Published in final edited form as:

Expert Rev Vaccines. 2016 June ; 15(6): 709–717. doi:10.1586/14760584.2016.1147354.

HIV Susceptibility of human antigen-specific CD4 T cells in AIDS pathogenesis and vaccine response

Haitao Hu^{1,*}, Fengliang Liu¹, Jerome Kim², and Silvia Ratto-Kim³

¹Department of Microbiology & Immunology and Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX 77555 USA

²International Vaccine Institute, Seoul, Republic of Korea

³U.S. Military HIV Research Program, Henry M. Jackson Foundation, Walter Reed Army Institute of Research, Silver Spring, MD, 20910 USA

Summary

HIV causes infection and progressive depletion of human CD4 T cells. Emerging data have shown that antigen-specific CD4 T-cell subsets manifest differential susceptibility to HIV, potentially leading to pathogen-specific immune failure and opportunistic infections. This concept was recently explored in context of vectors utilized in HIV vaccine trials, and the data suggest that adenovirus type 5(Ad5)-specific CD4 T cells elicited by Ad5-HIV vaccine may be particularly susceptible to HIV, potentially rendering Ad5 vaccine recipients susceptible to HIV acquisition. We here examined recent data regarding the HIV susceptibility of antigen-specific CD4 T cells induced during infection or HIV vaccination and discussed its potential impact on HIV acquisition risk posed by HIV vaccination.

Keywords

HIV vaccine; CD4 T cells; HIV susceptibility; antigen specificity; pathogenesis

Introduction

CD4 T cells orchestrate host immunity by differentiating into different lineages or subsets that function to recruit and activate other immune cells [1,2]. In HIV infection, however, CD4 T cells also serve as predominant HIV target cells. A hallmark for untreated HIV disease is progressive depletion of CD4 T cells, leading to impairment of cellular immunity and enhanced susceptibility to the opportunistic infections (OIs) that define the acquired immunodeficiency syndrome (AIDS) [3–5].

*Correspondence: Haitao Hu, MD, PhD, University of Texas Medical Branch, Department of Microbiology and Immunology, Sealy Center for Vaccine Development (SCVD), MRB 4.142A, 301 University Blvd, Galveston, TX 77555, Office: 409-747-0395, Fax: 409-772-5065, haihu@UTMB.edu.

Financial and competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Over the past three decades since the outbreak of AIDS, the overall impact of HIV on total CD4 T cells has been intensively investigated [6]. Of interest, early studies found that there were selective defects in antigen-reactive CD4 T cells in HIV-infected patients [7,8]. Subsequent evidence from various HIV infection cohorts showed that susceptibility to different OIs occurred at different disease stages and is not strictly associated with total CD4 depletion [9,10]. In support of these observations, recent studies have identified that different antigen-specific CD4 T cells manifest marked differences in their susceptibility to HIV and display distinct phenotypic, functional and gene-expression profiles [11–14].

HIV vaccine trials then, present an interesting question. Thus far only the vaccine platform tested in the RV144 trial demonstrated modest protection [15,16]. One of the barriers might lie in the fact that HIV targets the type of immune cells (CD4 T cells) that are crucial for the generation of robust and durable humoral and cellular response to HIV [17–20]. Notably, the recombinant Ad5-vectored HIV vaccines (Ad5-HIV) failed in multiple human trials, including STEP and Phambili, where the vaccines provided no protection but were unexpectedly associated with excess HIV infections [20–25]. Despite efforts to identify potential mechanisms for this observation in Step trial, including examinations of a range of parameters, such as cytokine-based magnitudes of CD4 T-cell response using different assays, none of these was found to correlate with HIV infection risk in STEP [26–30]. Different hypotheses have emerged, including antibody-dependent enhancement of infection (as has been suggested for Dengue) or vaccine-induced generalized increase in immune activation. These hypotheses do not seem to fully explain the results of STEP trial. Another hypothesis is that Ad5 vaccination may have induced a type of cellular (CD4) response that predisposes vaccinated subjects to infection. Indeed, recent analyses of cell samples from Ad5-vaccinated human subjects have shown that vector Ad5-elicited CD4 T cells appear to be particularly susceptible to HIV [11]. These findings from human trials and laboratory analyses serve to highlight the importance of better defining host CD4 responses to vaccine vectors used for HIV vaccination, for example, their susceptibility to HIV. In this review, we examine recent emerging data in these areas and discuss important future research that should be pursued.

HIV Susceptibility of antigen-specific CD4 T cells in AIDS pathogenesis

Human CD4 T cell compartment is highly heterogeneous and consists of various subsets defined by differentiation, phenotype and functional characteristics as well as antigen specificity [1,2]. Significant advances have been made in understanding HIV infectivity in different CD4 T-cell subsets based on differentiation [8,31–34], phenotypes and functional profiles [35]. More recently, a growing body of evidence suggests that human CD4 T-cell subsets specific for different antigens or pathogens are also differentially infected by HIV, which may underlie the clinical patterns of OIs seen with progressive HIV disease [11–14,36,37].

An important hypothesis initially examined was whether HIV-specific CD4 T cells are more susceptible to HIV, since HIV exposure induces activation and proliferation of HIV-specific CD4 T cells [13]. Using real-time PCR to measure HIV DNA as a quantitative record of infection history, Douek *et al.* showed that HIV-specific CD4 T cells harbored higher levels

of HIV DNA than total memory CD4 T cells, suggesting preferential *in vivo* infection of HIV-specific CD4 T cells in patients [13]. Following this important discovery, subsequent studies had focused on CD4 T-cell subsets specific for common HIV-related opportunistic pathogens. Geldmacher *et al.* comparatively examined frequency and *in vivo* cell-associated HIV loads for cytomegalovirus (CMV)- and *Mycobacterium tuberculosis* (MTB)-specific CD4 T cells in HIV infected patients; they showed that while CMV-specific CD4 T cells are readily identifiable at chronic stage of HIV infection, MTB-specific CD4 T cells are more susceptible to HIV and preferentially depleted [14,36–38].

Using a T-cell proliferation based assay, where antigen-specific CD4 T cells in PBMC were identified based on CFSE-dilution (CFSE-low), we directly compared HIV susceptibility of different antigen-specific CD4 T cells *in vitro* (Fig. 1). Our data showed that while the *Candida albicans* (*C. albicans*)- and tetanus toxoid (TT)-specific CD4 T cells are susceptible, the CMV-specific CD4 T cells are highly resistant to both R5 and $\times 4$ HIV; compared to *C. albicans*- and TT-specific cells, CMV-specific CD4 T cells can inhibit HIV at both entry and post-entry levels [12]. Microarray analysis performed with the sorted antigen-specific cells from PBMC identified distinct transcriptomic profiles that involved selective up-regulation of innate antiviral genes by CMV-specific CD4 T cells, whereas *C. albicans*- and TT-specific CD4 T cells mainly up-regulate Th17-associated inflammatory genes [12]. Based on this assay (Fig. 1), we have examined a range of antigen-specific CD4 populations for their susceptibility to HIV and associated cytokine profiles in PBMC of healthy donors (summarized in Table 1). Further investigation of *in vivo* HIV infectivity in these different antigen-specific CD4 T cells in HIV-infected patients should be pursued in future, which is important for improving our understanding of HIV pathogenesis in terms of host susceptibility to OIs.

HIV susceptibility of vaccine-elicited CD4 T cells in HIV vaccination

Overview of human HIV vaccine efficacy trials

Six efficacy trials testing different HIV vaccine approaches in humans have been completed to date (summarized in Table 2), among which only the RV144 trial that tested an ALVAC primed gp120 boost regimen demonstrated modest protection (~31%) [15,21,23,25,39,40]. Immune correlates analyses identified that vaccine-induced Env binding antibody (V1V2 IgG) inversely correlated with HIV infection risk in RV144 vaccine recipients [16]. Unlike RV144, earlier two trials (VAX003 and VAX004) testing the recombinant HIV gp120 only regimen failed with no protective efficacy reported; the HIV infection rates between vaccine and placebo groups in these two trials were comparable [39,40]. It has now become clear that for antibody-based vaccine approaches, induction of broadly neutralizing antibodies that can target constantly evolving HIV envelope might be critical [41]. Another three trials testing the Ad5 vector-based vaccine approaches (Step, Phambili and HVTN505) also failed [21–25]. In these trials, vaccine-induced immune response did not provide protection; there were even enhanced HIV infections in the Ad5 vaccine group [21–25]. Mechanisms for such Ad5-associated effect in human trials are not clear but one hypothesis is that a type of immune response (possibly CD4) may have been induced by Ad5 vaccination that predisposes vaccine recipients to HIV. Although the existence of a true effect on HIV

acquisition by Ad5 vectors remains hypothetical, independent observations from multiple trials certainly highlight the importance and necessity to better understand host responses induced during vector HIV vaccination, especially those to vaccine vectors.

1. HIV susceptibility of vector Ad5-specific CD4 T cells in HIV vaccination

In HIV vaccination, compared to vaccine-induced CD8 and antibody responses, which are known to be important for control of viral replication [42] and prevention of viral acquisition [43] respectively, roles of vaccine-induced CD4 response are less clear. While it is believed that certain type of CD4 response, e.g. follicular helper CD4 T cells (T_{FH}), is important for generation of anti-HIV immunity through multiple immunological functions [19,44], there has been concern about induction of strong CD4 response during HIV vaccination, especially at the portals of HIV entry, since HIV preferentially infects activated and proliferating CD4 T cells [6,31,45–47].

In related to the findings from Step and Phambili trials, recent studies have attempted to address the hypothesis whether certain viral vectors (e.g. Ad5) induce activation and expansion of vaccine responding CD4 T cells that are susceptible to HIV. Indeed, our recent study reported that a single dose of rAd5-HIV vaccination led to significant systemic expansion of Ad5-vector specific CD4 T cells in humans, which remained detectable and higher than the baseline level about one year post vaccination [11]. Also in support of the hypothesis, Benlahrech *et al.* re-stimulated memory CD4 T cells from Ad5 naturally exposed individuals *in vitro* using Ad5 vector-pulsed dendritic cells (DC), and showed that Ad5-reactive CD4 T cells are readily susceptible to HIV infection [48]. Similarly, Perreau *et al.* demonstrated that Ad5 vector-antibody immune complexes (IC) could enhance the maturation of DCs that in turn promote activation and HIV infectivity of Ad5-specific CD4 T-cells *in vitro* [49]. This result is consistent with the observation from Step trial that excess HIV infections were associated with pre-existing Ad5 seropositivity [21], although the impact of baseline Ad5 serostatus on HIV infection appeared to become less significant and diminished in those Ad5 vaccine recipients with longer term follow up [24].

More recently, we have examined the HIV susceptibility of vector Ad5-specific CD4 T cells using PBMC samples from rAd5-HIV vaccine recipients, based on the proliferation assay described in Fig. 1. [11,12]. Unlike previous reports where Ad5-reactive CD4 T cells were re-stimulated with vector-pulsed APC, we directly stimulated the CFSE-labeled PBMC with HIV vaccine vector (rAd5) or insert antigens. In HVTN505 trial testing a DNA prime Ad5 boost regimen, a non-significant increase in HIV infections was observed in the vaccine arm [25]. Using cell samples from an earlier phase I trial (RV156A) testing similar Ad5 vectors [50], we showed that vaccine-expanded, vector Ad5-specific CD4 T cells are highly susceptible to both R5 and $\times 4$ HIV infection [11] (Fig. 2). Altogether, these findings suggest that Ad5-specific CD4 T cells, induced during natural Ad5 exposure or by Ad5-HIV vaccination, might represent susceptible and/or preferential target CD4 T cells by HIV.

To extend these *in vitro* observations, we also measured the frequency of Ad5-specific CD4 T cells in untreated HIV-infected patients as compared to frequencies of CD4 T cells specific for a range of other viruses, and found that while the CMV-, EBV- and HIV-specific CD4 T cells persisted at high levels, the Ad5-specific CD4 T cells were much lower in

frequency or lost, even when compared with HIV-specific CD4 T cells [11]. This result supports the *in vitro* HIV infectivity observations and suggests that Ad5-specific CD4 T cells might be selectively depleted *in vivo* during HIV infection. Additional studies are needed to verify the *in vivo* HIV infectivity of antigen-specific CD4 T cells, including those specific for Ad5, in HIV-infected subjects following acute HIV exposure but before significant CD4 T-cell depletion occurs.

HIV susceptibility of other vector antigen-specific CD4 T cells

In addition to Ad5, a number of other HIV vaccine vectors have been developed, including those derived from poxvirus [51–54] and herpes virus [55–59]. Different viral vectors, like the cognate viruses from which they are derived, are biologically and immunologically distinct in terms of *in vivo* tropism [60–62], interaction with dendritic cells [63] and innate immune signatures [64–66]. The hypothesis tested would then be whether vaccine-specific CD4 T cells induced by different vectors might manifest differential susceptibility to HIV, thereby affecting HIV acquisition risk. Similar to Ad5, CMV is a DNA virus, belonging to the herpes virus family, and has been tested as an AIDS vaccine vector in non-human primate (NHP) models [67]. Our comparative analysis of HIV infectivity between Ad5- and CMV-specific CD4 T cells demonstrated that while the Ad5-specific cells are susceptible, the matched CMV-specific CD4 T cells from the same individuals are very resistant to HIV *in vitro* (Fig. 2) [11]. This result is not surprising but could potentially be significant, since pre-clinical studies have shown that rhesus CMV-vectored SIV vaccine (RhCMV-SIV) could stringently control SIV in rhesus macaques, and importantly, no enhanced SIV infection was observed in the immunized monkeys [55–57]. It should be interesting to further investigate in the NHP model if vector CMV-specific CD4 T cells elicited by vaccination are virtually resistant to SIV *in vivo* and if so, whether such resistance may contribute to the overall immune protection conferred by the Rh-CMV-SIV vaccines.

We are currently examining the HIV susceptibility of different candidate HIV vaccine-elicited, vector-specific CD4 T cells from different human trials. ALVAC is the canary poxvirus vector tested in RV144, where no increased HIV acquisition was observed as opposed to rAd5 in STEP trial [15,16]. Our ongoing work shows that in contrast to Ad5, ALVAC-specific CD4 T cells in PBMC of RV144 appear to be substantially more resistant to HIV (unpublished data). The mechanisms for such difference between Ad5 and ALVAC are still under investigation; however, the data, together with the findings about CMV, suggest that viral vectors may not simply serve as vehicles for passive delivery of HIV antigens, but may significantly impact host response to the vaccines, for example, by altering the predominant responding CD4 T cell phenotype, level of activation or HIV susceptibility of vaccine-induced (vector and/or insert) CD4 T cells.

Phenotypes and *in vivo* localization of antigen-specific CD4 T cells may be important for HIV acquisition risk during vaccination

Cellular phenotypes and molecular profiles are associated with the HIV susceptibility of antigen-specific CD4 T cells. Our recent study showed that vector Ad5-specific CD4 T cells selectively manifest a Th17-like pro-inflammatory phenotype with a significant fraction of them expressing IL-17, whereas the matched CMV-specific CD4 T cells display a polarized

Th1 phenotype, producing large amounts of IFN- γ , low levels of IL-2 and no IL-17 [11]. Microarray analysis identified that Ad5- and CMV-specific CD4 T cells display distinct transcriptional profiles; as compared to CMV, Ad5-specific CD4 T cells express higher levels of almost all currently known Th17-associated genes but lower levels of CCR5 ligand (CCL-3, CCL-4 and CCL-5). Flow cytometric analysis further identified that the IL-17- and IL-2-producing, Ad5-specific CD4 T cells are preferentially infected by HIV compared to the single IFN- γ -producing CD4 T cells [11]. These findings may have important implications for our understanding of what functional type of vaccine-specific CD4 T cells should be induced by HIV vaccine from the perspective of HIV susceptibility and HIV acquisition risk. Based on the data from our group and others [12,14,37], we have proposed a schematic model describing potential mechanisms involved in regulating HIV susceptibility of different Ag-specific human CD4 T cells at entry and post-entry levels (Fig. 3).

In vivo anatomic localization of vaccine-elicited CD4 T cells might represent another critical parameter. HIV transmission usually occurs through mucosal sites [68,69]. Preferential localization of vaccine-induced CD4 T cells in certain immune compartments, for example, mucosal portals of viral entry, may significantly impact the overall HIV infection risk for HIV vaccine recipients. Benlahrech *et al.* showed that Ad5-specific CD4 T cells from Ad5 naturally exposed individuals display a mucosal homing phenotype, expressing $\alpha 4\beta 7$ integrin and CCR9 [48]. In line with this observation, our study demonstrated that vaccine-expanded, vector Ad5-specific CD4 T cells from RV156 vaccine recipients also manifest a strong gut-homing potential, expressing high levels of $\alpha 4\beta 7$ and mucosal homing chemokine CCL-20 (MIP-3 α) [11]. These findings are in accordance with the Th17-like phenotype of Ad5-specific CD4 T cells and may reflect the fact that Ad5 is naturally acquired through nasopharynx tract or gut and can establish persistent infection in these mucosal tissues, which may lead to induction of a CD4 response typical of mucosal immunity [70]. The mucosal homing potential of vector Ad5-specific CD4 T cells is further supported by *in vivo* evidence obtained from both human volunteers and vaccinated NHP. Preliminary analysis of mucosal specimens from HVTN505 trial showed that a higher frequency of CD4 T cells was detected in rectum and colon of vaccine recipients as compared with placebo group [71]. A recent NHP study by Carnathan *et al.* demonstrated that Ad5 vaccination, when compared to other vaccine vectors, led to higher level of activated CD4⁺CCR5⁺ T cells in the rectal mucosa of rhesus macaques (RMs); importantly, pooled analysis of all vaccinated RMs after SIV challenge showed that SIV acquisition is associated with the levels of activated and proliferating CD4 T cells in rectal mucosa of RMs [72].

Different from Ad5 vectors, immunization of human subjects with a rare adenovirus serotype vector, Ad26, did not cause an increase in numbers or activation status of CD4 T cells in gut mucosa [71], suggesting that the mucosal homing potential, and possibly the HIV susceptibility as well, of Ad5-specific CD4 T cells may not extend to other Ad vectors. A similar difference exists between two herpesvirus vectors, CMV and varicella zoster (VZV) [59,73,74]. Unlike CMV vector that was shown to be associated with stringent SIV control, VZV-vectored SIV vaccination was found to be increase SIV infection and accelerate disease progression [59]. In addition, our examination of the HIV susceptibility of CD4 T cells specific to HSV-2, another herpes virus that can establish infection at genital

mucosa, showed that unlike CMV, HSV-2-specific CD4 T cells are susceptible to HIV [11] and express IL-17 cytokine (unpublished data; Table 1), indicating that HSV-2-specific CD4 T cells are qualitatively more like Ad5-specific, instead of CMV-specific, CD4 T cells. Altogether, these data further support the concept that closely related but different vectors may have distinct effects on SIV/HIV infection risk and vaccine efficacy.

Mechanisms for how closely related vectors stimulate qualitatively distinct adaptive CD4 responses (e.g. cytokine profile, *in vivo* localization and HIV susceptibility) are unknown but might be related to their innate immune properties [64–66]. For examples, it was shown that immunization with Ad35 and Ad26 vectors elicited more potent innate antiviral responses than Ad5 in rhesus macaques [64]; similarly, ALVAC vector (canary poxvirus) was found to induce distinct cytokine profile compared to the vaccinia poxvirus vectors MVA and NYVAC [65]. In a sense, viral vectors may be acting like adjuvants during vaccination. In an NHP study, Vaccaro and colleagues observed differential immune protection conferred by the same ALVAC/gp120 vaccination when different adjuvants (Alum and MF59) were used. These two adjuvants appeared to stimulate distinct transcriptional pathways and could alter *in vivo* localization of B lymphoblasts by regulating $\alpha\beta 7$ expression [75]. Therefore, it might be interesting to explore whether these adjuvants can possibly affect the phenotypes and SIV susceptibility of vaccine-induced CD4 T cells in this model as well.

Vector vs. insert-specific CD4 T cells and HIV infection risk in HIV vaccination

Immunization with recombinant vector HIV vaccines leads to the induction of both vector and insert-specific CD4 T cells. There has been evidence that vectors can modify insert-specific immune responses [27], making it possible that insert-specific CD4 T cells may vary in phenotypes and/or HIV susceptibility when delivered by different vector platforms. Hansen *et al.* showed in NHP models that vaccine-induced SIV Gag-specific CD4 T cells manifest a central memory phenotype (T_{CM}) when delivered by Ad5 vector, but an effector memory (T_{EM}) phenotype when delivered by CMV vector [58]. Relative to viral vectors, little information is currently available about HIV susceptibility of vaccine insert-specific CD4 T cells and its potential relationship with HIV acquisition risk. Two earlier human trials (VAX003 and VAX004) testing the recombinant HIV gp120 protein vaccine (in the absence of viral vectors) showed that despite induction of gp120-specific CD4 T cells, no enhancement of HIV infection was observed following vaccination [39,40], suggesting that gp120-specific CD4 T cells may either pose a negligible risk for HIV acquisition or that, in balance, the beneficial protective immunity induced by gp120 compensates for the potential negative effect posed by the vaccine-activated CD4 T cells. In contrast, an NHP study designed to recapitulate the Step trial showed a greater risk of SIV acquisition in rAd5 immunized monkeys, but the acquisition risk seemed to be more related to vaccine insert but not vaccine vectors [76]. Nevertheless, it is important in future studies to comparatively examine the HIV susceptibility of vaccine vector- vs. insert-specific CD4 T cells and to assess their relative roles in contributing to HIV acquisition in HIV vaccination.

Expert Commentary & Five-year View

CD4 T cells serve as both victim and enforcer in HIV pathogenesis and host immunity; the infection and destruction of CD4 T cells by HIV deprives the host of an effector that at once is critical to induction, sustainment, and regulation of anti-HIV immunity. If an effective CD4 response is required for the success of an HIV vaccine, which is most likely true, an ideal HIV vaccine strategy should be able to induce a type of vaccine-specific CD4 T cells that are functional and (relatively) resistant to HIV, minimizing potential HIV acquisition risk. Since there is increasing evidence that antigen-specific CD4 T cells elicited by different vaccine vectors or pathogens manifest marked differences in susceptibility to HIV as we discussed (Fig. 3), induction of “HIV resistant” CD4 populations through optimized vaccine approaches should be an achievable goal.

The failure of rAd5-HIV vaccines in human trials is disappointing. However, dissecting the underlying mechanisms would certainly provide important new insights into future immunogen, vector and trial design. First, results of these trials highlight the importance of defining host responses to vaccine vectors, including both innate and adaptive responses. Second, these results suggest that currently used immunological assays demonstrate limitation and do not fully predict vaccine outcome. It would be useful to include additional parameters, such as *in vivo* anatomic localization, pro-inflammatory vs. antiviral characteristics and HIV susceptibility of CD4 T cells induced by vaccines. Third, the trial results indicate the necessity to identify correlates of HIV acquisition in vector HIV vaccinated subjects. Although such effort has been pursued in recent years for Step/Phambili trials, no definitive parameters have yet been identified so far.

In the future, it might be helpful to introduce the concept of HIV susceptibility of vaccine-induced CD4 T cells to pre-clinical and clinical HIV vaccine studies as part of vaccine assessment. To achieve this goal, appropriate bioassays should be established first. In addition to the proliferation-based assay, other methods have also been used to measure human Ag-specific CD4 response induced in natural infection or vaccination. Some of these assays identify Ag-specific T cells in PBMC based on expression of certain activation markers on T cells following antigen recognition (interaction with APC), including CD154 (CD40L) [77] and CD25/CD134 (OX4) [78]. Potential adaptation of these assays for examining HIV susceptibility of CD4 T cells in vaccine studies should be explored but might have some challenges at this moment, since expression of these activation markers on Ag-experienced T cells is usually dynamic and can be modulated by HIV during *in vitro* infection. MHC class-II tetramer staining has also been used to identify Ag-specific T cells [79] and may represent another useful assay for HIV susceptibility analysis. A potential difficulty for this method is that since evaluation of HIV infectivity will probably focus on vector-induced CD4 T cells, complete MHC-II-restricted tetramers would be needed to recognize entire epitopes of fairly large viral vectors. In these regards, the CFSE-based proliferation assay described in our studies might be a feasible system to start with. Nevertheless, exploring and optimizing immune assays to evaluate HIV infectivity of vaccine-induced CD4 T cells should be a critical first step.

These *in vitro* immune assays can be tested using extant cell samples from completed human trials, including HVTN 502, 505, Phambili and RV144. In addition, the HIV susceptibility of CD4 T cells measured using the *in vitro* assays should be further validated *in vivo* in animal models (e.g. NHP) as well as in breakthrough HIV-infected vaccine recipients from human trials (e.g. Step vs. RV144). It is worth noting that while NHP models have contributed substantially to HIV vaccine studies, due to the recognized difference between macaques and humans, translation of findings from NHP models to human trials should be considered with caution. In this regard, parallel evaluation of *in vivo* HIV susceptibility in both NHP model and human vaccine recipients with breakthrough HIV infection would provide a good opportunity to verify the results from NHP. Moreover, it should be further determined whether the *in vitro* and *in vivo* HIV susceptibility of vaccine-induced CD4 T cells correlates with overall HIV acquisition risk in vaccine recipients, which could possibly be performed using case-control samples from the human efficacy trials. Lastly, thorough mucosal studies in animal models and using mucosal specimens from human vaccine recipients should also be considered in future. In summary, these steps should improve our understanding of vaccine-induced CD4 T cells resident or transmitting through mucosal portals of viral entry and the susceptibility of these cells to HIV in the context of vector HIV vaccination.

Acknowledgments

This work was funded by grants from NIH (R21AI110214) and the Robert Mapplethorpe Foundation (to H Hu).

References

Reference annotations

* Of interest

** Of considerable interest

1. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood*. 2008; 112(5):1557–1569. [PubMed: 18725574]
2. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). *Annu Rev Immunol*. 2010; 28:445–489. [PubMed: 20192806]
3. Moir S, Chun TW, Fauci AS. Pathogenic mechanisms of HIV disease. *Annu Rev Pathol*. 2011; 6:223–248. [PubMed: 21034222]
4. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet*. 2014; 384(9939):258–271. [PubMed: 24907868]
5. Stevenson M. HIV-1 pathogenesis. *Nat Med*. 2003; 9(7):853–860. [PubMed: 12835705]
6. Okoye AA, Picker LJ. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunol Rev*. 2013; 254(1):54–64. [PubMed: 23772614]
7. Lane HC, Fauci AS. Immunologic abnormalities in the acquired immunodeficiency syndrome. *Annu Rev Immunol*. 1985; 3:477–500. [PubMed: 2415141]
8. Schnittman SM, Lane HC, Greenhouse J, Justement JS, Baseler M, Fauci AS. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. *Proc Natl Acad Sci U S A*. 1990; 87(16):6058–6062. [PubMed: 2385584]
9. Munoz A, Schragger LK, Bacellar H, et al. Trends in the incidence of outcomes defining acquired immunodeficiency syndrome (AIDS) in the Multicenter AIDS Cohort Study: 1985–1991. *Am J Epidemiol*. 1993; 137(4):423–438. [PubMed: 8096356]

10. Mocroft A, Youle M, Phillips AN, et al. The incidence of AIDS-defining illnesses in 4883 patients with human immunodeficiency virus infection. Royal Free/Chelsea and Westminster Hospitals Collaborative Group. *Arch Intern Med.* 1998; 158(5):491–497. [PubMed: 9508227]
11. Hu H, Eller MA, Zafar S, et al. Preferential infection of human Ad5-specific CD4 T cells by HIV in Ad5 naturally exposed and recombinant Ad5-HIV vaccinated individuals. *Proc Natl Acad Sci U S A.* 2014; 111(37):13439–13444. [PubMed: 25197078] Demonstrate that vaccine expanded, vector Ad5-specific CD4 T cells from Ad5-HIV vaccine recipients are susceptible to HIV *in vitro*
12. Hu H, Nau M, Ehrenberg P, et al. Distinct gene-expression profiles associated with the susceptibility of pathogen-specific CD4 T cells to HIV-1 infection. *Blood.* 2013; 121(7):1136–1144. [PubMed: 23258923]
13. Douek DC, Brenchley JM, Betts MR, et al. HIV preferentially infects HIV-specific CD4⁺ T cells. *Nature.* 2002; 417(6884):95–98. [PubMed: 11986671] First demonstration of *in vivo* preferential HIV infection of an antigen-specific CD4 population in patients
14. Geldmacher C, Ngwenyama N, Schuetz A, et al. Preferential infection and depletion of Mycobacterium tuberculosis-specific CD4 T cells after HIV-1 infection. *J Exp Med.* 2010; 207(13):2869–2881. [PubMed: 21115690] Comparative analysis of HIV susceptibility and functional characteristics of opportunistic pathogen-specific CD4 T cells in HIV patients
15. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med.* 2009; 361(23):2209–2220. [PubMed: 19843557]
16. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med.* 2012; 366(14):1275–1286. [PubMed: 22475592]
17. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4⁺ T cell responses associated with control of viremia. *Science.* 1997; 278(5342):1447–1450. [PubMed: 9367954]
18. Wilson JD, Imami N, Watkins A, et al. Loss of CD4⁺ T cell proliferative ability but not loss of human immunodeficiency virus type 1 specificity equates with progression to disease. *J Infect Dis.* 2000; 182(3):792–798. [PubMed: 10950773]
19. Streeck H, D'Souza MP, Littman DR, Crotty S. Harnessing CD4(+) T cell responses in HIV vaccine development. *Nat Med.* 2013; 19(2):143–149. [PubMed: 23389614]
20. Huang Y, Follmann D, Nason M, et al. Effect of rAd5-Vector HIV-1 Preventive Vaccines on HIV-1 Acquisition: A Participant-Level Meta-Analysis of Randomized Trials. *PLoS One.* 2015; 10(9):e0136626. [PubMed: 26332672]
21. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet.* 2008; 372(9653):1881–1893. [PubMed: 19012954] The Step trial reporting enhanced HIV infections in sub-groups of Merck-type rAd5 vaccine recipients
22. Duerr A, Huang Y, Buchbinder S, et al. Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (Step Study). *J Infect Dis.* 2012; 206(2):258–266. [PubMed: 22561365]
23. Gray GE, Allen M, Moodie Z, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. *Lancet Infect Dis.* 2011; 11(7):507–515. [PubMed: 21570355]
24. Gray GE, Moodie Z, Metch B, et al. Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN 503/Phambili study. *Lancet Infect Dis.* 2014; 14(5):388–396. [PubMed: 24560541]
25. Hammer SM, Sobieszczyk ME, Janes H, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N Engl J Med.* 2013; 369(22):2083–2092. [PubMed: 24099601] Failure of another major efficacy trial testing a different rAd5-HIV vaccine (NIH type) with non-significant increase in HIV infections in vaccine group
26. McElrath MJ, De Rosa SC, Moodie Z, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet.* 2008; 372(9653):1894–1905. [PubMed: 19012957]

27. Frahm N, DeCamp AC, Friedrich DP, et al. Human adenovirus-specific T cells modulate HIV-specific T cell responses to an Ad5-vectored HIV-1 vaccine. *J Clin Invest*. 2012; 122(1):359–367. [PubMed: 22201684]
28. Huang Y, Duerr A, Frahm N, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial reveals an association of nonspecific interferon-gamma secretion with increased HIV-1 infection risk: a cohort-based modeling study. *PLoS One*. 2014; 9(11):e108631. [PubMed: 25369172]
29. Pandey JP, Nambodiri AM, Bu S, De Dieu Tapsoba J, Sato A, Dai JY. Immunoglobulin genes and the acquisition of HIV infection in a randomized trial of recombinant adenovirus HIV vaccine. *Virology*. 2013; 441(1):70–74. [PubMed: 23582638]
30. Fellay J, Frahm N, Shianna KV, et al. Host genetic determinants of T cell responses to the MRKAd5 HIV-1 gag/pol/nef vaccine in the step trial. *J Infect Dis*. 2011; 203(6):773–779. [PubMed: 21278214]
31. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen IS. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell*. 1990; 61(2):213–222. [PubMed: 2331748]
32. Zack JA, Cann AJ, Lugo JP, Chen IS. HIV-1 production from infected peripheral blood T cells after HTLV-I induced mitogenic stimulation. *Science*. 1988; 240(4855):1026–1029. [PubMed: 2835813]
33. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009; 15(8):893–900. [PubMed: 19543283]
34. Chun TW, Chadwick K, Margolick J, Siliciano RF. Differential susceptibility of naive and memory CD4+ T cells to the cytopathic effects of infection with human immunodeficiency virus type 1 strain LAI. *J Virol*. 1997; 71(6):4436–4444. [PubMed: 9151834]
35. Geldmacher C, Koup RA. Pathogen-specific T cell depletion and reactivation of opportunistic pathogens in HIV infection. *Trends Immunol*. 2012; 33(5):207–214. [PubMed: 22398371]
36. Geldmacher C, Schuetz A, Ngwenyama N, et al. Early depletion of Mycobacterium tuberculosis-specific T helper 1 cell responses after HIV-1 infection. *J Infect Dis*. 2008; 198(11):1590–1598. [PubMed: 19000013]
37. Casazza JP, Brenchley JM, Hill BJ, et al. Autocrine production of beta-chemokines protects CMV-Specific CD4 T cells from HIV infection. *PLoS Pathog*. 2009; 5(10):e1000646. [PubMed: 19876388]
38. Casazza JP, Betts MR, Price DA, et al. Acquisition of direct antiviral effector functions by CMV-specific CD4+ T lymphocytes with cellular maturation. *J Exp Med*. 2006; 203(13):2865–2877. [PubMed: 17158960]
39. Flynn NM, Forthal DN, Harro CD, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis*. 2005; 191(5):654–665. [PubMed: 15688278]
40. Pitisuttithum P, Gilbert P, Gurwith M, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis*. 2006; 194(12):1661–1671. [PubMed: 17109337]
41. Klein F, Mouquet H, Dosenovic P, Scheid JF, Scharf L, Nussenzweig MC. Antibodies in HIV-1 vaccine development and therapy. *Science*. 2013; 341(6151):1199–1204. [PubMed: 24031012]
42. Koup RA, Douek DC. Vaccine design for CD8 T lymphocyte responses. *Cold Spring Harb Perspect Med*. 2011; 1(1):a007252. [PubMed: 22229122]
43. Kwong PD, Mascola JR, Nabel GJ. Broadly neutralizing antibodies and the search for an HIV-1 vaccine: the end of the beginning. *Nat Rev Immunol*. 2013; 13(9):693–701. [PubMed: 23969737]
44. Porichis F, Kaufmann DE. HIV-specific CD4 T cells and immune control of viral replication. *Curr Opin HIV AIDS*. 2011; 6(3):174–180. [PubMed: 21502921]
45. McCune JM. The dynamics of CD4+ T-cell depletion in HIV disease. *Nature*. 2001; 410(6831):974–979. [PubMed: 11309627]
46. Rowland-Jones S. HIV infection: where have all the T cells gone? *The Lancet*. 354(9172):5–7.
47. Chou CS, Ramilo O, Vitetta ES. Highly purified CD25- resting T cells cannot be infected de novo with HIV-1. *Proc Natl Acad Sci U S A*. 1997; 94(4):1361–1365. [PubMed: 9037058]

48. Benlahrech A, Harris J, Meiser A, et al. Adenovirus vector vaccination induces expansion of memory CD4 T cells with a mucosal homing phenotype that are readily susceptible to HIV-1. *Proc Natl Acad Sci U S A*. 2009; 106(47):19940–19945. [PubMed: 19918060] Demonstration that *in vitro* activated Ad5-reactive CD4 T cells from naturally exposed individuals are readily susceptible to HIV
49. Perreau M, Pantaleo G, Kremer EJ. Activation of a dendritic cell-T cell axis by Ad5 immune complexes creates an improved environment for replication of HIV in T cells. *J Exp Med*. 2008; 205(12):2717–2725. [PubMed: 18981239]
50. Eller MA, Eller LA, Opollo MS, et al. Induction of HIV-specific functional immune responses by a multiclade HIV-1 DNA vaccine candidate in healthy Ugandans. *Vaccine*. 2007; 25(45):7737–7742. [PubMed: 17920731]
51. Pantaleo G, Esteban M, Jacobs B, Tartaglia J. Poxvirus vector-based HIV vaccines. *Curr Opin HIV AIDS*. 2010; 5(5):391–396. [PubMed: 20978379]
52. Franchini G, Gurunathan S, Baglyos L, Plotkin S, Tartaglia J. Poxvirus-based vaccine candidates for HIV: two decades of experience with special emphasis on canarypox vectors. *Expert Rev Vaccines*. 2004; 3(4 Suppl):S75–S88. [PubMed: 15285707]
53. Marovich MA. ALVAC-HIV vaccines: clinical trial experience focusing on progress in vaccine development. *Expert Rev Vaccines*. 2004; 3(4 Suppl):S99–S104. [PubMed: 15285709]
54. de Bruyn G, Rossini AJ, Chiu YL, et al. Safety profile of recombinant canarypox HIV vaccines. *Vaccine*. 2004; 22(5–6):704–713. [PubMed: 14741163]
55. Hansen SG, Ford JC, Lewis MS, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature*. 2011; 473(7348):523–527. [PubMed: 21562493] * Demonstrate stringent control SIV by CMV vectored-SIV vaccination in NHP
56. Hansen SG, Piatak M Jr, Ventura AB, et al. Immune clearance of highly pathogenic SIV infection. *Nature*. 2013; 502(7469):100–104. [PubMed: 24025770]
57. Hansen SG, Sacha JB, Hughes CM, et al. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. *Science*. 2013; 340(6135):1237874. [PubMed: 23704576]
58. Hansen SG, Vieville C, Whizin N, et al. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat Med*. 2009; 15(3):293–299. [PubMed: 19219024]
59. Staprans SI, Barry AP, Silvestri G, et al. Enhanced SIV replication and accelerated progression to AIDS in macaques primed to mount a CD4 T cell response to the SIV envelope protein. *Proc Natl Acad Sci U S A*. 2004; 101(35):13026–13031. [PubMed: 15326293] Report in NHP that candidate Env vaccine based on VZV vector was associated enhanced infection and accelerated disease progression
60. Vogels R, Zuijdgheest D, van Rijnsoever R, et al. Replication-deficient human adenovirus type 35 vectors for gene transfer and vaccination: efficient human cell infection and bypass of preexisting adenovirus immunity. *J Virol*. 2003; 77(15):8263–8271. [PubMed: 12857895]
61. Abbink P, Lemckert AA, Ewald BA, et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J Virol*. 2007; 81(9):4654–4663. [PubMed: 17329340]
62. McFadden G. Poxvirus tropism. *Nat Rev Microbiol*. 2005; 3(3):201–213. [PubMed: 15738948]
63. Lore K, Adams WC, Havenga MJ, et al. Myeloid and plasmacytoid dendritic cells are susceptible to recombinant adenovirus vectors and stimulate polyfunctional memory T cell responses. *J Immunol*. 2007; 179(3):1721–1729. [PubMed: 17641038]
64. Teigler JE, Iampietro MJ, Barouch DH. Vaccination with adenovirus serotypes 35, 26, and 48 elicits higher levels of innate cytokine responses than adenovirus serotype 5 in rhesus monkeys. *J Virol*. 2012; 86(18):9590–9598. [PubMed: 22787208]
65. Teigler JE, Phogat S, Franchini G, Hirsch VM, Michael NL, Barouch DH. The canarypox virus vector ALVAC induces distinct cytokine responses compared to the vaccinia virus-based vectors MVA and NYVAC in rhesus monkeys. *J Virol*. 2014; 88(3):1809–1814. [PubMed: 24257612]
66. Teigler JE, Kagan JC, Barouch DH. Late endosomal trafficking of alternative serotype adenovirus vaccine vectors augments antiviral innate immunity. *J Virol*. 2014; 88(18):10354–10363. [PubMed: 24991003]

67. Barouch DH, Picker LJ. Novel vaccine vectors for HIV-1. *Nat Rev Microbiol.* 2014; 12(11):765–771. [PubMed: 25296195]
68. Haase AT. Targeting early infection to prevent HIV-1 mucosal transmission. *Nature.* 2010; 464(7286):217–223. [PubMed: 20220840]
69. Wu L, KewalRamani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. *Nat Rev Immunol.* 2006; 6(11):859–868. [PubMed: 17063186]
70. Garnett CT, Erdman D, Xu W, Gooding LR. Prevalence and quantitation of species C adenovirus DNA in human mucosal lymphocytes. *J Virol.* 2002; 76(21):10608–10616. [PubMed: 12368303]
71. NIAID. Mini-Summit on Adenovirus Platforms for HIV Vaccines. Bethesda, MD: National Institutes of Health; 2013. 2013
72. Carnathan DG, Wetzel KS, Yu J, et al. Activated CD4+CCR5+ T cells in the rectum predict increased SIV acquisition in SIVGag/Tat-vaccinated rhesus macaques. *Proc Natl Acad Sci U S A.* 2015; 112(2):518–523. [PubMed: 25550504]
73. Hansen SG, Ford JC, Lewis MS, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature.* 2011; 473(7348):523–527. [PubMed: 21562493]
74. Hansen SG, Jr MP, Ventura AB, et al. Immune clearance of highly pathogenic SIV infection. *Nature.* 2013; 502(7469):100–104. [PubMed: 24025770]
75. Vaccari M, Gordon SN, Fourati S, et al. Adjuvant Dependent Mucosal V2 Responses and RAS Activation in Vaccine Induced Protection from SIV(mac251) Acquisition. *AIDS Research and Human Retroviruses.* 2014; 30(Suppl 1):A64–A65.
76. Qureshi H, Ma ZM, Huang Y, et al. Low-dose penile SIVmac251 exposure of rhesus macaques infected with adenovirus type 5 (Ad5) and then immunized with a replication-defective Ad5-based SIV gag/pol/nef vaccine recapitulates the results of the phase IIb step trial of a similar HIV-1 vaccine. *J Virol.* 2012; 86(4):2239–2250. [PubMed: 22156519]
77. Chattopadhyay PK, Yu J, Roederer M. Live-cell assay to detect antigen-specific CD4+ T-cell responses by CD154 expression. *Nat Protoc.* 2006; 1(1):1–6. [PubMed: 17406204]
78. Zaunders JJ, Munier ML, Seddiki N, et al. High levels of human antigen-specific CD4+ T cells in peripheral blood revealed by stimulated coexpression of CD25 and CD134 (OX40). *J Immunol.* 2009; 183(4):2827–2836. [PubMed: 19635903]
79. Nepom GT. MHC class II tetramers. *J Immunol.* 2012; 188(6):2477–2482. [PubMed: 22389204]

Key issues

- Untreated HIV disease is characterized by infection and progressively depletion of human CD4 T cells, leading to resurgence of opportunistic infections (OIs) and development of AIDS.
- Recent data have shown that CD4 T cells specific for different antigens manifest differential susceptibility to HIV, which has important implications for AIDS pathogenesis and vaccine responses
- Failure of Ad5-vectored HIV vaccines in humans (STEP, Phambili and HVTN 505), where the vaccines were unexpectedly associated with excess HIV infections, highlights the importance of better defining host innate and adaptive responses to vaccine vectors, especially the potential HIV susceptibility of vaccine insert or vaccine-vector elicited CD4 T cells.
- Recent studies demonstrated that vaccine-elicited, vector Ad5-specific CD4 T cells are particularly susceptible to HIV, manifesting a pro-inflammatory phenotype with gut-mucosal homing potential.
- More thorough evaluation of vaccine (including vector)-specific CD4 T-cell response in terms of HIV susceptibility and mucosal homing (e.g. portals of viral entry) is needed in future pre-clinical and clinical HIV vaccine studies.
- Development of appropriate animal models and new assays for assessing in vitro and in vivo HIV susceptibility of vaccine-induced CD4 T cells should be helpful.
- Regarding AIDS pathogenesis, it is important to further examine whether HIV susceptibility of pathogen-specific CD4 T cells correlates with pathogen-specific immune failure and risk of opportunistic infections in HIV-infected patient.

Figure 1. Summary of the *in vitro* HIV susceptibility assay

(A) Mononuclear cells (MNCs) from blood or tissues of individuals are labeled with CFSE, a fluorescent dye to track T cell division, and then stimulated with antigens of interest (pathogen or vaccine antigens) for 3 days, followed by exposure to HIV. Productive HIV infection of antigen (vaccine)-specific CD4 T cells in MNCs is determined based on intracellular p24 expression in the CFSE-low CD4 T cell population by flow cytometry. (B) CFSE-low CD4 T cells were confirmed to be antigen specific (using CMV as an example) based on the observation that >91% of CFSE-low CD4 T cells produced IFN- γ in response to CMV antigen re-stimulation.

Figure 2. HIV susceptibility of human Ad5- and CMV-specific CD4 T cells induced by natural exposure or rAd5 vaccination

The assay was performed as described in Figure 1. Shown is the representative data for PBMC cells from Ad5 naturally exposed (top) or rAd5-HIV vaccinated individual (Bottom) infected with R5 (left) or $\times 4$ HIV (right) *in vitro*. Flow cytometric plots (CD3+CD8⁻ T cells) show p24+ percentages in CFSE-low and CFSE-high CD4 T cells between Ad5 and CMV stimulation (left).

Figure 3. A schematic model for differential susceptibility of Ag-specific CD4 T cell populations to HIV

Pathogenic exposure or vector HIV vaccination (Ad5) induces expansion of antigen-specific CD4 T cells (Red: CMV; Blue: Ad5/tetanus toxoid/*C. albicans*). For CMV-specific cells, exposure to HIV leads to limited HIV entry due to high level of autocrine MIP-1β production, which blocks majority of CCR5 receptor on the cells. In addition, although a small number of HIV may successfully enter, their post-entry replication in CMV-specific cells remains inhibited by a broad array of innate antiviral mechanisms such as restriction factors and Interferon-stimulated genes (ISG), leading to low susceptibility of CMV-specific CD4 T cells to HIV. In contrast, for CD4 T cells specific for *C. albicans*, tetanus toxoid and Ad5, HIV can more readily enter into these cells due to lower level of MIP-1β production. Following viral entry, HIV replication in *C. albicans*-, tetanus toxoid- or Ad5-specific CD4 T cells may be further enhanced by IL-17 and IL-2 signaling, leading to high susceptibility of these Ag-specific CD4 T cells to HIV.

Table 1

In vitro HIV infectivity and cytokine profiles of antigen-specific CD4 T cells

Antigen	HIV infectivity	IL-17	IL-2	MIP-1 β	IFN- γ
CMV	+	-	+	+++	+++
<i>C. Albicans</i>	+++	+++	++	++	++
T. Toxoid	++	++	++	++	++
HSV-2	++	+	++	++	++
Ad5	+++	+	++	++	++

1. Data are adapted from our recent publications [11,12] and unpublished results.

2. Negative: -; Detectable level: +; Medium level: ++; High level: +++

Table 2

Human HIV vaccine efficacy trials and the reported efficacy

Trial	Platform	Vaccines	Phase	Status	Results	Reference
STEP	Ad5	Merck-Ad5 HIV	IIb	Early termination (2007)	Increased HIV Infections	[21,22]
Phambili	Ad5	Merck-Ad5 HIV	IIb	Early termination (2007)	Increased HIV Infections	[23,24]
HVTN 505	DNA/Ad5	VRC- DNA HIV/ VRC-rAd5 HIV	IIb	Early termination (2013)	Increased HIV Infections (non-significant)	[25]
RV144	ALVAC-Protein	ALVAC-HIV /AIDSVAX	III	Completed (2006)	~31% protection	[15]
VAX 003	Protein	AIDSVAX B/E	III	Completed (2003)	No protection; No increased infections	[40]
VAX 004	Protein	AIDSVAX B/B	III	Completed (2003)	No protection; No increased infections	[39]