

# Immune mechanisms associated with protection from vaginal SIV challenge in rhesus monkeys infected with virulence-attenuated SHIV 89.6

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**Abstract:** Although live-attenuated human immunodeficiency virus-1 (HIV) vaccines may never be used clinically, these vaccines have provided the most durable protection from intravenous (IV) challenge in the simian immunodeficiency virus (SIV)/rhesus macaque model. Systemic infection with virulence attenuated-simian–human immunodeficiency virus (SHIV) 89.6 provides protection against vaginal SIV challenge. This paper reviews the findings related to the innate and adaptive immune responses and the role of inflammation associated with protection in the SHIV 89.6/SIVmac239 model. By an as yet undefined mechanism, most monkeys vaccinated with live-attenuated SHIV 89.6 mounted effective anti-viral CD8+ T cell responses while avoiding the self-destructive inflammatory cycle found in the lymphoid tissues of unprotected and unvaccinated monkeys.

**Christopher J. Miller<sup>1,2,3,4</sup>,  
Kristina Abel<sup>1,2</sup>**

<sup>1</sup>California National Primate Research Center, <sup>2</sup>Center for Comparative Medicine, and <sup>3</sup>Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, <sup>4</sup>Division of Infectious Diseases, School of Medicine, University of California, Davis, CA, USA

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Dr Christopher J. Miller, UC Davis–CNPRC/CCM, One Shields Ave., Davis, CA 95616, USA.  
Tel.: 530 752 1229; fax: 530 754 4411;  
e-mail: cjmill@ucdavis.edu

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## Introduction

Sexual transmission of human immunodeficiency virus (HIV) is the most common route of HIV dissemination throughout most of the world. To be effective against sexual transmission, a vaccine must prevent HIV transmission across the oral, rectal and vaginal mucosal surfaces. Each of these mucosal surfaces has a distinct mucosa-associated lymphoid tissue (MALT). It is possible that the induction of immunity, and the nature of the cellular and innate immune responses, may differ at each of these mucosal sites. A number of preclinical studies in macaques have been published demonstrating vaccine-mediated protection from intravenous (IV) simian immunodeficiency virus (SIV) or simian–human immunodeficiency virus (SHIV) challenge, but there are very few

studies that have achieved reproducible protection against intravaginal viral challenge. In nonhuman primate models of AIDS, live-attenuated lentiviruses provide the most reliable protection from systemic and mucosal challenge with pathogenic SIV. Although live-attenuated lentiviruses may never be used in humans because of safety concerns, understanding the nature of the protective immune mechanisms induced by live-attenuated vaccines in primate models is thought to be critical for developing other vaccine approaches. In the monkey AIDS model, live-attenuated vaccines have provided the most consistent protection from vaginal challenge [1, 65]. This paper will review the data generated in these, and related, studies that were undertaken to define the immune mechanisms associated with protection in nonhuman primate live-attenuated AIDS vaccine systems.

Understanding how attenuated viruses protect monkeys from superinfection after vaginal SIV inoculation may provide critical insight into the nature of effective anti-HIV immunity.

### Mechanisms of Protection Induced by Attenuated Lentivirus Vaccines

As previously pointed out by others [96], the time-dependent nature of the attenuated virus protection is most consistent with immune-mediated control of challenge virus replication rather than vaccine virus interference [5, 95] with challenge virus replication. Further, the level of protection induced by an attenuated SIV is related to its ability to replicate in the animal, better *in vivo* replication capacity correlates with increased protection [54, 96]. The reason for this is not understood and although the above conclusions are based on IV immunization and challenge, we have now shown consistent protection against vaginal SIV challenge using a live-attenuated SHIV as a 'vaccine' [1, 65]. Systemic infection with an attenuated-SHIV (SHIV 89.6) which replicates well after intravaginal (IVAG), IV or intranasal (IN) inoculation [1, 55], provides protection against vaginal SIV challenge [1, 65]. However, immunization with a DNA plasmid encoding SHIV DNA results in a delayed systemic infection and reduced protection from IVAG SIV challenge [10, 11]. Further, IVAG immunization with an extremely attenuated-SIV (SIVmac1A11) does not protect from vaginal SIV challenge [58]. Thus, the relative ability of attenuated viruses to protect monkeys against vaginal SIV challenge is proportional to the replicative capacity of the attenuated virus.

HIV vaccines may need to elicit genital immune responses to effectively prevent heterosexual transmission [50, 52, 64, 66]. However, we found that the SHIV 89.6-induced protection against IVAG challenge with pathogenic SIVmac239 was achieved independent of the route of immunization and in the absence of strong anti-SIV antibody responses in the genital tract secretions [1]. The proportion of protected monkeys after IV, IN or IVAG was similar [1]. In addition, protection of macaques from mucosal challenge has also been achieved by systemic immunization with other live-attenuated lentiviruses [43, 71, 80]. The route of immunization may have been irrelevant in the challenge outcome because the animals were infected systemically with the vaccine virus for a prolonged period of time (>6 months). The route of immunization may be more important for non-replicating vaccines that do not propagate and

disseminate in a manner analogous to an attenuated lentiviral vaccines.

### Innate Antiviral Immune Responses

One of the reasons attenuated virus vaccines are more effective than DNA, subunit or whole-killed vaccines may be because they elicit non-specific antiviral responses including type I interferons and/or chemokines in the lymphoid tissues of animals. In fact, type I interferons [36, 79] and chemokines [13] significantly suppress HIV replication *in vitro* and HIV-infected long-term non-progressors have high serum levels of chemokines, interleukin (IL)-16 and cellular anti-viral factors [87]. We found that SHIV 89.6 vaccinated-protected monkeys had increased interferon (IFN)-alpha mRNA levels in peripheral blood mononuclear cell (PBMC) during the acute phase (week 1–5) post-challenge compared with vaccinated-unprotected monkeys. Almost 90% of vaccinated-protected animals had higher IFN-alphaPBMC mRNA levels during the acute phase post-challenge compared with their baseline levels at the time of challenge [1]. This suggests that the ability to increase IFN-alpha mRNA levels post-challenge contributed to the observed protection against challenge with pathogenic SIVmac239 in these animals. Thus post-challenge increase in the PBMC IFN-alpha mRNA levels of vaccinated-protected monkeys is a likely response to a minimal level of challenge virus replication and this may contribute to vaccine-mediated protection.

Only a few SHIV 89.6 immunized animals had elevated beta-chemokine (MIP-1alpha and MIP-1beta) PBMC mRNA levels at the time of challenge and, during the first 5 weeks post-challenge, a similar percentage of vaccinated and naïve monkeys had elevated beta-chemokine PBMC mRNA levels [1]. Thus, increased PBMC beta-chemokine mRNA levels post-challenge were not predictive of challenge outcome, but seemed to be part of an innate, proinflammatory response to pathogen exposure. In contrast to our results, some vaccine studies in rhesus and cynomolgous macaques found that immunization produced increased chemokine levels in PBMC cultures and this *in vitro* response correlated with protection [4, 23, 34, 51]. However, the technical differences in the methods used in these studies and our own likely contributed to the disparate results. In the studies reported by others, beta-chemokine levels were measured in supernatants of *in vitro* stimulated PBMC, whereas in our study beta-chemokine mRNA levels were measured directly *ex vivo* from

cells isolated from vaccinated and naïve monkeys without *in vitro* mitogen stimulation. Our data are consistent with studies of HIV-1 infected patients in which a lack of correlation between beta-chemokine levels and disease progression has been described [12, 69, 70] and SIV-infected rhesus macaques [39].

### Antiviral Antibody Responses

Given that the SHIV 89.6 vaccine and the SIVmac239 challenge virus used in our studies have completely different envelope genes, it is not surprising that we found no association between serum anti-SIV binding or neutralizing antibody titers with challenge outcome in the SHIV 89.6/SIVmac239 system [1]. This is consistent with earlier studies that have shown that protection from pathogenic virus can be achieved in the absence of strong antibody responses with attenuated SHIV vaccines and SIV challenge [16, 65, 89] or with attenuated SIV vaccines and SHIV challenge [16, 89]. Similar data have also been reported after infection with other attenuated SIV viruses [15, 19, 20, 91].

In the SHIV 89.6/SIVmac239 system, neutralizing antibodies to SHIV 89.6 were detectable in the serum of 23 of 37 animals tested at the time of challenge, and 15 of these 23 monkeys were subsequently categorized as vaccinated-protected [1]. However, post-challenge, neutralizing antibodies to the challenge virus, SIVmac239, were detectable in only two of 37 SHIV-immunized monkeys tested and neither of these animals was protected from challenge [1]. Thus, anti-SIVmac239 neutralizing antibodies did not play role in the challenge outcome in this system [1]. This is consistent with earlier reports showing potent neutralizing antibody responses in SHIV 89.6 infected monkeys to related laboratory-adapted HIV-1 strains, but not to primary HIV-1 isolates [68]. Also consistent with our results, the protection achieved by immunization with attenuated SIV (SIVmac $\Delta$ 3 or SIVmac $\Delta$ nef) against pathogenic SIVmac251 [17, 96], two viruses with closely related envelope genes, cannot be explained by the induction of neutralizing antibodies.

While neutralizing antibodies are likely to be critical in the protection achieved with many lentiviral vaccines, it is worth noting that protection has also been found in vaccine systems in which anti-env neutralizing antibodies could not be elicited or could not be documented. Thus, some protection from IV SIV challenge has been achieved with vaccinia vectors that did not include

env as an antigen [74] and with subunit protein vaccines containing only gag and pol antigens [41]. Further, an association has been noted between the strength of anti-gag antibody responses and relative protection levels from IV SIV challenge in a recombinant vaccinia vaccine system [35]. In addition, it has been reported that Modified Vaccinia Ankara (MVA) and Canarypox Virus (ALVAC) vaccines that elicited anti-env antibodies that could neutralize tissue culture-adapted virus but not the autologous challenge virus strains, protected monkeys from mucosal pathogenic SIV inoculation [93] and a similar finding has been described with recombinant adenoviral vector SIV vaccines and gp120 protein vaccines [78].

One explanation for these observations is that antibody functions other than neutralization, including ADCC [8, 26, 22] contributed to protection. Further, non-neutralizing antibodies may contribute to formation of immunogenic immune complexes and these complexes enhance the immune response to SIV in the challenge inoculum that leads to very rapid generation of neutralizing antibodies after challenge. This enhanced antibody response mediated by immune complexes may contribute to the observed vaccine protection in some cases [18, 31, 32, 53, 84].

### Antiviral T Cell Responses

Previous studies have provided conflicting data about the role cytotoxic T lymphocytes (CTL) play in the protective immunity induced by live-attenuated vaccines [16, 42, 43]. In the SHIV 89.6 vaccine model, we compared CTL precursor frequencies, the frequency of IFN-gamma secreting cells and the levels of IFN-gamma mRNA in PBMC of vaccinated-protected and vaccinated-unprotected animals at the time of challenge and during the acute phase post-challenge. At the time of challenge, more than 80% of all SHIV 89.6 vaccinated monkeys had detectable SIV-specific CTL activity, but only the vaccinated-protected monkeys showed a significant increase in CTL precursor frequencies in the first 5 weeks post-challenge. We previously reported [65] that anamnestic CTL responses were apparently an important part of the protective immune response in SHIV 89.6 vaccinated-protected monkeys [1]. The induction of strong CTL responses has also been observed after immunization with attenuated SIVmac deletion mutants [43]. The observed role of CTL in vaccine-mediated protection is consistent with the well documented role of CD8 T cells in the control of virus replication in HIV [61] and SIV infection [72, 73, 88].

It has been demonstrated in mice that cytokines, especially IFN-gamma and tumor necrosis factor (TNF)-alpha, contribute to the clearance of viral infections via non-cytolytic mechanisms [27–29]. More recently, it has also been shown that IFN-gamma is involved in the non-cytolytic clearance of Hepatitis B virus (HBV) in the chimpanzee [30]. Although *in vitro* CD8-mediated inhibition of SIV replication was detected in PBMC of approximately 60% of all SHIV 89.6 vaccinated monkeys at the time of challenge, the ability of CD8+ T cells of an individual monkey to inhibit virus replication *in vitro* was not predictive of challenge outcome in a monkey. Thus, SHIV 89.6-induced control of post-challenge viremia was associated with CD8+ T cell mediated cytolytic activity, but not with non-cytolytic CD8+ T cell mediated suppression of viral replication in our studies.

The role of anti-HIV/SIV cellular immune responses in disease progression and vaccine-mediated protection has been the focus of intense study in recent years. Immunization with SHIV 89.6 led to the induction of persistent anti-SIV proliferative responses in the majority of animals [1], as has been observed after immunization with other live-attenuated viruses [21]. However, during the acute phase post-challenge, the T-cell proliferative responses were similar in SHIV 89.6 vaccinated-protected and SHIV 89.6 vaccinated-unprotected animals and we did not detect polarized Th1 or Th2 responses in any monkeys and a specific pattern of Th1/Th2 cytokine expression in PBMC was not associated with challenge outcome [1]. Similarly, simultaneous production of IFN-gamma, IL-2 and IL-4 was observed in PBMC of vaccinated rhesus macaques after challenge with a pathogenic SHIV [33]. Thus the role of antigen-specific CD4+ T cell cytokines in live-attenuated vaccine mediated protection remains to be defined.

#### **The Role of Inflammation in Long-Term Control of Challenge Virus Replication in Live-Attenuated Systems and HIV Pathogenesis**

In the SHIV 89.6/SIVmac239 system, we determined the relationship between virus replication, inflammation, and SIV-specific IFN-gamma T cell responses in the lymphoid tissues of SHIV 89.6 vaccinated and unvaccinated monkeys 6 months after challenge with pathogenic SIVmac239. We found that the lymphoid tissue vRNA levels in the study animals at 6 months post-challenge [2] supported the original categorization of monkeys into vaccinated-protected and vaccinated-unprotected groups based on post-challenge plasma vRNA levels [1]. Thus, relative tissue vRNA levels among

the animals were consistent with the relative plasma vRNA levels [2]. The high levels of vRNA in spleen and peripheral and genital lymph nodes of unvaccinated and vaccinated-unprotected animals were associated with a concomitant and severe loss of CD4+ T cells in blood and tissues. In the blood and lymphoid tissues of vaccinated-unprotected and unvaccinated monkeys, there was a trend towards increased frequencies of activated CD4+ and CD8+ T cells expressing HLA-DR and CD38 [2]. Several other investigators have found that higher frequencies of HLA-DR and CD38 positive T cells in the blood are associated with disease progression [25, 47, 48]. The presence of higher frequencies of activated CD4+ T cells in lymphoid tissues of unprotected monkeys at 6 months post-challenge is consistent with the fact that CD4+ T cell activation facilitates SIV replication. Increased expression of CD38 and HLA-DR on CD8+ T cells in blood and lymphoid tissues reflects the activation of these cells as they develop into CTL effector cells [37]. However, increased immune activation in SIV-infected monkeys and HIV-infected humans is associated with disease progression [86]. Consistent with this notion we found that at 6 months post-challenge the SHIV vaccinated-unprotected animals had higher numbers of CD8+CD38+ T cells than the vaccinated-protected animals in PBMC and lymphoid tissues. This finding may reflect a decreased effectiveness of the adaptive T cell response in the SHIV-vaccinated unprotected monkeys and interpretation that is consistent with the finding that although activated, these CD8+CD38+ T cells are not effective anti-HIV CTL [25]. Further, in African monkeys with non-pathogenic SIV infections there is little immune activation [90]. Thus SHIV 89.6 vaccination may allow some rhesus monkeys to respond to the SIV challenge in a manner that is similar to the response of African monkeys to SIV (Fig. 1).

To clarify the role of IFN-gamma in SHIV 89.6-mediated protection and disease progression, IFN-gamma mRNA levels and SIV-specific IFN-gamma T cell responses were determined in the same samples [2]. Vaccinated-protected monkeys with low vRNA levels in lymphoid tissues had only slightly increased tissue IFN-gamma mRNA levels and strong IFN-gamma T cell responses to SIVgag peptide stimulation *in vitro* [2]. Tissue IFN-gamma mRNA levels and *in vitro* SIV-specific IFN-gamma T cell responses to peptide stimulation were both consistently increased in vaccinated-unprotected monkeys with intermediated to high vRNA levels. In contrast, unvaccinated animals had high levels of vRNA in lymphoid tissues, increased tissue IFN-gamma mRNA levels, and T cells that did not

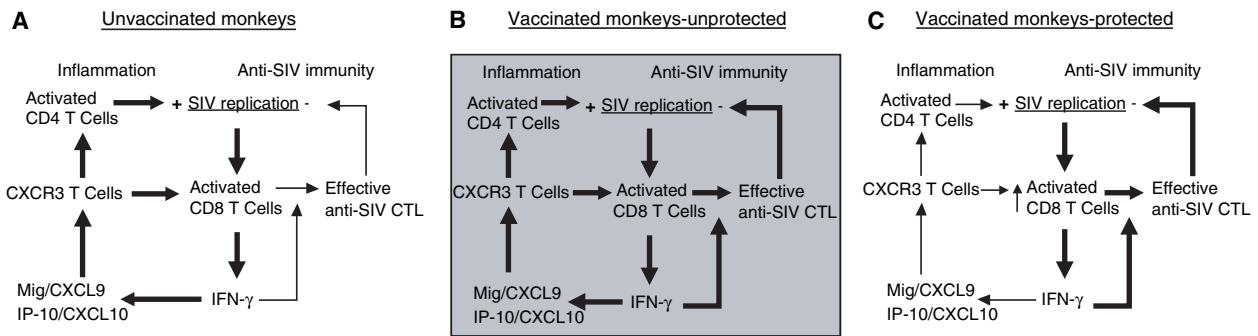


Fig. 1. A diagrammatic representation of the proposed relationship between effective anti-viral T cell responses and the associated inflammation that supports viral replication in (A). In unvaccinated-SIV-infected rhesus monkeys inflammation far exceeds the modest ant-viral T cell responses. (B) Monkeys immunized with non-pathogenic-SHIV-89.6 but unprotected from subsequent intravaginal SIV challenge have strong anti-viral T cell responses and considerable IFN-gamma driven inflammation. (C) Monkeys immunized with non-pathogenic-SHIV-89.6 and protected from subsequent intravaginal SIV challenge have strong anti-viral T cell responses but little IFN-gamma driven inflammation to support viral replication.

respond to *in vitro* SIVgag peptide stimulation [2]. This discordance between T cell responses and constitutive IFN-gamma mRNA expression was confirmed by statistical analysis and was most obvious when the vRNA levels in the lymphoid tissues were either very high or very low [2]. Based on these findings, we proposed that *in vitro* antigen-specific T cell responses reflect the potential of an animal to develop anti-SIV CD8+ T cell responses in lymphoid tissues, and that the tissue IFN-gamma mRNA levels reflect the amount of ongoing virus-induced inflammation in the same tissues [2]. This interpretation is consistent with the recently proposed model that infection with SIV might initiate IFN-gamma driven chronic inflammation [82]. Thus, low levels of virus replication are associated with effective anti-SIV T cell immunity (robust SIV p27-specific T cell IFN-gamma responses) without any inflammation contributing to pathogenesis. The most robust T cell IFN-gamma responses are induced in tissues with intermediate levels of virus replication.

This is the first study to our knowledge showing that there is an increasing frequency (positive correlation) of IFN-gamma secreting cells at low to intermediate vRNA levels (up to 4.2 log<sub>10</sub> vRNA copies/ $\mu$ g RNA), but a decrease (negative correlation) in the number of SIV-specific IFN-gamma secreting T cells at higher vRNA levels [2] in lymphoid tissues of SIV-infected monkeys. In tissues with very high vRNA levels, inflammation (increased IFN-gamma mRNA levels, see also below) may drive pathogenesis, and thereby result in suppression of effective anti-SIV immunity. Most SHIV 89.6 vaccinated animals did not develop the inflammatory response to SIV challenge that is associated with uncontrolled virus replication and vaccine failure.

The IFN-gamma induces several chemokines [7] including Mig/CXCL9 and IP-10/CXCL10 [14]. Increased levels of Mig/CXCL9 and IP-10/CXCL10 in lymphoid tissues of SIV-infected monkeys are associated with inflammation and disease progression [82]. We found that the increased IFN-gamma mRNA levels in lymphoid tissues of SHIV 89.6 vaccinated-unprotected monkeys were associated with the induction of Mig/CXCL9 and IP-10/CXCL10 mRNA. Vaccinated-unprotected animals had the highest IFN-gamma mRNA levels in spleen and lymph nodes, and Mig/CXCL9 and IP-10/CXCL10 mRNA levels were also increased [2]. Mig/CXCL9 and IP-10/CXCL10 attract activated, but not resting T cells expressing CXCR3, a chemokine receptor [7]. Increased CXCR3 mRNA levels were found in matched tissue samples from animals with elevated Mig/CXCL9 and IP-10/CXCL10 mRNA levels. Thus, Mig/CXCL9 and IP-10/CXCL10 likely contributed to the recruitment of activated T cells to lymphoid tissues.

As Mig/CXCL9 and IP-10/CXCL10 recruit both activated CD8+ and CD4+ T cells, they can promote ongoing viral replication (Fig. 1). In the case of the SHIV 89.6-immunized animals, the net effect of increased Mig/CXCL9 and IP-10/CXCL10 expression was deleterious because the highest levels of these pro-inflammatory chemokines were found in lymphoid tissues with higher vRNA levels [2]. The critical role of virus-induced inflammation in promoting SIV pathogenesis is underlined by the fact that in sooty mangabeys, natural hosts for SIV, moderate plasma vRNA levels are maintained in the absence of generalized immune activation and associated bystander immunopathology [90] and these animals do not develop AIDS for extended periods.

Importantly, this is the first study showing that vaccination with an attenuated lentivirus can prevent the IFN-gamma driven inflammation that occurs in essentially all of the unvaccinated monkeys after pathogenic SIV challenge [2]. In SHIV 89.6 vaccinated monkeys, IFN-gamma driven inflammation was present only in lymphoid tissues of the animals that were not protected from the SIV challenge. Further, the onset of uncontrolled viral replication occurred much later in vaccinated-unprotected monkeys than in unvaccinated animals indicating that the immune escape occurred relatively late after challenge [1]. Thus, IFN-gamma driven inflammation can directly contribute to viral replication and to the observed inability of prior lentiviral infection to protect some individuals from subsequent challenge with a pathogenic virus (Fig. 1).

We also found that the level of CD8+ T cell proliferation and the extent and nature of lymph node histopathology correlated with lymph node vRNA levels and these parameters were significantly different in vaccinated-protected compared with vaccinated-unprotected animals [49]. The frequency of proliferating CD8+ T cells was significantly higher in the lymph nodes of vaccinated-unprotected and unvaccinated monkeys compared with vaccinated-protected monkeys [49]. High lymph node vRNA levels also corresponded with increased levels of MIP-1 alpha and MIP-1 beta. Although there were CD8+ T cells expressing MIP-1 alpha or MIP-1 beta, the frequency of these cells negatively correlated with levels of protection [49]. These results are consistent with the conclusion that beta-chemokine expression by cells including CD8+ T cells and non-CD8+ T cells is a feature of the immune activation and inflammation associated with robust SIV replication. Thus, the beta-chemokine mediated control of viral replication does not seem to play a role in SHIV 89.6 vaccine outcome [49]. In PMBC at 6 months post-challenge, beta-chemokine levels were near baseline and there was no difference between the levels among vaccinated-protected, vaccinated-unprotected and unvaccinated animals [1]. This result underlines the importance of the proposed analysis of tissue responses in SHIV vaccinated monkeys.

#### **Hormonal Manipulation of Protection Levels in the Live-Attenuated SHIV 89.6 Vaccine System**

Hormonal contraception is widely used by women and exogenous progesterone enhances vaginal SIV transmission [60]. Thus it is critical to determine the effect of hormonal contraceptives on AIDS vaccines designed to prevent sexual transmission of

HIV. We have recently shown that Depo-Provera administration to SHIV-89.6 vaccinated female rhesus macaques prior to challenge significantly lowers the efficacy of the live-attenuated vaccine against IVAG challenge with virulent SIVmac239 [3]. The higher plasma vRNA levels in the Depo-Provera treated SHIV monkeys were associated with the loss of SIVgag-specific IFN-gamma T cell responses in PBMC at 1 week post-challenge [3]. This result is especially troubling because live-attenuated lentiviral vaccines, although inherently unsafe because of the potential for retention of virulence and integration-induced carcinogenesis [38, 85], provide the most consistent protection from systemic and mucosal challenge with virulent viruses in the monkey models of AIDS. The impact of Depo-Provera may be even greater with vaccines that are less effective than live-attenuated viruses.

At least three mechanisms can explain the deleterious effect of Depo-Provera on live-attenuated 'vaccine' effectiveness. Depo-Provera could increase the 'effective' dose of the challenge inoculum or Depo-Provera could blunt the development of anamnestic antiviral immune responses, or Depo-Provera could elicit a pro-inflammatory environment in the host that facilitates virus replication after genital transmission. There is abundant evidence that exogenous progestins increase susceptibility to genital tract infections in unvaccinated women and monkeys. Use of exogenous hormones for contraception, especially injectable progestins, increases a woman's susceptibility to HIV and other STD infections [6, 59]. Progesterone-dependent thinning of the vaginal epithelium [60] is unlikely to be the complete explanation for why women that use injectable progestins are at increased risk for HIV and STD infection. Although, Depo-Provera may increase the effective dose of the inoculum by increasing the probability of replication competent virion/target cell interaction, Depo-Provera also seems to suppress the initial immune response to vaginally transmitted HIV/SIV.

Exogenous progesterone has profound immunosuppressive effects and a single dose enhances renal allograft survival in dogs and skin allograft survival in rabbits [92] and produces uncontrolled growth of Moloney sarcoma virus-induced tumors in mice [57]. Further, progesterone enhances susceptibility and decreases immune responses to vaginal herpes infection in mice [44, 62, 77]. Exposure to progesterone for more than 15 days prevents the induction of protective mucosal immune responses in mice immunized intravaginally with an attenuated HSV-2 [24]. We noted that Depo-Provera-SHIV monkeys, in contrast to pro-

tected SHIV monkeys, were incapable of making anti-SIVgag ELISPOT responses at 1 week post-challenge [3]. Studies to assess the effect of Depo-Provera on innate antiviral immunity are underway. In addition, in the mouse model of cervical chlamydia infection, exogenous progesterone increases tissue inflammation and the severity of disease [45]. Although we did not conclusively document the nature of the Depo-Provera effect in our previous studies, it seems likely that Depo-Provera induced immune suppression and inflammation affects multiple steps in viral transmission, including the initial susceptibility to infection, the initial immune response to the virus invasion in the naïve host, and the ability of the immune host to mount effective anti-viral recall responses.

### Analysis of Antiviral Immunity in Tissues

Despite the analysis of multiple immune effector mechanisms in a large number of SHIV vaccinated animals (Table 1), no single immune response was found to be responsible for protection against IVAG challenge with pathogenic SIVmac239 in all animals [1, 2, 49]. It is possible that immune mechanisms other than the ones we measured contribute to the observed protection against infection with pathogenic virus. Thus far, the analysis of effective immune effector mechanisms in the acute stage of infection has been limited to immune responses measured in PBMC, and this may not accurately reflect the immune responses in the gut [9, 63, 94] the main site of CD4 + T cell depletion, and in the other lymphoid tissues that are the main sites of HIV and SIV replication [75, 76]. Further, the results of the current study support the fact that although live-attenuated vaccines provide the most consistent protection from SIV challenge, the protection is neither

uniform nor absolute. The coexistence of the vaccine and challenge virus after challenge has been described [46, 56], and recent reports indicate that superinfection with HIV can occur [81]. Immune responses in tissues need further evaluation and will be critical in our understanding of the observed vaccine-mediated protection. In fact we have found that studies that seek to define protection based only on assessment of antiviral immune responses in blood are of limited utility because lentiviral infection is focused on the lymphoid tissues of the intestine and lymph nodes and thus effector mechanisms responsible for controlling viral replication are likely to be in these tissues. In addition, as the challenge virus must cross the genital mucosa to gain access to these target tissues, we hypothesize that the antiviral effector mechanisms responsible for reducing or eliminating the challenge inoculum are located in genital secretions and the genital mucosa.

If the current paradigm of stepwise dissemination of HIV from mucosal surface to regional lymph nodes and then to systemic lymphoid tissue is correct [40], then the best vaccine strategy is to elicit anti-HIV immune responses at the site of virus entry and to limit or block viral dissemination and replication. The fact that SIV-specific cytotoxic T lymphocytes can be consistently detected in, genital tract, lymphoid tissues and gut during acute SIV infection [83], implies that a full range of anti-viral immunity can be brought to bear in the attempt to blunt each stage of virus transmission and dissemination. We believe that the outcome of SIV challenge in monkeys vaccinated with the live, virulence-attenuated SHIV 89.6 is controlled, at least in part, by the balance between SIV-specific IFN-gamma T cell responses vs. non-specific IFN-gamma driven inflammation (Fig. 1). In most monkeys, SHIV 89.6 vaccination reverses this balance between inflammation and anti-SIV immunity, so that little inflammation occurs, but strong anti-SIV T cell immunity develops after SIV challenge [2]. Vaccinated-protected monkeys had little inflammation in lymphoid tissues and strong *in vitro* T cell responses to SIV peptide stimulation. One manifestation of the increased inflammation in tissues of vaccinated-unprotected and unvaccinated monkeys was increased IFN-gamma-induced expression of Mig/CXCL90 and IP-10/CXCL10 and recruitment of activated CXCR3+ T cells. Thus, the inflammatory response that is critical for the induction of effective anti-viral cellular immune responses enhances SIV replication in unvaccinated and vaccinated-unprotected monkeys. By an as yet undefined mechanism, most monkeys vaccinated

Table 1. Summary of the relative level of the immune responses in the acute post-SIV challenge stage of the study in SHIV 89.6 vaccinated-protected monkeys, SHIV 89.6 vaccinated-unprotected monkeys and vaccine naïve SIV-infected control monkeys

	Shiv 89.6 vaccinated monkeys		Naïve monkeys
	Protected	Unprotected	
Anti-SIV serum antibodies	Moderate	High	High
T cell proliferative response	Moderate	Moderate	Low
Anti-SIVgag pCTL (>700/10 <sup>6</sup> CD8 <sup>+</sup> T)	High	Moderate	Not done
SIVgag-specific IFN-g T cells	High	High	Low
b-Chemokine PBMC mRNA levels	High	High	High
IFN-α PBMC mRNA Levels	High	Moderate	Moderate
Mx PBMC mRNA levels	Moderate	Moderate	High

with live-attenuated SHIV 89.6 mounted effective anti-viral CD8+ T cell responses while avoiding the self-destructive inflammatory cycle found in the lymphoid tissues of unprotected and unvaccinated monkeys. Recent data suggests that T regulatory cells could contribute to the controlling the inflammation that contributes to immune-mediated damage in chronic viral diseases (reviewed in [67]) Defining the mechanism(s) by which the vaccine induces this condition and prevents disease progression is a major goal of ongoing studies.

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