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Antigenic requirement for Gag in a vaccine that protects against high-dose mucosal challenge with simian immunodeficiency virus

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ABSTRACT

We reported previously on a vaccine approach that conferred apparent sterilizing immunity to SIVsmE660. The vaccine regimen employed a prime–boost using vectors based on recombinant vesicular stomatitis virus (VSV) and an alphavirus replicon expressing either SIV Gag or SIV Env. In the current study, we tested the ability of vectors expressing only the SIVsmE660 Env protein to protect macaques against the same high-dose mucosal challenge. Animals developed neutralizing antibody levels comparable to or greater than seen in the previous vaccine study. When the vaccinated animals were challenged with the same high-dose of SIVsmE660, all became infected. While average peak viral loads in animals were slightly lower than those of previous controls, the viral set points were not significantly different. These data indicate that Gag, or the combination of Gag and Env are required for the generation of apparent sterilizing immunity to the SIVsmE660 challenge.

Introduction

Recent reports have demonstrated that "functional" cures for HIV infection might sometimes be possible (Saez-Cirion et al., 2013; Hutter et al., 2009). Cessation of antiretroviral therapy, with or without allogeneic bone marrow transplantation, however, often leads only to a delay in the rebound of viral loads (Henrich et al., 2013; Persaud et al., 2013). Even if some individuals can maintain control of their viral loads throughout their lifetime, the limitations of using these strategies globally are apparent. Development of a prophylactic vaccine is still an essential component of any strategy to eradicate HIV infection worldwide.

Recombinant rhesus cytomegalovirus (RhCMV) vaccine vectors expressing Gag, Pol, Env, and a Rev–Tat–Nef fusion protein have been shown to induce immunity that can control and eventually clear a pathogenic SIVmac239 challenge virus in roughly half of the vaccinated rhesus macaques (Hansen et al., 2011; Hansen et al., 2013). A variety of other vaccine vectors have also achieved varying levels of apparent sterilizing protection in multiple low dose challenges (Barouch et al., 2012; Patel et al., 2013; Flatz et al., 2012; Letvin et al., 2011). Multiple low-dose challenge models closely mimic a heterosexual exposure to HIV because of the transmission of a small number of founder viruses from the challenge stock (Keele et al., 2009; Li et al., 2010). In men who have sex with men (MSM), however, multiple founder viruses are often transmitted during the time of initial exposure (Li et al., 2010; Tully et al., 2012). Since a greater number of founder viruses are transmitted during a high-dose challenge, this model may more accurately predict the efficacy of vaccines to prevent the transmission of HIV in MSM.

We previously described a vaccine regimen that resulted in apparent sterilizing immunity to a high-dose mucosal challenge with the pathogenic SIVsmE660 quasispecies. This vaccine used a heterologous prime–boost vaccine strategy with vectors based on recombinant vesicular stomatitis virus (VSV) and virus-like vesicles (VLVs) derived from a Semliki Forest Virus (SFV) replicon packaged with VSV G (SFV-G). These vectors encoded Gag and Env proteins derived from the SIVsmE660 viral swarm (Schell et al., 2011). Four out of six animals challenged never showed detectable viral loads, and the two that were infected rapidly controlled their viral loads to below the limit of detection. Control of viral loads in the infected animals was mediated by $CD8^+$ T cells, as evidenced by the rebound in viral loads when $CD8^+$ T cells were depleted from these animals (Schell et al., 2011). The 4 protected animals showed no rebound in viral loads upon $CD8^+$ T cell depletion implying that the vaccine generated sterilizing immunity. These animals had high neutralizing antibody (nAb) to E660 Envs prior to challenge, but

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only marginal or undetectable $CD8^+$ T-cell responses to Env and Gag. These results suggested that antibody to Env might be sufficient for protection. Therefore, in the current study, we asked if the same vaccine regimen expressing Env alone would generate similar sterilizing immunity.

Results

Rechallenge of protected animals

We decided to first test if the previously protected animals would maintain their sterilizing immunity against an additional high-dose mucosal challenge with SIVsmE660 (Schell et al., 2011; Schell et al., 2012). Because of the high titers of neutralizing antibodies, and relatively low levels of cell-mediated responses produced by these animals, we suspected that the sterilizing protection seen was antibody mediated. Therefore, prior to the re-challenge (650 days after the initial challenge), the protected animals received a boost with a recombinant VSV vector expressing an SIVsmE660 Env protein that has its cytoplasmic domain replaced with the cytoplasmic domain of VSV G (EnvG). A full historical timeline for these animals is shown in Fig. 1A. The boost increased neutralizing antibody titers to the tier 1 Env, E660.11, as well as two transmitted founder virus Envs (DF38.21.33C and EN82.57C) in each animal to levels higher than those at the time of initial challenge (Fig. 1B). Only one animal, CM17 showed high levels of Env responsive PBMCs at the time of boost (Fig. 1C) as assayed by IFN-γ ELISPOT. After the boost, two animals, CJ98 and DT03, showed an increase in the numbers of circulating Env responsive PBMCs. Only one animal, CJ98, showed an appreciable level of Gag responsive circulating PBMCs at the time of re-challenge (Fig. 1D). The animals were re-challenged with the original stock of SIVsmE660 at a high dose ($TCID_{50}$=4000) and all remained protected (Fig. 1E).

Vaccination schedule, SIVsmE660 challenge, and plasma viral loads of naïve animals

We next wanted to test the hypothesis that the sterilizing protection we previously demonstrated for a VSV/SFV-G heterologous prime boost vaccine regimen (Schell et al., 2011; Schell et al., 2012) was provided by the high level of neutralizing antibodies to Env induced by the vaccine. Eight rhesus macaques that lacked SIVsmE660-restrictive Trim 5α TFP/CypA (Reynolds et al., 2011) and MHC alleles were selected for the study. Their genotypes are shown in Table 1. The vaccination scheme was the same as in the previous study (Schell et al., 2011), except that we omitted vectors encoding Gag (Fig. 2A). Animals were primed (day 0) with a VSV vector expressing an SIVsmE660 Env protein that has its cytoplasmic domain replaced with the cytoplasmic domain of VSV G (EnvG). They were boosted with the SFV-G virus-like vesicle (VLV) vector encoding EnvG (day 56) and then given a second boost with a VSV vector encoding EnvG (day 119).

Table 1
Genotypes of animals in the Env-only study. The sex of each animal is denoted in parentheses.

Animal ID	A01	A02	A08	A11	B01	B03	B04	B08	B17	DRB* w201	Trim 5α
BJ42 (M)	–	+	–	–	–	–	–	–	–	–	TFP/ TFP
BV15 (M)	–	–	+	–	–	–	–	–	–	–	TFP/Q
CC61 (M)	–	–	–	–	–	–	–	+	–	–	TFP/Q
CD71 (M)	–	–	–	+	–	–	–	–	–	+	TFP/Q
CK48 (F)	–	–	–	–	–	–	–	–	–	–	TFP/Q
CR43 (F)	–	–	–	–	–	–	–	–	–	–	TFP/Q
DR07 (F)	–	–	+	–	+	–	–	–	–	+	TFP/Q
EL27 (F)	–	+	–	–	–	–	–	–	–	–	TFP/Q

The animals were then challenged intrarectally at day 147 with the same high dose ($TCID_{50}$=4000) of the SIVsmE660 quasispecies used above and in the previous study (Schell et al., 2011; Schell et al., 2012). As shown in Fig. 2B, all eight animals became infected after the challenge. Average plasma viral loads for the previously published vaccine and control groups are compared in Fig. 2C. The Env-only (Fig. 2C, green line) vaccine regimen tested here suppressed peak viral loads about 10-fold, but the peak loads were about 10-fold higher than the infected animals from the previous Gag+Env vaccine group (Fig. 2C, red line). None of the animals in the Env-only vaccine group controlled their infection, and their viral set points were even slightly higher than control animals (Fig. 2C, blue line).

ELISA titers of gp140 specific antibodies

To determine if the animals receiving the Env-only vaccine generated levels of Env-specific antibodies comparable to animals from the previous study using Env and Gag antigens (Schell et al., 2011), we initially performed ELISA assays using sera collected at the day of challenge and 28 days post-challenge. At the time of challenge, the animals that received the Env-only vaccine (Fig. 3, left panel) generated comparable or higher titers of antibodies to gp140 to those that received the Gag+Env vaccine (Fig. 3, right panel). The average titers were significantly higher in the Env-only group ($p<0.04$). Consistent with the animals from the Env-only vaccine group becoming infected, all showed an anamnestic antibody response to gp140 28 days post-challenge. Only those animals that were not protected from infection in the previous Gag+Env vaccine study (DF38 and DG21, denoted by an asterisk in Fig. 3, right panel) showed an anamnestic antibody response.

The Env-only vaccine generated high titers of neutralizing antibodies

We next tested the ability of the sera from the Env-only vaccine animals to neutralize an SIV envelope from the viral swarm (E660.11), and transmitted/founder (T/F) envelopes isolated from infected animals from a previous study (EN82.57C and DF38.21.33C) (Schell et al., 2011). As shown in Fig. 4A, animals from the Env-only vaccine group had neutralizing antibodies (nAbs) against the tier 1 E660.11 envelope by the time of the first boost (day 56). Following the boost with the VLV-EnvG vector E660.11 at day 56, nAbs titers rose approximately 100-fold by day 119. The final VSV boost at day 119 did not increase the titers of nAb against E660.11.

When the Env-only animals were compared to the previous Gag+Env animals, similar neutralization titers against E660.11 were seen over the vaccination course. In Fig. 4D, the average neutralization of E660.11 envelope is shown for the Env-only (green line) and Gag+Env vaccinated animals. The Gag+Env group was divided into protected (orange line) and infected (red line) animals. By the time of the VSV boost (day 119), the Env-only animals had developed significantly higher titers of nAbs against E660.11 envelope than either the protected or infected animals of the Gag+Env group as determined by a two-tailed T test ($p<0.0006$ and $p<0.02$, respectively). This significant difference in nAb titers between the two groups was maintained through the day of challenge (day 147). Only those animals that were unprotected (green and red lines) showed an anamnestic nAb response after the challenge.