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No Acquisition: a New Ambition for HIV Vaccine Development?

Samir K. Lakhache^{1,2}, Guido Silvestri³, and Ruth M. Ruprecht^{1,2}

¹Dana-Farber Cancer Institute, Boston MA 02115 USA

²Harvard Medical School, Boston MA 02115, USA

³Yerkes National Primate Research Center, Emory University, Atlanta GA 30329 USA

Abstract

Development of a safe and effective prophylactic HIV-1 vaccine presents unique challenges. The pessimism following the failure of two HIV-1 vaccine concepts in clinical trials, HIV-1 gp120 and an adenovirus-based approach to induce only cellular immune responses, has been replaced by cautious optimism engendered by the RV144 trial outcome, the isolation of several new broadly reactive neutralizing monoclonal antibodies, and recent primate model data indicating prevention of viral acquisition by active or passive immunization. Intense efforts are underway to optimize immunogen design, adjuvants, and the tools for preclinical evaluation of candidate vaccines in primates, where correlates of protection can be examined in detail – as proof-of-concept for clinical trials.

The development of a safe and effective prophylactic HIV-1 vaccine presents unique challenges related to the genetic diversity of the virus, its peculiar ability to escape host immune responses, and our limited understanding of the immunological correlates of protection from virus transmission. Despite these challenges, the recently completed RV144 AIDS vaccine efficacy trial has raised the tantalizing possibility that HIV transmission may be preventable by vaccination. RV144 was a phase III trial based upon a prime/boost regimen involving ALVAC and AIDSVAX; it was designed to either induce cytotoxic T lymphocytes (CTL) or neutralizing antibody (nAb) responses, respectively [1]. However, the individual vaccine components by themselves were deemed to be insufficient on their own. The vaccinations induced binding antibodies and modest cellular immune responses; neutralizing antibodies (nAbs) have not been observed. Currently, intense efforts are underway to find potential correlates of protection. While according to a modified intention-to-treat analysis, RV144 had an efficacy of 31.2% [1], Gilbert et al. [2] recently voiced statistical concerns: "...RV144 data provide moderate evidence of low-level positive vaccine efficacy – with ≥22% chance remaining for no efficacy under a range of prior assumptions – an inference that reflects greater uncertainty than has much of the discussion about this trial". This review focuses on advances in AIDS vaccine development in the post RV144 era.

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Address for correspondence: Ruth M. Ruprecht, MD, PhD, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02115, USA, Phone: 617-632-3719; Fax: 617-632-3112, ruth_ruprecht@dfci.harvard.edu.

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Correlates of vaccine protection

Vaccine development against viral infections has relied historically on nAb titers as read-out. However, currently available HIV envelope immunogens have been unable to induce nAbs with sufficient breadth/potency to neutralize most primary HIV isolates in vitro.

New data have emerged regarding the negative result of the STEP trial [3] designed to induce only T-cell immunity using recombinant human type 5 adenovirus vectors encoding HIV *gag*, *pol* and *nef*: Vaccine-induced cellular immune responses exerted selective pressure on breakthrough viruses in vaccinees when compared to placebo recipients [4]. Thus, although the STEP trial had to be terminated prematurely, the in vivo effects of vaccine-induced cellular immunity imply that combining a T-cell vaccine with immunogens designed to induce protective antibody responses may hold promise.

Since only few efficacy trials have been conducted in humans [1,3,5,6], with only one showing moderate protection [1], finding correlates of vaccine protection is challenging. While important information has been derived from studying long-term non-progressors of HIV infection [7] and elite controllers [8], the biological situation of vaccinated naïve hosts confronting infectious virus differs from that of systemically infected, seropositive hosts who have brought the virus under control.

Studies of possible correlates of vaccine-induced protection have also relied on nonhuman primate models. Recently, several such vaccine challenge studies have yielded not only suppression of viremia [9-11], but prevention of viremia – at least in some vaccinees [12-16]. Such primate studies will be important in dissecting the correlates of protection.

The role of primate models in evaluating AIDS vaccine concepts

Non-human primate (NHP) models for AIDS vaccines have evolved and currently include the use of several Asian macaque species challenged with simian immunodeficiency virus (SIV) and simian-human immunodeficiency virus (SHIV). All currently available SHIVs are built from the SIVmac239 backbone and encode HIV-1 *vpu* (most strains), *tat*, *rev*, and *env*. NHP model studies should attempt to replicate the biology of HIV-1 transmission among humans as closely as possible. Therefore, vaccine challenge studies should consider the following: 1) mucosal transmission is the predominant route of HIV-1 transmission among humans; 2) almost all new HIV-1 acquisitions world-wide involve R5 strains; 3) most recently transmitted viruses have a tier 2 neutralization profile (i.e. viruses that are relatively difficult to neutralize); 4) relatively low HIV-1 inocula are thought to be involved in mucosal transmission events in humans; 5) NHP challenge viruses should be grown in peripheral blood mononuclear cells of same species to avoid potentially confounding xenoresponses. The latter may arise due to impurities associated with immunogens produced in cells of a foreign species (e.g., human) or during the phase of multiple low-dose virus challenges if the virus is produced in cells of a foreign species (xenoresponses have been shown to provide protection against challenges involving SIV or SHIV grown in human cells [17-20]); and 6) human AIDS vaccine recipients will unlikely be exposed to “homologous” virus, i.e., HIV-1 strains exactly matching the immunogen(s). The last point implies that AIDS vaccine efficacy studies in primate models should not use “homologous” virus challenges. While some reports [21,22] have cited a lack of correlation between the outcome of human efficacy trials and preclinical vaccine studies in macaques, many of the primate studies involved homologous virus challenges. Furthermore, challenges involving a single, high virus dose and/or viruses with a tropism that does not reflect the typical mucosally transmitted R5 virus involved in most HIV-1 acquisitions in humans should no longer be used to assess the potential efficacy of vaccines aimed at preventing HIV-1 infection.

Current NHP viral challenge models and HIV-1 infection in humans

To date, no perfect animal model exists that mimics all aspects of HIV-1 infection and disease progression in humans. Table 1 compares important biological characteristics of different primate challenge viruses with the corresponding parameters of HIV-1 infection in humans. The commonly used SIV challenge strains have an extended coreceptor usage [23,24] – which may influence the earliest stages of viral invasion of mucosal tissues and viral spread. Furthermore, SIVmac251 and SIVmac239 are more virulent in primates than HIV-1 in humans. Typically, about 20% of monkeys infected with these SIV strains have a rapid downhill course and fail to seroconvert [25]. The latter has not been described in HIV-1-infected humans.

The once popular SHIV89.6P strain differs drastically in tropism and pathogenicity from newly transmitted HIV-1. SHIV89.6P, although dualtropic in vitro, acts X4-tropic in vivo and predominantly replicates in naïve CD4⁺ T cells, resulting in a dramatic depletion of circulating CD4⁺ T cells within a few weeks from infection [26,27]. In contrast, recently transmitted HIV-1s are almost always R5-tropic and preferentially replicate in memory CD4⁺ T cells (Table 1). As preclinical vaccine efficacy studies performed with SHIV89.6P have no predictive value for HIV-1 transmission in humans, this acutely pathogenic SHIV is no longer recommended [28,29].

Of note, SHIV89.6P should not be regarded as the representative of all SHIVs. Indeed, the initial SHIV constructs encoded HIV-1 *env* genes derived from laboratory-adapted, X4-tropic HIV-1 strains (reviewed in [30]). The next-generation SHIVs encoded dualtropic HIV89.6 *env* which, after adaptation to macaques, resulted in the highly virulent SHIV89.6P [26].

Newer SHIVs have been constructed encoding R5-tropic *env* genes [31–34]. Our group has generated a panel of R5 SHIVs containing recently transmitted *env* genes of African HIV-1 clade C isolates (Table 1). The resulting clade C SHIVs (SHIV-Cs) have been used successfully to test the efficacy of passive and active immunization strategies [15,16,35,36]. With one such SHIV-C, SHIV-1157ipd3N4, the relative transmissibility of an exclusively R5-tropic virus through different mucosal routes was examined in macaques. Unlike SIV [37], SHIV-1157ipd3N4 reflected the relative risks of HIV-1 acquisition among humans following different modes of sexual exposure [38]. While R5 SHIVs show gradual disease progression like HIV-1 in humans, R5 SHIV-C-infected animals did not show typical viral setpoints but rather exhibited gradual downward sloping over extended time, which may be due to maintaining the animals in biocontainment suites. In contrast, HIV-1-infected individuals are exposed to environmental antigens and/or other pathogens with the resulting immune activation facilitating virus replication.

SHIVs have played an important role in determining correlates of immune protection. They are especially attractive tools to analyze humoral immune responses directed against HIV-1 Env. SHIVs could be employed to assess the potential role of RV144 vaccine-induced humoral immune responses in providing protection against mucosal virus transmission.

Preclinical efficacy studies of candidate AIDS vaccine: consider the real world

—Sexually transmitted diseases increase the risk of HIV-1 acquisition [39]. Acute parasite infection or mucosal inflammation rendered macaques significantly more susceptible to SHIV-C acquisition [38,40,41]. Current vaccine efficacy studies in primates do not consider cofactors/co-infections and this may compromise the predictive value of such studies. For instance, parasite-induced cytokine skewing may negatively affect the immunogenicity of vaccines designed to induce cellular immunity [42,43]. Hence, NHP vaccine challenge

models should also consider those co-factors (local infections, microtrauma, etc) that appear to favor HIV-1 transmission in humans.

Antibodies as protective principle

Passive immunization with neutralizing monoclonal antibodies (nmAbs): cause of protection

Passively administered human anti-HIV-1 nmAbs completely protected NHPs against intravenous, oral, intrarectal or intravaginal SHIV challenges with either X4, and X4/R5 or R5 tropism (reviewed in [30]). Recently, the anti-HIV-1 V3 loop nmAb HGN194 showed cross-clade protection in infant macaques, identifying the V3 epitope as protective and thus as a potential vaccine target [35]. This is an important finding since, unlike targets of other broadly neutralizing mAbs (bnmAbs), V3 is immunodominant and it may be feasible to induce nAbs that recognize relatively conserved features of this target. It will be of great interest to assess the in vivo protection that the recently described human nmAbs [44-47] will provide in passive immunization studies when used either as single-agents or in novel nmAb combinations.

Antibodies: action beyond neutralization

Antibodies may not only directly block virus entry (classical neutralization), but also mediate effector functions through the Fc portion of IgG. NHP studies involving SHIV89.6P have revealed a partially protective role of antibody-dependent cellular cytotoxicity (ADCC) [48,49]. Interestingly, the anti-CD4 binding site bnmAb b12 exerted most of its protective effects through ADCC rather than direct neutralization as was shown in an elegant study [50]. A related function is antibody-dependent cell-mediated viral inhibition (ADCVI) (reviewed in [51]). Recently, a third effector function, inhibition of transcytosis, was linked to strong protection against repeated low-dose vaginal challenges [13]. In this study, virosomes displaying gp41 fragments were administered by different mucosal routes to macaques that were repeatedly challenged with a heterologous, tier 2 R5 SHIV.

Benefits of preexisting anti-Env antibodies: accelerated induction of nAb responses with increased breadth and priming of cellular immunity

More rapid induction of nAbs with greater breadth was demonstrated in newly SIV-infected macaques by passive immunization with polyclonal anti-SIV IgG [52]. A similar finding was reported for passive immunization of SHIV_{SF162P3}-challenged monkeys with breakthrough infection compared to SHIV infection in naïve animals [53]. Rapid induction of high-titer nAbs against a heterologous second challenge virus was also demonstrated for vaccine-induced nAb responses targeting the V3 loop [54].

Passively administered antibodies not only bind and neutralize incoming virus, but also form antigen-antibody complexes which may be taken up by Fc receptor-expressing cells. In the absence of productive infection, such cells could cross-present viral antigens, which may even prime T-cell responses, as was recently shown [35]. After successful passive immunization, CD4⁺ and CD8⁺ T-cell proliferative responses were induced in all monkeys that were completely protected from systemic infection with high-dose nmAb, in contrast to monkeys given a 50-fold lower dose. These data imply that the concentration of antigen-antibody complexes needs to exceed a certain threshold in order to prime T-cell responses.

Cellular immunity as a correlate of protection

T cell-mediated immune responses to HIV/SIV involve the generation of cytotoxic T lymphocytes (CTL), which lyse autologous cells that present viral peptides on cell surface

MHC class-I molecules, as well as a number of non-cytolytic mechanisms (i.e., production of antiviral cytokines and chemokines). After the initial failure of phase III trials involving gp120, induction of virus-specific T- cell responses became the central theme for HIV-1 vaccine development. Although the negative result of the STEP trial was viewed initially as a setback for CTL-based vaccine strategies, recent primate studies have yielded more encouraging results [11,55-57]. Most notably, recombinant rhesus cytomegalovirus (RhCMV) encoding different SIV antigens induced long-lasting effector memory T-cell responses in macaques. Although all monkeys became viremic, vaccine-induced immunity suppressed SIV replication to undetectable levels [10], possibly leading to clearance [9].

Cellular plus humoral Immunity as correlates of protection

The disappointing STEP trial results implied a need to include humoral anti-HIV-1 responses; the notion has been further supported by results of RV144 trial. In our recent study [16], macaques were immunized to induce SHIV-C-specific cellular as well as nAb responses and repeatedly challenged with heterologous R5 SHIV-C. We noted complete or partial protection in half of the vaccinees. For the first time, both nAb and cellular immune responses were linked to the degree of protection: highly significant inverse correlations were observed between peak viremia and cross-nAb titers and the level of T cell responses measured as interferon- γ producing cells by ELISPOT assay.

Prevention of HIV acquisition: a new ambition

Almost 20 years ago, the goal for HIV-1 vaccines was curtailed to prevention of disease progression rather than prevention of infection (reviewed in [58]). However, the RV144 trial has given hope that vaccination may prevent HIV-1 acquisition. Recent primate model studies [12-16] imply that this is feasible by various immunization strategies, although complete protection of all vaccinees remains an ambitious goal. Is it reachable? Over the past few years, several new immunogen designs [59-61], vectors (reviewed in [62,63]) and adjuvants (reviewed in [64]) have been developed. Significant advances have also been made towards the development of tools to assess preclinical efficacy and understanding the correlates of protection in NHP models. Together, these advances have laid foundation for the new ambitious goal, i.e., prevention of HIV-1 acquisition in most vaccinees. Changing the long-term goal from modulating viremia and slowing HIV-1 disease progression to preventing virus acquisition by an AIDS vaccine is an important milestone.

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Highlights

- RV144 trial data and recent non-human primate studies suggest that HIV-1 acquisition may be preventable by vaccination
- Induction of virus-specific humoral as well as cellular immune responses is an important goal for AIDS vaccine development
- Recently, several new tools have been developed to determine preclinical efficacy of candidate HIV-1 vaccines in non-human primates

Table 1
Biological characteristics of HIV-1 infection in humans versus those of challenge viruses in macaques

	HIV-1	SIV		Dual-tropic SHIV	R5 SHIV-B	R5 SHIV-C
		SIVmac239	SIVmac251	SIVsmE660	SHIV89.6P	SHIV-1157ipd3N4 [38] SHIV-1157ipEL-p [31] SHIV-2873Nip [33]
Co-receptor (ref/review)	CCR5 ^a	CCR5/Bob/ Bonzo [23,24]	CCR5/Bob/ Bonzo [23,24]	CCR5/Bob [24]	CCR5 [65]	CCR5
Time to viral set point (ref/review)	~14 weeks [21]	6 weeks [21]	6 weeks [21]	6 weeks [21]	15-20 weeks [65]	gradual downward sloping over extended time
Viral set point (copies/ml) (ref/review)	10,000 to 50,000 [1,66]	100,000 to 500,000 [10,67,68]	100,000 to 500,000 [11,69,70]	1,000 to 500,000 [55,71]	>100,000 (few animals only) [65]	>10,000 or gradual downward sloping ^b
Disease progression	gradual	fast	fast	fast	gradual	gradual
Time to AIDS onset (ref/review)	8-10 years [21,75]	0.5 to 3 years [21,75]	0.5 to 3 years [21,75]	0.5 to 3 years [21,75]	0.5 to 2 years [65]	2 to 3 years (earliest cases) follow-up ongoing for SHIV-1157ipEL-p and SHIV-2873Nip
Seroconversion after infection (ref/review)	all infected hosts	20-30% RM progress rapidly without seroconversion [25]	20-30% RM progress rapidly without seroconversion [25]	rapid disease progression without seroconversion was reported [76]	most RM show seroconversion [65]	all infected hosts

^a Almost all transmitted viruses have been R5 tropic

^b based on data of 17 adult Indian RM. Lower viral loads may be expected in macaques maintained in biocontainment suite facilities compared to humans leading normal lives with continued exposure to environmental antigens and/or other pathogens