5 vaccines differed in inserts (Table 1), further raising the possibility that different inserts played roles, such as Env-induced antibodies reducing risk of infection as suggested by macaque vaccine studies [19,20].

Role of preexisting adenovirus immunity in increased HIV-1 infection risk after recombinant adenovirus serotype 5 vaccination?

The increased infection rate in men with baseline Ad5 seropositivity in Step is further supported by observed Ad5-specific cellular immune boosting in vaccines [21,22], data that Ad5 exposure of PBMC from Ad5-seropositive persons causes proliferation of mucosal-homed HIV-1-susceptible $CD4^+$ T cells [23], and the observation that Ad5-specific $CD4^+$ T cells (generated either by natural infection or rAd5 vaccination) appear to be more susceptible to infection than cytomegalovirus (CMV)-specific $CD4^+$ T cells [24]. However, none of the trials included a control vector-only group to distinguish between contributions of responses against rAd5 versus HIV-1 inserts. Indeed, an SIV-macaque study recapitulating Step observed that increased susceptibility to penile SIV challenge was seen for rAd5 delivering SIV *gag/pol/nef* but not empty rAd5 vector, suggesting a role for inserts [25]. Further evidence against rAd5 as the sole cause of increased susceptibility to HIV-1 infection is that Ad5-seronegative Step vaccines had an unchanged incidence of HIV-1 infection despite observed expansion of Ad5-specific $CD4^+$ T lymphocytes [13,26], although this expansion was not noted in another MRK rAd5 vaccine study [27].

Poorly understood effects of recombinant adenovirus serotype 5 vaccines in the mucosal compartment

Mucosal compartments as major sites for HIV-1 transmission and viral replication have been understudied in humans. Gastrointestinal mucosa is also a major reservoir of chronic adenoviral infections and Ad-specific $CD4^+$ T lymphocytes [28]. A VRC rAd5 vaccine study (HVTN 076) demonstrated increased CCR5-expressing

$CD4^+$ T lymphocytes in rectal mucosal after vaccination, suggesting a role for increased target cell availability in the increased HIV-1 infections in Step vaccines [18]. When HVTN 505 was modified posthoc to examine rectal mucosa, no HIV-1-specific cells were detected in that compartment, although more than a year had elapsed after last vaccination. Although collecting and analysing mucosal samples is operationally and technically challenging, this will be important for safety and efficacy evaluation in future efficacy studies of HIV-1 preventive vaccines.

Consideration of other recombinant Adenovirus vaccine vectors

Concern regarding preexisting vector immunity prompted development of other rAd serotype vectors with lower seroprevalence than Ad5, including Ad26 and Ad35, which have shown promise in macaques [29,30] and early human trials [31,32]. Given concern over cellular immune cross-reactivity across different human adenovirus serotypes, nonhuman primate rAd types have also been considered, although cellular immune cross-reactivity has been noted across human and chimpanzee adenoviruses [33]. Further human studies will be required to examine the risk of Ad cross-reactive cellular immunity and risk for increased HIV-1 susceptibility. Although including a vector-only control vaccination would be ideal to address these issues, the potential risk without any benefit would make it ethically difficult to justify, particularly in high-risk populations.

Another consideration is the use of alternative serotype replication competent recombinant Adenoviruses as vaccine vectors. In particular, serotypes 4 and 7 have a substantial track record of being well tolerated as oral vaccines administered to more than 10 million persons [34], and have been engineered as recombinant vaccine candidates for hepatitis B [35] and influenza [36]. Whether these would have the same effects on HIV-1 acquisition as rAd5 is unclear.

The rAd5 trials inadequately reflect the utility of $CD8^+$ T cells in an HIV-1 vaccine: caveats to peptide-based immunogenicity testing

The poor outcome of rAd5 vaccine trials despite being 'immunogenic' for HIV-1-specific $CD8^+$ T cells has raised questions about the utility of this arm of immunity for a vaccine. However, a major caveat is that there is little evidence that the vaccine-elicited HIV-1-specific $CD8^+$ T cells had the capacity to recognize HIV-1-infected cells. Despite early reservations in this regard about the IFN-γELISpot assay and other exogenous peptide loaded target cell assays [37], ELISpot was the primary tool to prioritize vaccine candidates due to its simplicity, reproducibility and high throughput capacity. Unfortunately, HIV-1-specific $CD8^+$ T-cell ELISpot magnitudes were similar in Step and HVTN 204 (phase IIA trial of the VRC rAd5 vaccine preceding HVTN 505) vaccines who subsequently became HIV-1-infected versus those who did not [38,39].

A likely explanation is the discordance between ELISpot and the capacity of $CD8^+$ T cells to recognize virus-infected cells; HIV-1-specific $CD8^+$ T cells can have sufficient avidity to be triggered by excess exogenous peptides, but insufficient avidity for physiologic levels of endogenously presented epitopes [40–42]. Notably, vaccination with low versus high epitope levels yields $CD8^+$ T-cell responses with high versus low avidities, respectively [43]. It is conceivable that CMV promoter-driven expression of codon-optimized HIV-1 genes in both rAd5 vaccines yielded supraphysiologic epitope levels generating low-avidity $CD8^+$ T-cell responses detectable by ELISpot detection but unable to recognize HIV-1-infected cells. Finally, another related possibility is that sequence mismatch between the vaccine and infecting HIV-1 resulted in nonrecognition by vaccine-generated HIV-1-specific $CD8^+$ T cells. These points are supported by observations that some HIV-1 specific $CD8^+$ T cells from MRK rAd5-vaccinees have no antiviral activity against HIV-1-infected cells with vaccine-matched epitope sequences, or common epitope variants (O.O. Yang, unpublished observation).

Evidence for some recombinant adenovirus serotype 5 vaccine-induced $CD8^+$ T-cell anti-HIV-1 activity

Successful containment of SIV by recombinant cytomegalovirus vaccine-generated $CD8^+$ T cells in macaques supports the utility of this arm of immunity [44], and there are hints that rAd5 vaccination also produced some antiviral activity. HIV-1-infected Step vaccines had lower plasma viremia if they had more than two Gag epitope vaccine responses preinfection [45], and their HIV-1 sequences demonstrated a 'sieve effect' of greater viral evolution in epitopes targeted by vaccine-induced $CD8^+$ T cells [46]. These findings highlight the importance of considering sequence conservation and expression level in insert design to generate antiviral $CD8^+$ T cells [47].

Balancing vaccine-induced immune activation versus vaccine-induced antiviral benefit

By its very nature, adaptive immunity requires activation of responding $CD4^+$ T-helper cells. Given the dependence of

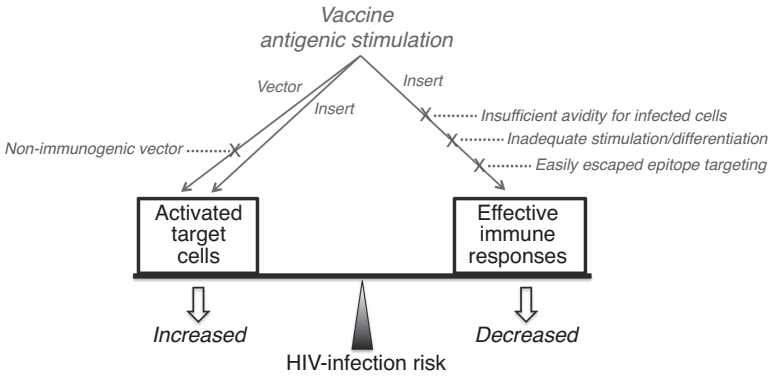

Fig. 1. Balance between HIV-1 acquisition risk and benefit due to vaccination-induced immune activation.

HIV-1 replication on activation-associated transcriptional factors, this process is a critical driver of HIV-1 pathogenesis. Mucosal immune activation in response to HIV-1 itself likely increases target cell availability for early viral dissemination [48], and activation of responding HIV-1-specific $CD4^+$ T cells leads to their clonal deletion [49]. Furthermore, vaccinations to recall antigens in general transiently increases viral replication in infected persons [50]. Thus, it is unclear whether enhanced susceptibility to HIV-1 infection in some rAd5 vaccines is specific to the vector, or more likely an effect of any immunogenic vaccine [51].

Balancing risk versus benefit in HIV-1 vaccine approaches

Thus, there is a balance between vaccine-driven immune activation and efficacy of vaccine-elicited responses (Fig. 1). Although vector-specific immune activation is ideally avoided, response to vaccine-delivered HIV-1 antigens is unavoidable. At the other end of this balance is the efficacy of the HIV-1-specific vaccine responses, for which there is currently no good assay to reflect both the recognition of HIV-1-infected cells and the capacity to avoid the viral escape that leads to ineffectiveness of the $CD8^+$ T-cell response in infected persons [47]. The relative weights of the factors determining the failure of rAd5 vaccines are unclear, but the hints of antiviral activity in some vaccines suggest that this balance could be manipulated favourably by insert and/or vector redesign.

Unresolved issues regarding recombinant adenovirus serotype 5 vaccines

It remains to be confirmed whether other serotype rAd vectors will have the generous insert capacity and immunogenicity of rAd5, which has dendritic cell tropism and maturational effects [52]; different Adenovirus serotypes vary substantially in properties such as cell receptor usage/cell tropism and immunomodulatory effects. Second, the effects of rAd vectors on the mucosal sites of HIV-1 transmission and their capacity to generate deleterious mucosal vector-specific and deleterious versus beneficial insert-specific responses are unknown. Third, it is unclear whether maximizing insert gene expression favours ineffective low-avidity responses, and how to tune expression to generate higher avidity responses while also increasing the magnitude and breadth of $CD8^+$ T-cell responses. Fourth, how best to design inserts generating responses that recognize common epitope polymorphisms and avoid viral escape mutation remains to be determined; it is increasingly clear that simplistic inserts such as whole *gag* are inadequate [53–55].

Conclusion

It is likely that $CD8^+$ T-cell response-generating vectors will be an important complement to current humoral-based vaccines. To date, rAd5 vectors have been the most promising, but lack of a clear explanation for increased HIV-1 acquisition in Step urges caution for the path forward for rAd vectors in general. A circumspect approach to human testing of the safety of novel vectors and adjuvants is required, with a focus on immune activation, vector-specific responses and insert-specific responses at key effector sites at which HIV-1 is transmitted. Given the potential advantages of rAd vectors and the likely inevitability of a component of increased HIV-1 infection risk for all vaccines, cautious pursuit of alternative Ad serotypes is a reasonable path in the stepwise scientific foundation of an effective HIV-1 vaccine.

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Conflicts of interest

There are no potential conflicts of interest for the authors.

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