Local Control of Repeated-Dose Rectal Challenges in DNA/MVA-Vaccinated Macaques Protected against a First Series of Simian Immunodeficiency Virus Challenges

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Here, we report the results of a late boost and three additional series of simian immunodeficiency virus (SIV) challenges in seven DNA/modified vaccinia virus Ankara (MVA)-vaccinated rhesus macaques who resisted a first series of rectal challenges. During 29 additional challenges delivered over 2.3 years, all animals became infected. However, 13 blips of virus in six macaques and anamnestic Env-specific rectal IgA responses in three of the six suggested that local control of infections was occurring during the serial challenge.

In 2011, we reported on the prevention of infection by 12 repeated simian immunodeficiency virus (SIV) strain SIVE660 challenges in 5 rhesus macaques receiving a granulocyte-macrophage colony-stimulating factor (GM-CSF)-adjuvanted DNA/modified vaccinia virus Ankara (MVA) vaccine and in 2 animals receiving a nonadjuvanted vaccine (Fig. 1A). Animals had been vaccinated with two intramuscular inoculations of 3 mg of a SIV239 Gag-, Pol-, and Env-expressing DNA vaccine (D) or two inoculations of the same vaccine that coexpressed GM-CSF (Dg), followed by two intramuscular boosts with 1 × 10⁶ PFU of a SIV239 Gag-, Pol-, and Env-expressing MVA vaccine (M) in regimens designated DDMM or DgDgMM (1). The 1st series of SIVE660 challenges was undertaken 6 months after the initial series of immunizations. In the work presented here, we monitored the protected animals for 1 year for the presence of an occult infection, gave a late MVA boost, waited 6 months, and then subjected the animals to a 2nd series of 12 rectal SIV2660 challenges followed by a 3rd and 4th series of rectal SIV251 challenges (Fig. 1A). The goals of the study were to learn more about the effects of late boosts on vaccine responses and the longevity of vaccine-mediated protection.

Impressively, 6 of the 7 animals were protected from the 2nd series of SIVE660 challenges (Fig. 1B). The animal in the DgDgMM group with both the lowest and a falling avidity for Env-specific antibody (Ab) (Fig. 2A) was infected at the 10th challenge (Fig. 1C). The first series of SIV251 challenges was initiated with a dose of 120 50% tissue culture infective doses (TCID₅₀). When the 5 unvaccinated controls were slow to become infected (one infection in 5 challenges), the challenge dose was increased to 650 TCID₅₀. The higher dose infected the 4 uninfected controls within 4 challenges. All 6 of the vaccinated animals became infected, with the last animal becoming infected at challenge 12.

Figure 2 shows the patterns of immune responses through the initial series of immunizations, the first series of challenges, the 1-year observation period before the late MVA boost, the third late MVA boost, and 6 months past the late MVA boost.

Consistent with the macaques not becoming infected, animals that were protected during the 1st series of challenges showed no changes in the magnitudes of their SIV-specific serum IgG or rectal IgA responses during the 1st series of challenges or during the 1-year observation period prior to the late boost (Fig. 2B and C).

The magnitudes of Ab and T cell responses expanded and contracted with the MVA boosts, while the quality of the Ab, as measured by avidity, tended to increase with boosts (Fig. 2). Following the late MVA boost, the magnitudes of elicited Ab, CD4, and CD8 T cell responses expanded to peak values similar to those observed in the first series of immunizations (Fig. 2B to E). The only parameter to show a trend for a significant increase was the avidity of the Env-specific IgG response, which rose in 6 of the 7 rhesus macaques from indices in the mid-50s after the first series of immunizations to indices in the mid-60s after the late MVA boost (P = 0.06) (Fig. 2A). The contractions of the serum IgG, rectal IgA, and T cell responses were overall similar following the 1st series of immunizations and the late MVA boost. The one exception was the contraction of CD4 T cells, which was less acute after the late MVA boost than after the initial series of immunizations (P = 0.05) (Fig. 2D). Most of the contraction of responses occurred in the 1st 12 weeks postimmunization.

Throughout the 2nd series of E660 challenges and the two additional series of SIV251 challenges, transient blips of RNA (<1,000 copies, verified by independent repeat assays) were detected in the 6 serially challenged animals that entered the 3rd and 4th
A series of challenges. Thirteen of the 14 blips were not associated with an anamnestic Env-specific IgG response in serum and are considered not to have established systemic infections (Fig. 3A and B). Three of the animals with blips had significant expansions of Env-specific IgA but not IgG in rectal secretions (rhesus macaques RLe11, RWg11, and RFm11) (Fig. 3C). This would be consistent with a rectally contained infection stimulating a local IgA response without stimulating a local IgG response (2). For one animal, RKk11, the final blip of SIV251 RNA was associated with an anamnestic serum IgG response but there was a failure to establish viremia (Fig. 3). This animal was considered infected and represented the last animal to become infected.

The postinfection levels of viral RNA revealed some control of postinfection viremia. These studies considered week one of infection to be the 1st week in which viral RNA was detected in plasma (Fig. 4). The one animal infected by the 2nd series of SIVE660 challenges had 1,000-fold- and 100-fold-lower levels of viral RNA at peak and set point (24 weeks postinfection) than those of unvaccinated controls. The SIV251-infected animals had 30- to 300-fold-lower levels of peak viral RNA (P = 0.02, Mann-Whitney test) but no significant reduction at 24 weeks postinfection compared to the unvaccinated controls (Fig. 4C to F). By 24 weeks postinfection, RKk11, which had shown a blip associated with seroconversion in the absence of detectable viremia, had become viremic (Fig. 3A and B and 4D).

The serial challenges revealed the DNA/MVA vaccines plus a late MVA boost eliciting remarkably durable protective immunity for SIVE660 (Fig. 1). In addition, the vaccines markedly delayed the acquisition of SIV251 during the challenges. The poorer protection observed against the SIV251 challenges than against the SIVE660 challenges was anticipated because SIV251 is hard to neutralize, whereas SIVE660 is a virus swarm that includes viruses that are neutralization sensitive (3).

The data suggest that all six of the extensively challenged animals exhibited local control of SIVE660 infection. This is suggested by transient blips of virus that were associated in three of the six animals with anamnestic mucosal IgA but not IgG responses (Fig. 3). One of the desirable characteristics for a vaccine is the ability to establish a memory response that can undergo a protective anamnestic expansion in response to an infection. Because of the ability of HIV to rapidly establish latent reservoirs and undergo immune escape, it has been proposed that an HIV vaccine will need to be a “sterilizing” vaccine, or a vaccine that prevents infection in the absence of an anamnestic response. To achieve sterilizing immunity, a vaccine needs to elicit persisting

**FIG 1 Temporal immunizations, challenges, and infection outcomes.** (A) Immunization and challenge schematic. The schematic presents the timing of vaccinations and challenges and the doses of the serial rectal challenges. Age-matched controls were added to the study at the time of each serial challenge. The SIVE660 challenge was the SIVsmE660–Hirsch200 stock, grown in pigtail macaque PBMC, provided by Vanessa Hirsch. The SIV251 stock was the Desrosiers-2006 stock, day 9 harvest, grown in rhesus macaque PBMC, provided by DAIDS, NIH. (B to D) Kaplan-Meier curves for percentages of animals uninfected by the indicated serial challenges. The numbers of animals in each group are indicated in the keys. Ch., challenge.
FIG 2 Effect of a late boost on temporal immune responses in vaccinated and challenged animals. See Fig. 1 and the text for details on the vaccination and challenge regimen. (A) Avidity of the elicited Env-specific Ab for the SIV239 Env. Env gp160 was concanavalin A-captured from Triton X-100-disrupted virions produced by transient transfection and used for ELISAs as described previously (1). (B) Titer of binding Ab for SIV239 gp140 (Immune Technology Corp) estimated against a standard curve of rhesus IgG (1). (C) Specific activities (S.A.) for Env-specific IgA (ng of Env-specific IgA per μg of total IgA) in rectal secretions for SIV251 gp130 (Immune Diagnostics, Woburn, MA) (1). (D and E) Magnitudes of SIV-specific CD4 and CD8 T cells responding to Gag and Env determined using intracellular cytokine staining for gamma interferon (IFN-γ) following stimulation with pools of SIV239 peptides (15-mers overlapping by 11) (12). Vaccination groups are indicated above line graphs, and the time in trial above box plots. DgDgMMM and DDMMM indicate two DNA and three MVA immunizations. In line graphs, error bars show standard deviations. Box plots (shown are medians, 25th and 75th percentiles, and minimum-maximum whiskers) are in fuchsia for the DgDgMM and turquoise for the DDMMM regimens. Statistics for box plots use a paired t test and are two sided. Pre-Ch, 2 to 3 weeks prechallenge.
levels of Ab that are sufficient to block an incoming infection. Here, in a series of repeated challenges over a period of 3 1/2 years, we report findings that suggest that a simian DNA/MVA vaccine may not require sterilizing immunity to provide protection but, rather, can control rectal challenges by local responses, some of which can be scored as anamnestic IgA responses in rectal secretions.

The occurrence of low-level blips of virus during serial challenge has also been reported for intravaginal exposures following vaccination with a gp41 virosome vaccine (4). This vaccine elicited Ab in cervical secretions that inhibited transcytosis and showed antibody-dependent cellular cytotoxicity (4). The ability of host immune responses to control local infections has also been suggested for exposed uninfected commercial sex workers and discordant couples (5–7). Analyses for immune responses in these cohorts have revealed adaptive mechanisms for protection in some individuals (8–11). In the case of vaccinated people, adaptive responses elicited by the vaccine, both in the presence and absence of anamnestic expansion, could contribute to local control of infection. We suggest that this is happening in macaques for...

FIG 3 The data consistent with the control of local infections during serial challenges are presented. (A) Temporal levels of viral RNA in plasma during the 2nd through 4th series of challenges (challenges 13–24, 25–31, and 32–43). Levels of viral RNA were tested using quantitative PCR with sensitivities of 40 and 80 copies per ml for SIVE660 and SIV251, respectively. All blips were confirmed in independent assays. (B) Results of tests for postinfection anamnestic serum IgG responses during serial challenges (see Fig. 2). (C) Numbers of blips in different animals during the serial challenges, and specific activities (S.A.) of rectal IgA and IgG at the indicated times. The cutoff value for the specific activity of IgA was 0.145. Fold increases in the specific activities were calculated for week 165 relative to week 137. A 3-fold increase in specific activity is considered significant. Mucosal Ab responses were measured against SIV251 gp130 as previously described (1, 12).

<table>
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<th>Macaque</th>
<th>Vaccine</th>
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<th>Blip with seroconversion</th>
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<th>Rectal IgG, S.A.</th>
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<td></td>
<td></td>
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<td>Wk 151, Pre Ch-25</td>
<td>Wk 165, Pre Ch-32</td>
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both GM-CSF-adjuvanted and non-GM-CSF-adjuvanted DNA/MVA SIV vaccines.

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