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Limited dissemination of pathogenic SIV after vaginal challenge of rhesus monkeys immunized with a live, attenuated lentivirus

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ABSTRACT

In non-human primate models of AIDS, attenuated lentiviruses provide the most reliable protection from challenge with pathogenic virus but the extent to which the vaccine virus replicates after challenge is unclear. At 7 and 14 days after vaginal challenge with pathogenic SIVmac239, plasma SIV*env* RNA levels were significantly lower in female macaques immunized 6 months earlier with live, attenuated SHIV89.6 compared to unimmunized control animals. In 2 SHIV-immunized, unprotected macaques SIV replication produced moderate-level plasma viremia with dissemination of challenge virus to all tissues on day 14 after challenge. In protected, SHIV-immunized monkeys, SIV replication was controlled in all tissues, from the day of challenge through 14 days post-challenge. Further, in $CD8^+$ T cell-depleted SHIV-immunized animals, SIV replication and dissemination were more rapid than in control animals. These findings suggest that replication of a pathogenic AIDS virus can be controlled at the site of mucosal inoculation by live-attenuated lentivirus immunization.

Introduction

HIV is a sexually transmitted disease and a vaccine that can stop HIV transmission offers the best opportunity to stop the AIDS pandemic. In the simian immunodeficiency virus mac (SIVmac) rhesus macaque model of HIV infection, attenuated lentivirus vaccines have consistently provided the most effective protection against systemic and mucosal challenge with pathogenic SIV (Abel et al., 2003; Abel et al., 2004; Almond et al., 1995; Miller et al., 1997). The degree of protection conferred by an attenuated lentivirus vaccine is related to the ability of the vaccine virus to replicate in the host (Lohman et al., 1994) and highly attenuated viruses do not replicate sufficiently to generate a protective immune response (Ruprecht, 1999; Busch et al., 2005). Although reversion to virulence and integration-based carcinogenesis preclude the use of attenuated lentivirus vaccines in humans, identifying the mechanisms by which attenuated lentiviruses confer protection will be useful for developing other vaccine approaches.

The route of immunization with nonpathogenic SHIV 89.6 does not alter the level of protection, as either vaginal, intranasal or intravenous inoculation of SHIV 89.6 produces protection from uncontrolled virus replication after vaginal SIV challenge in about 60% of the immunized animals (Abel et al., 2003). The similar level of protection following mucosal and systemic inoculation of SHIV is likely due to SIV-specific T cell responses in the vagina that are primed and maintained by vaccine virus replication in the genital tract during the 6–8 months of systemic SHIV infection, even after IV inoculation (Genescà et al., 2008b). Thus in this model, the immunizing virus establishes a persistent, disseminated infection and antiviral immune responses are distributed to all tissues.

We recently established a correlation between protection from uncontrolled viral replication after vaginal SIV challenge and the presence of $CD8^+$ T cell responses in the vagina (Genesca, McChesney, and Miller, 2009). The SHIV-induced, antiviral $CD8^+$ T cell response is characterized by cytolytic T cells in the vagina of 60% of immunized animals, but these antiviral T cell responses are much more inconsistent in cervix and other tissues (Genescà et al., 2008a). $CD8^+$ T cell depletion at the time of SIV challenge abrogates the protective effect of SHIV immunization confirming the critical role of $CD8^+$ T cells in the observed protection (Genescà et al., 2008b). Unexpectedly, after SIV challenge, the protective antiviral T cell response does not significantly expand in blood or tissues, except in the vagina (Genescà et al., 2008b).

While the nature of immunologic responses to infection with attenuated SHIV is being elucidated, characterizing the viral populations

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present in blood and tissues after SIV challenge has not been undertaken. In fact, in other attenuated lentivirus vaccine models it is unclear if "vaccine failure" is due to replication of the vaccine virus, the challenge virus, or both (Almond et al., 1995; Berry et al., 2008; Kwofie et al., 2002; Mackay et al., 2004; Reynolds et al., 2008; Rose et al., 1995; Shibata et al., 1997; Silverstein et al., 2000b; Ui et al., 1999; Wakrim et al., 1996). Recombination between vaccine and challenge virus (Gundlach et al., 2000; Reynolds et al., 2008), persistence of vaccine virus after challenge (Mackay et al., 2004; Silverstein et al., 2000a) or persistence of both vaccine and challenge virus (Khatissian et al., 2001) have been reported. The assays commonly used to assess levels of viral replication do not distinguish between vaccine and challenge virus. Further, the relative contribution of the vaccine and challenge viruses to vaccine failure, the extent of SIV dissemination and the sites of SIV replication after challenge of SHIV89.6 immunized monkeys are not known. As infection of $CD4^+$ T cells by an "attenuated" virus vaccine could deplete target cells in tissues interfering with challenge virus replication, it is critical to understand the extent to which vaccine virus contributes to uncontrolled virus replication after pathogenic virus challenge. Thus, to better understand the anatomic sites of vaccine and challenge virus replication, we sought to determine the extent of SIV dissemination and replication in tissues of SHIV-immunized animals after challenge.

Results

The relative contribution of the vaccine and challenge viruses to plasma viremia after vaginal SIVmac239 challenge

The overall design and challenge outcome of this study based on plasma vRNA levels have been recently reported (Genescà et al., 2008a). Briefly, 26 animals previously infected with SHIV89.6 for 6–8 months were challenged vaginally with SIVmac239 along with 21 unvaccinated control animals that received only challenge virus. The SHIV-immunized animals were necropsied at the time of challenge ($n=9$), 3 days p.c. ($n=3$), 7 days p.c. ($n=6$), and 14 days p.c. ($n=12$), while the unvaccinated control animals were necropsied at 3 days p.c. ($n=3$), 7 days p.c. ($n=9$), and 14 days p.c. ($n=9$). We have previously shown that approximately 60% SHIV-immunized rhesus macaques maintain plasma vRNA levels below 10^4 copies/ml and normal $CD4^+$ T cell numbers in blood for 6 months after intravaginal challenge with SIV (Abel et al., 2003; Busch et al., 2005; Genesca et al., 2007). These animals are defined as "protected". However in SHIV-immunized rhesus macaques that have plasma vRNA levels above 10^4 copies/ml at any time during the 6-month observation period after vaginal SIVmac239 challenge, $CD4^+$ T cell numbers steadily decline and these animals are defined as "unprotected" (Abel et al., 2003; Busch et al., 2005; Genesca et al., 2007).

As previously reported using the SIV*pol*-specific bDNA assay to measure viral load (Genescà et al., 2008a), the mean plasma *gag* vRNA level of the unimmunized control animals was significantly higher than the mean plasma *gag* vRNA level of the SHIV-immunized animals at both 7 and 14 days p.c. using an SIV*gag* RT-PCR assay (Figs. 1A and B). Of note, 2 of the SHIV-immunized animals had plasma SIV*gag* RNA levels $>10^4$ copies/ml at 14 days PI, thus these 2 animals are defined as unprotected and would be expected to have steadily declining numbers of $CD4^+$ T cells (Abel et al., 2003; Busch et al., 2005; Genesca et al., 2007).

To determine if the vaccine or challenge virus was the source of the plasma vRNA, plasma SIV*env* vRNA levels were measured by RT-PCR (Fig. 1). At 7 days p.c. SIV*env* RNA was detected in plasma of 10 of 18 SHIV-naïve control animals (ranging from 10^2 to $>10^5$ vRNA copies/ml), but SIV*env* RNA was detected in only 4 of 18 SHIV-immunized monkeys (less than 10^3 vRNA copies/ml). The difference in the mean plasma SIV*env* RNA level of the 2 groups was statistically significant (Figs. 1C and D). By day 14 p.c. all 9 of the control animals had high SIV*env* vRNA in plasma (10^5–10^8 vRNA copies/ml), but only 5 of 12 SHIV-immunized

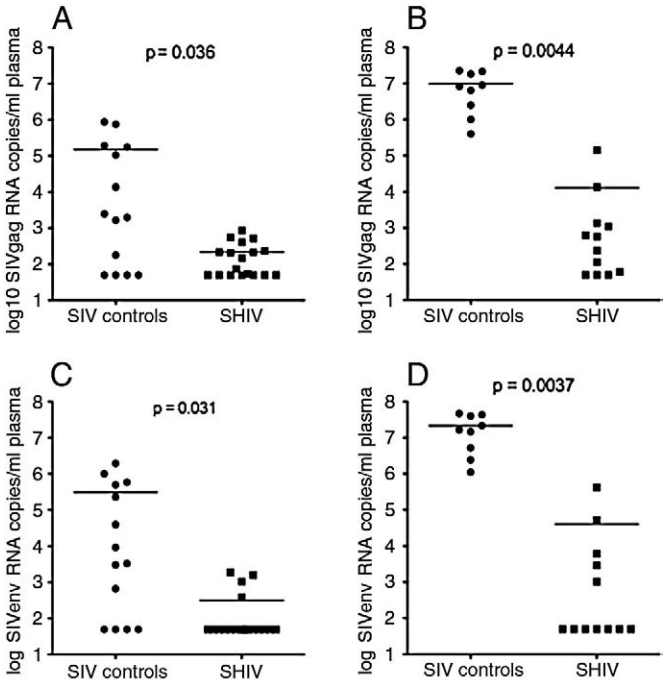

Fig. 1. Plasma vRNA levels in naive control and SHIV-immunized monkeys after vaginal challenge with SIVmac239. Plasma SIV*gag* concentration (copies/ml) at (A) 7 days and (B) 14 days p.c. Plasma SIV*env* concentration (copies/ml) at (C) 7 days and (D) 14 days p.c. Each symbol represents the result for an individual animal. The horizontal line indicates the mean plasma vRNA level in each animal group. The *p* values were calculated using a unpaired one-tailed *T*-test.

monkeys had moderate plasma SIV*env* RNA^+ (10^3–10^6 vRNA copies/ml), and the mean plasma SIV*env* RNA levels of the 2 groups were significantly different (Figs. 1C and D). As with SIV*gag* RNA, the plasma SIV*env* RNA levels in the 2 "unprotected" SHIV-immunized animals were $>10^4$ copies/ml at 14 days PI.

SIVgag and SIVenv RNA levels in the tissues of naïve control animals after vaginal SIV challenge

The RT-PCR assay was used to quantify the number of SIV*gag* copies in a tissue sample and results are reported using vRNA copies/μg tissue RNA. We classified samples with $<10^4$ vRNA copies/μg tissue RNA to have low levels of vRNA; samples with 10^4–10^6 vRNA copies/μg tissue RNA to have moderate levels of vRNA; and samples with $>10^6$ vRNA copies/μg tissue RNA to have high levels of vRNA.

SIV*gag* RNA was not detected in any tissue sample collected from the 3 control animals necropsied at 3 days p.c., (Fig. 2). At 7 days p.c. SIV*gag* RNA was detected in at least one tissue collected from 7 of 9 control animals (Fig. 2). Of these 7 $vRNA^+$ animals, moderate SIV*gag* RNA levels were found in multiple tissues of 5 animals while 2 animals had only a low level of SIV*gag* RNA in one tissue. By day 14 after vaginal SIVmac239 inoculation, moderate to high levels of SIV*gag* vRNA were detected in the tissues of all 9 unimmunized control animals necropsied (Fig. 2).

Among the 7 SIV*gag* RNA^+ control animals at day 7 PC after vaginal SIV challenge, the genital lymph nodes had the highest SIV*gag* RNA levels in 2 animals, and in the remaining 5 animals the tissue with the highest SIV*gag* RNA levels was unique to each animal (Fig. 2). At 7 days p.c., the lowest SIV*gag* RNA levels in the control animals as a group were in the genital tract (cervix, vagina) (Fig. 2). Among the 9 control animals necropised at day 14 p.c., the highest levels of SIV*gag* RNA were seen in the colon and mesenteric lymph nodes draining the intestinal tract, with intermediate vRNA levels in the systemic lymphoid tissues (axillary lymph node and spleen), and the lowest