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Use of Nonhuman Primate Models to Develop Mucosal AIDS Vaccines

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Abstract The HIV vaccines tested in the halted Step efficacy trial and the modestly successful phase 3 RV144 trial were designed to elicit strong systemic immune responses; therefore, strategies to direct immune responses into mucosal sites should be tested in an effort to improve AIDS vaccine efficacy. However, as increased CD4⁺ T-cell activation and recruitment to mucosal sites have the potential to enhance HIV transmission, mucosal immune responses to HIV vaccines should primarily consist of effector CD8⁺ T cells and plasma cells. Controlling the level of mucosal T-cell activation may be a critical factor in developing an effective mucosal AIDS vaccine. Immunization routes and adjuvants that can boost antiviral immunity in mucosal surfaces offer a reasonable opportunity to improve AIDS vaccine efficacy. Nonhuman primate models offer the best system for preclinical evaluation of these approaches.

Keywords HIV · Female genital tract · Immune activation · SIV

Introduction

The recent inability to complete the efficacy trial of an AIDS vaccine designed to elicit systemic T-cell responses [1–3] and the modest success in a human phase 3 trial of a

second AIDS vaccine designed to elicit both systemic antiviral T-cell and antibody responses [4••] highlight the need for AIDS vaccines that induce antiviral immunity at mucosal surfaces that are the portal of entry for HIV. The mucosal immune system represents a highly compartmentalized immunological system that in many ways functions independently from the systemic immune system, although the systems do interact. The mucosal immune system is a specialized subset of lymphoid tissues and cells that preferentially reside within the wide variety of mucosal surfaces [5–7]. Along with the skin, these mucosal surfaces form the primary barrier between pathogens and the vertebrate host. Thus, the mucosal immune system is the first line of immunologic recognition and defense against the vast majority of microbial pathogens, including HIV. As with the systemic immune system, distinguishing self from nonself antigens is a critical feature of the mucosal immune system. However, a further challenge exists at mucosal surfaces, as they are populated with a large number of beneficial microorganisms. Thus, to maintain a normal mucosal flora, it is critical that the mucosal system is able to promote immune recognition of pathogens and maintain immune tolerance to commensal organisms [5–7].

The nature of the antigen, the specific antigen-presenting cells (APCs) involved, and the presence of inflammation in the tissue shape mucosal immune responses. With most antigens (eg, food proteins), the “default” pathway for mucosal dendritic cells (DCs) and other APCs generates T helper 2 (T_H2) and regulatory T-cell responses that result in active suppression of systemic immunity or “oral tolerance” to food antigens [6]. Pathogens are recognized by mucosal APCs detecting pathogen-associated molecular patterns that bind to Toll-like receptors (TLRs), initiating innate immune and inflammatory responses. Proinflammatory conditions favor the development of stronger and broader immune

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responses promoting strong humoral and cellular immune responses [6]. Although it had been widely assumed that the commensal microbes were not recognized by the TLRs of mucosal APCs, microbial commensals are indeed recognized by TLRs under normal conditions, and this helps maintain epithelial homeostasis in the gut [6].

B and T cells, sensitized to antigen in mucosal inductive sites, leave the site of antigen presentation in the mucosa, move through the lymphatics to enter the blood to recirculate and re-enter mucosal tissues. The majority of these cells re-enter the mucosa of origin, where they differentiate into memory or effector lymphocytes [5, 6]. The anatomic localization of mucosal lymphocytes is determined by expression of homing receptors (integrins) on their surface and complementary mucosal “addressins” on vascular endothelial cells [5, 6]. Additionally, mucosal DCs influence the homing properties of mucosal T cells. Intestinal DCs produce retinoic acid, which increases the expression of the mucosal-homing receptor $\alpha 4\beta 7$ and CCR9, the receptor for the gut-associated chemokine CCL25 [5, 6].

Taken together, these observations may explain the somewhat archaic notion of a “common mucosal immune system” [5, 6]. Although early studies in mice suggested that the mucosal surfaces share a common set of mucosal lymphocytes and that immune responses induced at one site disseminate to all mucosal surfaces, the common mucosal immune system is more restricted than previously thought [5, 6]. In humans, immunization studies with cholera toxin B subunit by different mucosal routes have clearly shown that the strongest response takes place at the immunized mucosa, with weaker responses at anatomically adjacent mucosal sites [5]. The differential expression of chemokines, integrins, and cytokines among mucosal tissues may explain the linkage between some mucosal inductive sites and particular distal effector sites (eg, the nose and female genital tract) [5].

Mucosal HIV Transmission

HIV is transmitted primarily by sexual contact, and the female genital tract, male genital tract, and rectum are the anatomic sites of virus transmission [8]. Nonhuman primate (NHP) models have been critical for understanding how the virus enters these mucosal surfaces, infects target cells, and disseminates from mucosal surfaces [9, 10]. HIV and simian immunodeficiency virus (SIV) rapidly penetrate the mucus covering the epithelial surface of the vagina and ectocervix and infect intraepithelial dendritic Langerhans cells and $CD4^+$ T cells in the epithelium and lamina propria [11]. These infected cells enter draining lymphatics and can be found in the lymph nodes of the genital tract 18 to 24 h after exposure [11]. However, there is a little detectable viral replication in tissues until 5 days after

infection, when there are simultaneous and dramatic increases in viral replication and innate antiviral immune responses (Type 1 interferon expression) in all tissues [9, 12]. Further, recent studies have conclusively demonstrated that immediately after mucosal transmission, many systemic HIV and many systemic SIV infections are established by a very limited number (1 or 2) of viral envelope glycoprotein variants [13, 14]. Thus, vaccine-induced immune responses may only need to prevent infection or replication of a small number of virions transmitted during an exposure. Importantly, although the virus that establishes the systemic infection is more fit than chronic phase virus, acute phase virus is extremely sensitive to neutralization by chronic phase plasma, suggesting that transmitted virus could be similarly sensitive to vaccine-induced antibody responses [15]. Thus, there is a period between mucosal transmission and the onset of massive viral replication at day 5 postinfection that may provide an opportunity for vaccine-induced immune responses to limit or even eliminate a nascent HIV infection established by a limited number of viral variants before an infection becomes established in systemic lymphoid tissues.

Engaging the Mucosal Immune System to Prevent HIV Transmission

Vaccine-elicited antiviral effector mechanisms of the mucosal immune system have the potential to provide three layers of protection from mucosal-transmitted pathogens, such as HIV: 1) dimeric secretory IgA and monomeric IgG and IgA in mucosal secretions can neutralize virions in the lumen prior to binding to target cells; 2) dimeric IgA can neutralize virions inside epithelial cells, and monomeric IgG and IgA can neutralize virions in the lamina propria; and, 3) virus-infected cells in the mucosa can be killed by mucosal cytotoxic T cells and by virus-specific IgG mediating antibody-dependent cell-mediated cytotoxicity. If any invading virus should overcome these mucosal immune effector mechanisms, then the vaccine-elicited systemic immune responses provide a final opportunity to eliminate HIV infection during the dissemination phase of infection. However, once an HIV infection becomes established in systemic lymphoid tissues, the best outcome that can be expected is enhanced immune control of viral replication.

To generate mucosal immune responses to HIV vaccines, two general strategies are available. Adjuvants can be used to stimulate the mucosal immune system, and specific routes of immunization can be used to direct immune responses toward specific mucosal surfaces. Mucosal immunization strategies, using either novel adjuvants codelivered with antigens or replication-defective viral vectors, represent an important next step in the development of improved AIDS vaccines. In fact, it seems likely

that the efficacy of the vaccines tested in the RV144 and Step HIV vaccine trials could be improved by incorporating one or both of these strategies to induce antiviral immunity of mucosal surfaces. NHPs are critical in the effort to develop these strategies, as they are the only species that express the appropriate mucosal T-cell homing receptors and vascular addressins for modeling human mucosal immune responses to vaccines.

Mucosal Challenge Models for AIDS Vaccines

A number of challenge models exist for assessing the protective potential of candidate AIDS vaccines, including NHP models using either SIV or laboratory-engineered chimeric SIV/HIV (SHIV) viruses. Although the chimeric SHIVs do not adequately reproduce AIDS pathogenesis and are not adequate to assess the ability of a candidate vaccine to alter disease progression, they are adequate to determine if a vaccine increases resistance to acquiring infection. The SIV models are excellent models of AIDS pathogenesis and can be used to assess the effect of vaccination on both resistance to acquiring infection and the rate of disease progression in immunized animals that become infected. Mucosal SIV challenge faithfully reproduces the key features of mucosal HIV transmission, including the transmission of a few variants from complex quasispecies of viral variants [13, 14]. In addition, NHP models can employ either a repeated low-dose virus challenge system or a single high-dose challenge system to meet the specific objectives of the experiment. NHP SHIV models of mucosal HIV transmission have been used to demonstrate that passive transfer of neutralizing monoclonal antibodies can prevent virus transmission [16], providing support for the concept that vaccine-elicited antibody responses can prevent transmission. Recently, a small animal model of HIV transmission was developed by transplanting human bone marrow, liver, and thymus (BLT) into severe combined immunodeficient mice [17]. Consequently, human APCs and lymphocytes populate the mucosal surfaces, and the model can be used to assess some strategies to prevent vaginal HIV transmission [18]. Further, once infected, the BLT mice generate humoral and cellular HIV-specific immune responses [19]. However, because TLR ligands are expressed on a different set of murine cells compared with human cells, it will be difficult to evaluate the utility of vaccines and adjuvants that elicit innate immunity in the BLT mouse. In addition, the existence of a common mucosal immune system in mice but not in humans is due to fundamental differences in the distribution of vascular addressins in mice and humans. Finally, murine mucosal immune responses do not reflect mucosal immune responses in NHPs or humans [6].

Review of NHP Mucosal AIDS Vaccine Studies

Although studying mucosal immunity is technically challenging due to difficulty in obtaining samples of sufficient quality and quantity for analysis, the need to improve the relatively low efficacy of the systemic AIDS vaccines currently in development demands a focus on eliciting mucosal immune responses through vaccination. Thus, there is a renewed effort to study mucosal immunology in preclinical studies of AIDS vaccines. The remainder of this review summarizes some recently published NHP studies using a mucosal route of virus challenge. The decision to focus primarily on the results of mucosal challenge studies is based on the fact that correlates of protection against HIV are unknown and thus, immunogenicity studies provide little insight into the protective potential of the candidate HIV vaccines [20]. Only studies with mucosal virus challenge provide an opportunity to assess the potential immune correlates of protection from mucosal challenge. In fact, NHP mucosal challenge studies [21] predicted the lack of immunogenicity and partial efficacy seen in the RV144 phase 3 trial of an ALVAC (recombinant canarypox vector)-based HIV vaccine in Thailand [4•]. Critically, these preclinical studies of ALVAC-based vaccines studies used an appropriate NHP model and repeated low-dose mucosal challenge with highly pathogenic SIVmac251. Clearly, a similar approach to preclinical evaluation of HIV candidate vaccines should be used going forward.

Considerable literature documents the efforts of numerous investigators to elicit anti-SIV/SHIV mucosal immunity in NHP models. NHP have been immunized with recombinant SIV proteins or peptides, live-attenuated SIV or SHIV, viral or bacterial vectors encoding SIV genes, and DNA vaccines; this literature up to 2004 was summarized in the introduction of an article by Yoshino et al. [22]. An excellent review of more recent NHP mucosal AIDS vaccine studies was published earlier this year [23•]. In this article, we focus on some of the NHP AIDS vaccine studies with mucosal virus challenges published from 2007 to 2009, which are summarized in Tables 1 and 2. Of the 12 articles using mucosal immunization routes, animals were immunized by the intranasal (IN) route in five articles, by the tonsillar route in four, by the intratracheal route in two, and by the rectal route in one (Table 1). Further, two articles used what was described as oral immunization, but the immunization actually consisted of placing enteric-coated capsules containing live Ad5 vectors into the stomach using gastric feeding tube (Table 1). Although IN immunization has been studied extensively, it is unlikely to ever be used clinically, as IN immunization in humans is rarely associated with onset of Bell's Palsy [6]. Of the nine articles using systemic immunization routes, the animals were immunized by the intramuscular route in six articles, by

Table 1 Summary of recent (2007–2009) prophylactic AIDS vaccine studies employing mucosal immunization and mucosal virus challenge in NHP models^a

Study	Vaccine	Vaccine route	Challenge/ route	Level of protection	Immune correlates
Vagenas et al. [25]	AT-2 SIV + CpG-C	Palatine/lingual tonsils	SIVmac239/IR	Lower frequency of infection; lower peak plasma vRNA levels	Antiviral Ab in rectal secretions
Copeland [34]	Prime SIV + IL-2 + IL-15 DNA/boost SIV-MVA	IN or IM	SIVmac251/IR	CD4 T-cell preservation and delayed disease	Systemic and colorectal T-cell responses
Manrique et al. [35]	Prime multigenic DNA/boost MVA adjuvanted by IL-12 DNA	IN/IN; IM + IN/IM + IN or IM/IN	SHIV89.6P/IR	Lower peak/set point plasma vRNA levels; no AIDS progression	SHIV-specific T-cell responses in blood
Falkensammer et al. [36]	SCIV/-SIV genes / Ad5-SIV or SCIV-MULV <i>env</i> boost	Tonsils	SIVmac239/tonsil	Lower peak/set point plasma vRNA levels	Neutralizing Ab; complement C3-deposition on viral particles in plasma
Stahl-Hennig et al. [37]	Prime-boost regimen of SCIV and adenoviral vector vaccines	Tonsils	SIVmac239/tonsil	lower plasma vRNA levels	Strong T cell and antibody responses in blood
Bogers et al. [38]	Prime Ad5hr-HIV-1 (89.6p) <i>env</i> /boost heterologous <i>Env</i> protein or alphavirus replicons	IN + IT/IM	SHIV _{SF162p4} /IR	Lower plasma vRNA levels	Titer of neutralizing antibodies in sera
Hidajat et al. [39]	Prime Ad5hr-SIV with <i>env-gag- nef</i> /boost SIV gp120 protein (MPL-SE adjuvant)	Oral (tablets + stomach tube) + oral or IN + oral/IM+ IM	SIVmac251/IR	Lower peak plasma vRNA levels	ADCVI activity and transcytosis inhibition activity in plasma
Demberg et al. [40]	Prime Ad5hr-SIV DNA ± IL-12 or IL-15/boost with SIV gp140 + SIV <i>nef</i> protein	IM+IT/IM	SIVmac251/IR	No protection	None
Zhou et al. [41]	Prime Ad5 with SIV <i>env/rev</i> , <i>gag</i> , and <i>nef</i> genes/boost with SIV gp120 protein	Oral (tablets + stomach tube) + oral or IN + oral/IM+ IM	SIVmac251/IR	Lower peak/setpoint plasma vRNA levels	T cell responses to Gag and Nef
Stolte-Leeb et al. [42]	Prime multigenic DNA/boost MVA	ID/IM-ID + IM-ID or IM-ID + palatine tonsils	SHIV89.6P/IR	Lower peak/setpoint plasma vRNA levels; CD4 ⁺ T-cell preservation	None (better protection from mucosal and systemic than systemic vaccination alone)
Barnett et al. [26]	HIV-1 SF162 envelope protein vaccine	IM/IM or IM/IN	SHIV _{SF162p4} /IVAG	Protected from infection	Serum-neutralizing antibodies
Wang et al. [24]	HSP70 + SIVgp120 + SIVp27 + CCR5; HSP70 + SIVgp120 + SIVp27; HSP70 + CCR5	Rectal	SIVmac251/IR	6/15 protected from infection	Increased A3G mRNA in the CD4 ⁺ CCR5 ⁺ blood and lymph node T cells

Ab antibody; *ADCVI* antibody-dependent cell-mediated viral inhibition; *Env* envelope; *Gag* group-specific antigen; *HSP* heat shock protein; *ID* intradermal; *IL* interleukin; *IM* intramuscular; *IN* intranasal; *IR* intrarectal; *IT* intratracheal; *IVAG* intravaginal; *MPL-SE* monophosphoril lipid A stable emulsion; *MULV* Moloney murine leukemia virus; *MVA* modified vaccinia virus Ankara; *Nef* negative factor; *SCIV* single-cycle viral vectors; *SF* San Francisco; *SHIV* simian-human immunodeficiency virus; *SIV* simian immunodeficiency virus; *vRNA* viral RNA

^a Does not include live-attenuated virus studies

the intradermal route in three, by the subcutaneous (subQ) route in two, and by the transdermal route in one (Table 2).

Among the 21 articles reviewed, the vaccinated animals were challenged by intrarectal (IR) inoculation in 15 articles, by intravaginal (IVAG) inoculation in three, by tonsil inoculation in two, and by oral virus inoculation in one (Tables 1 and 2). Of the 12 articles utilizing mucosal

immunization routes, complete protection from, or increased resistance to, infection was reported in three articles [24–26] (Table 1). This includes two articles reporting protection from IR challenge with highly pathogenic SIVmac239 and one from IVAG challenge with variably pathogenic SHIV162P4. The animals in these three articles were immunized with various viral antigens, but all three

vaccines included the viral envelope. However, there was no commonality in the immunization routes in these three studies, as either rectal, IN, or tonsillar immunization routes were used. In two of these three articles, the route of immunization and challenge was matched [24, 25]. Although many of the other mucosally administered vaccines decreased viral replication after immunized animals became infected, none could block infection (Table 1).

Of the nine articles utilizing systemic immunization routes, complete protection from, or increased resistance to, infection was reported in one article (Table 2). This article reported increased resistance to IR challenge with a highly pathogenic SIVmac239 and used subQ immunization with a replication competent cytomegalovirus (CMV) vector expressing multiple SIV antigens, including envelope. Although the vaccine increased resistance to rectal SIV challenge, virus replication in immunized animals that became infected was not altered [27]. The immunologic basis for the all-or-none protection phenomena seen in this

study remains to be defined, but the results are similar to the all-or-none protection seen in both recently completed human AIDS vaccine efficacy trials [1–3, 4••]. Although the CMV vector was systemically administered, it is a replicating viral vector that disseminates throughout the body and produces antigen continuously, albeit at a low level, in mucosal tissues. Thus, it may not be surprising that the only study reporting protection from mucosal challenge after systemic immunization used a replicating viral vector as a vaccine [27]. Although many of the other systemically administered vaccines decreased viral replication after immunized animals became infected, none could block infection (Table 2).

Lessons from Live-Attenuated AIDS Vaccine Models

We recently completed a series of studies that defined antiviral T-cell responses in the mucosal and systemic

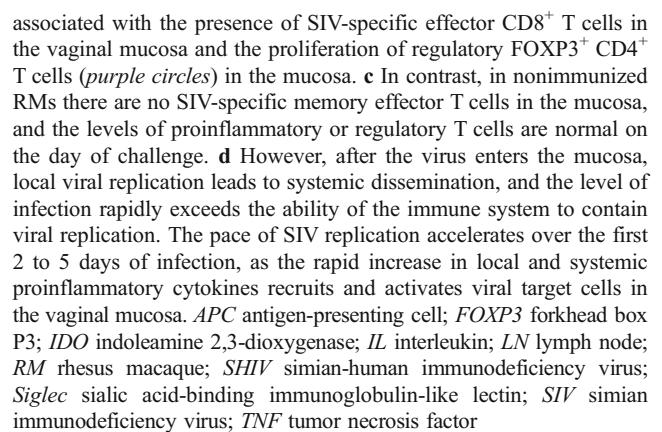
Table 2 Summary of recent (2007–2009) prophylactic AIDS vaccine studies employing systemic immunization and mucosal virus challenge in NHP models^a

Study	Vaccine	Vaccine route	Challenge/route	Level of protection	Immune correlates
Beignon et al. [43]	Lentiviral vector: TRIP-SIVmac239 <i>gag</i>	subQ	SIVmac251/IR	Reduction of acute viremia	T-cell responses in PBMC
Zhao et al. [44]	DNA/MVA HIV-1 immunogens	IM	SHIV162P/IR	Lower peak and total plasma vRNA levels	Non-neutralizing but high-avidity Ab in plasma
Suh et al. [45]	Multigenic DNA and recombinant adenovirus vaccine	IM	SIVmac239/oral	Lower plasma vRNA levels; prolonged survival	Gag-specific IFN- γ ELISPOT T-cell responses in PBMC
Sparger et al. [46]	Δ <i>vif</i> SIVmac239 DNA vaccine boosted with SIV/CMV Δ <i>vif</i> plasmid DNA	IM	SIVmac251/vaginal	Transient decrease in plasma vRNA levels; prolonged survival	SIV-specific T-cell proliferative responses and antiviral antibody titers in blood
Dubie et al. [47]	SIV/CMV Δ <i>vif</i> DNA + (rIL)-15 expression plasmid	IM/ID	SIVmac251/vaginal	Sustained suppression of plasma virus loads	SIV-specific cellular responses greater in blood at 12-wk PC
Lai et al. [48]	DNA/MVA + GM-CSF	IM or ID	SHIV89.6P/IR	Lower peak viremia and virus shedding	High avidity anti-Env IgG in blood and long-lasting antiviral IgA in rectal secretions
Cristillo et al. [49]	DNA boosted with HIV-1 gp120 Env and p41	Transdermal	SHIV162P3/IR	Lower plasma viremia (4/5 animals)	Gag- and Env-specific central memory T-cell responses on the day of challenge
Hansen et al. [27]	RhCMV vectors expressing SIV Gag, Rev/Nef/Tat, and Env	subQ	SIVmac239/IR	Increased resistance to infection	SIV-specific, T _{EM} responses and accumulation in lung
Vaccari et al. [50]	DNA-poxvirus-based vaccines	IM + ID/IM	SIVmac251/IR	Lower vRNA levels in mucosal sites; preservation of mucosal CD4 ⁺ CCR5 ⁺ T cells	Delayed or no expression of T-cell activation markers in mucosal sites

Ab antibody; *CMV* cytomegalovirus; *ELISPOT* enzyme-linked immunosorbent spot; *Env* envelope; *Gag* group-specific antigen; *GMCSF* granulocyte-macrophage colony-stimulating factor; *ID* intradermal; *IFN* interferon; *IM* intramuscular; *IR* intrarectal; *MVA* modified vaccinia virus Ankara; *Nef* negative factor; *PBMC* peripheral blood mononuclear cell; *PC* postchallenge; *Rev* regulator of virion protein expression; *RhCMV* rhesus cytomegalovirus; *rIL* recombinant interleukin; *SHIV* simian-human immunodeficiency virus; *SIV* simian immunodeficiency virus; *subQ* subcutaneous; *Tat* trans-activator of tTranscription; *T_{EM}* effector memory T cells; *TRIP* triplicate

^a Does not include live-attenuated virus studies

evidence for the critical role of SIV-specific CD8⁺ T-cell responses in SHIV-immunized monkeys, expansion of SIV-specific CD8⁺ T cells is limited to the vaginal mucosa, and there is minimal immune activation after the SIV challenge [29]. The extent of host inflammation and immune activation affects viral transcription directly and determines the number of target cells available for virus replication. HIV and SIV replication are regulated by a complex



network of cytokines and chemokines, as these soluble factors directly influence reverse transcription, HIV RNA expression, and expression of viral receptors and coreceptors [31–33]. Cytokine and chemokines also regulate migration and activation of viral target cells, amplifying HIV infection and replication [31–33]. Thus, both the strength of the CD8⁺ T-cell response and the degree of immune activation and inflammation can influence the level of viral replication. After vaginal SIV challenge, immune activation in the SHIV-immunized animals was controlled and limited, in contrast to the aberrant T-cell activation in the unimmunized animals [29]. On the day of SIV challenge, the antiviral CD8⁺ T-cell responses of SHIV-immunized animals existed in a relatively quiescent tissue environment [28] (Fig. 1). After SIV challenge, this quiescent tissue environment was actively maintained by a T-regulatory cell response that rapidly expanded to suppress any immune activation and prevent the generation of more activated target cells to support SIV replication (Genescà and Miller, unpublished data) (Fig. 1). The decreased levels of proinflammatory cytokines and indoleamine 2,3-dioxygenase (IDO⁺) cells in SHIV-immunized animals after vaginal SIV challenge are consistent with immunoregulatory mechanisms playing an active role in achieving this condition (Genescà and Miller, unpublished data) (Fig. 1).

Conclusions

The goal of conventional HIV vaccines is to elicit a strong systemic neutralizing antibody response that can limit infection upon HIV exposure and CD8⁺ T-cell responses to clear the infection after transmission. To improve the efficacy of these vaccines against mucosally transmitted HIV infections, new strategies for directing immune responses into mucosal sites are needed. Further, as immune activation and T-cell expansion counter balance the benefits of strong antiviral mucosal immune responses (Genescà and Miller, unpublished data), understanding and controlling the relationship between immune activation and protective mucosal immune responses may be a critical factor in developing an effective mucosal AIDS vaccine. An effective vaccine against HIV will require broadly neutralizing antibody responses to block infection of new target cells and antiviral T cells to control viral spread and eliminate infected cells. However, the results of efficacy trials of systemic AIDS vaccines suggest that antiviral immune responses at mucosal surfaces will be required to increase protection levels in AIDS vaccines. New immunization routes and adjuvants that can boost antiviral immunity in mucosal surfaces offer the best hope for improving AIDS vaccine efficacy in the near term, and NHP models offer the best system for preclinical evaluation of these approaches.

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