Protection of Rhesus Macaques Against High-dose SIVsmE660 Re-challenge Following CD8 T-cell Depletion and Boosting with Env Only

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Summary

We reported previously on a vaccine approach conferring apparent sterilizing immunity to high-dose mucosal challenge with the SIVsmE660 quasispecies (Schell et al., J. Virol. 85: 5764-5772). Four of six macaques were protected by vaccination with a prime-boost regimen using vectors based on recombinant vesicular stomatitis virus (VSV) and propagating Semliki Forest virus/VSVG replicons (SFVG) expressing SIV Gag and Env proteins. This vaccine regimen generated high-level serum neutralizing antibody (NAb) responses (in some cases > $1:10^6$) to tier 1 SIVsmE660 Envs, but did not induce detectable NAb to the SIV challenge stock. The lack of anamnestic antibody or T cell responses following challenge suggested sterilizing protection of the four animals. Protection from infection was also confirmed by CD8 T cell depletion at nine months post-challenge. Because the only correlate of protection we have been able to detect so far is a low level NAb to a tier 2 SIVsmE660 Env, we boosted the four CD8-depleted animals with a VSV vector expressing only SIV Env prior to re-challenge. Waning NAb titers to tier 1 SIVsmE660 Envs were increased approximately ten-fold following the boost. Some animals lacked detectable T-cell responses to Env or Gag at the time of re-challenge. The boosted animals were then given a high-dose (4000 $TCID_{50}$) mucosal challenge with the SIVsmE660. All four protected animals from the previous study and one from another study remained completely protected from the re-challenge. Our studies suggest that immune responses to SIV Env, most likely antibody, are responsible for the protection of these animals.

Background

Two groups of six rhesus macaques each were primed with VSV vectors encoding SIVsmE660 Gag and Env proteins (Vaccine) or irrelevant influenza HA (control) genes. Animals were boosted twice with SFV-G and serotype switch VSV vectors expressing the same Gag and Env proteins. All groups were then challenged with an intrarectal high-dose of SIVsmE660 ($TCID_{50}$=4000).

Figure 1. Previous Vaccine Protection from SIV Quasispecies Challenge

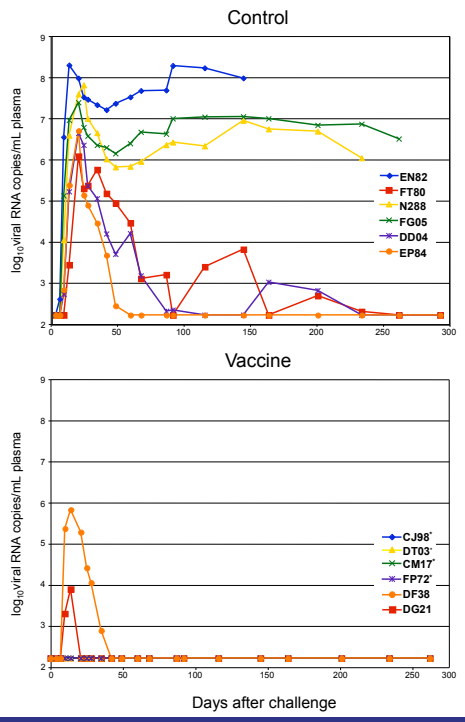

Figure 1. Viral load data (Schell et al., J. Virol. 85: 5764-5772) are shown. Four out of six macaques were completely protected from infection by high-dose rectal challenge with SIVsmE660. All control animals became infected and three developed high viral load and AIDS.

Figure 2. Correlation of NAbs with Protection in Vaccinated Animals

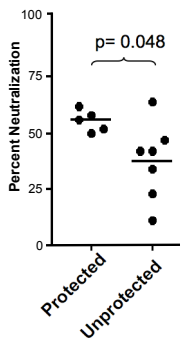

Figure 2. Comparison of NAbs between protected animals and unprotected animals from two combined vaccine studies. The animals were from the published study (Schell et al., J. Virol. 85: 5764-5772) and from a second study in which protection was largely prevented through expression of GM-CSF during the priming immunization (Schell et al., submitted). The final dilution of serum was 1:10. A tier 2 SIVsmE660 (CR54-PK-2A5) was used in the TZM-bl cell assay. The average percent neutralization is represented by the black horizontal bar for each group. Percent neutralization was calculated as the reduction in RLU by post-immunization serum relative to the corresponding pre-immunization serum sample for each animal. A significant difference in neutralization titers was seen between protected versus unprotected animals using the Mann-Whitney test (B, p=0.048).

Figure 3. CD8 Depletion Confirms Absence of Infection in Protected Vaccine Group Animals

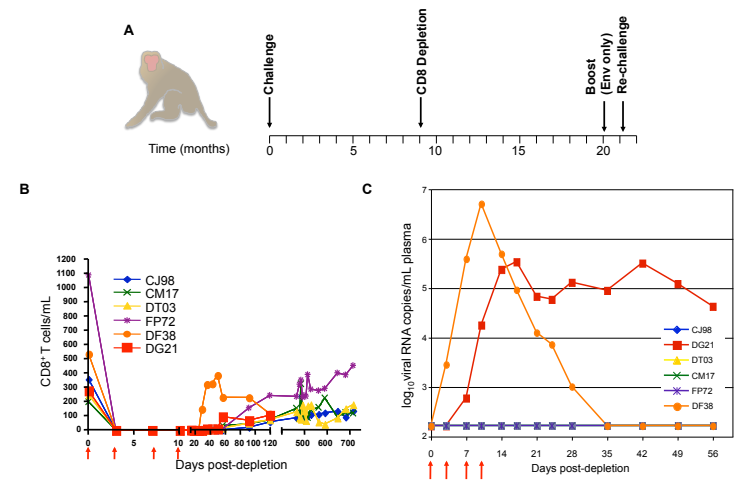

Figure 3. A) Timeline showing SIVsmE660 challenge (day 0), CD8 depletion, VSV(Ch)EnvG boost, and re-challenge. **B)** CD8+ T cell levels in animals treated with four doses of anti-rhesus CD8 antibody are shown at times after depletion. The CD8 cell depletion was complete in all animals by day 3 following the initial treatment. **C)** Viral RNA levels at times after depletion. Viral loads returned only in the two animals that showed initial viral loads. Red arrows indicate days when anti-CD8 antibody was administered.

Figure 4. Lack of Infection Following High-dose Re-challenge

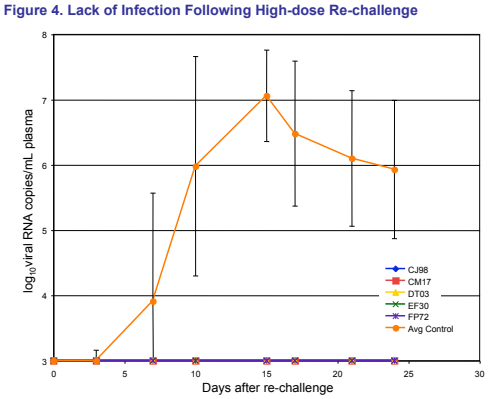

Figure 4. Five protected animals were re-challenged with a second intrarectal high-dose ($TCID_{50}$=4000) of SIVsmE660. No viral RNA was detected in any of the animals. Average loads of control animals challenged with the same stock are presented for comparison.

Figure 5. Env-only Boost Increased Nab Titers to the Vaccine Env Approximately 10-fold

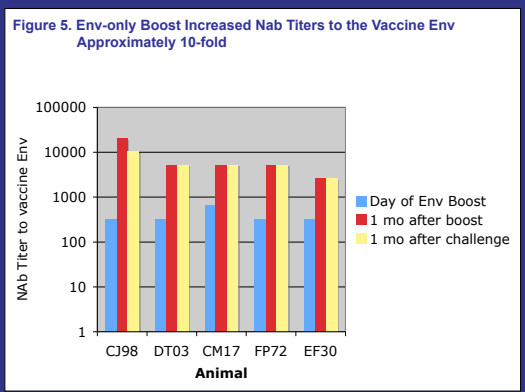

Figure 5. NAb titers were measured against a VSVΔG-EGFP-E660EnvG virus that expresses the same EnvG protein used in the vaccine (Schell et al., J. Virol. 85: 5764-5772). Titers were measured on the day of boost, one month after boost (day of re-challenge) and one month after challenge as indicated. Note that after challenge, there was no anamnestic increase in the NAb titer, consistent with lack of infection.

Figure 6. Two CD8-depleted Animals Lacked Significant T Cell Responses to Env or Gag at the Time of Re-challenge

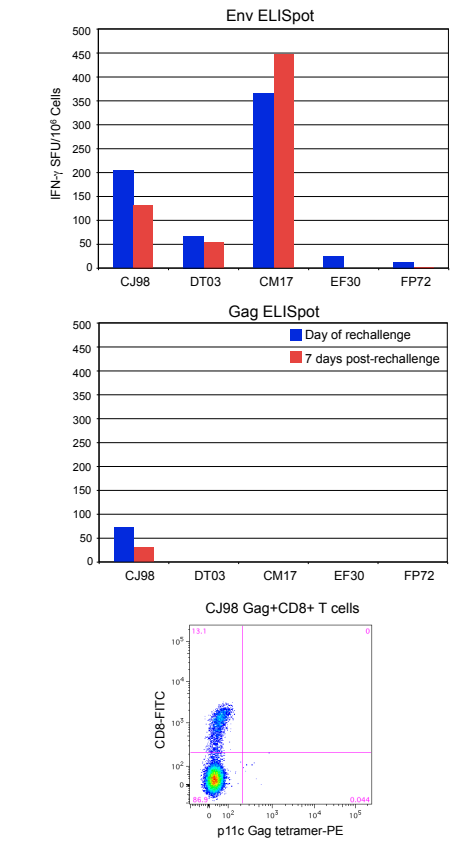

Figure 6. The bar graphs show IFN-γ production by PBMCs in response to Env and Gag antigens on the day of challenge and seven days following the challenge. Note that two of the animals (EF30 and and FP72) did not make significant responses to Env or Gag on the day of challenge. There was no anamnestic increase in the responses following challenge, consistent with lack of infection in all animals. Only one animal, CJ98, responded weakly to Gag antigen after depletion. Even though CJ98 is MamuA*01+, we failed to detect Gag-specific (p11c tetramer+) T cells by FACS at any time after depletion. The day of re-challenge is shown in the FACS plot.

Conclusions

VSV-based vectors combined with SFVG propagating replicons expressing SIV Env and Gag proteins can provide apparently sterilizing protection against high-dose mucosal challenge with an SIVsmE660 quasispecies. Protection correlated with the presence of low levels of serum antibody to a tier 2 SIVsmE660 Env, but not with CD8 T-cell responses to SIV Env or Gag. CD8 T-cell depletion, "Env only" boosting and high dose re-challenge, supported a role for antibody to Env in the complete protection of five macaques following re-challenge. Although our experiments do not rule out a role for mucosal T-cells in protection, we think it is most likely that rectal mucosal antibodies to Env are playing a major role. Experiments are underway to determine if vaccine vectors expressing "Env" only can provide protection against high dose mucosal challenge with SIV, and to determine the immune correlates of mucosal protection.

References & Acknowledgements

Schell, J. B., N. F. Rose, K. Bahl, K. Diller, L. Buonocore, M. Hunter, P. A. Marx, R. Gambhira, H. Tang, D. C. Montefiori, W. E. Johnson, and J. K. Rose. 2011. Significant protection against high-dose simian immunodeficiency virus challenge conferred by a new prime-boost vaccine regimen. J Virol **85:**5764-72.

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