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Preserved CD4⁺ Central Memory T Cells and Survival in Vaccinated SIV-Challenged Monkeys

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Abstract

Vaccine-induced cellular immunity controls virus replication in simian immunodeficiency virus (SIV)-infected monkeys only transiently, leading to the question of whether such vaccines for AIDS will be effective. We immunized monkeys with plasmid DNA and replication-defective adenoviral vectors encoding SIV proteins and then challenged them with pathogenic SIV. Although these monkeys demonstrated a reduction in viremia restricted to the early phase of SIV infection, they showed a prolonged survival. This survival was associated with preserved central memory CD4⁺ T lymphocytes and could be predicted by the magnitude of the vaccine-induced cellular immune response. These immune correlates of vaccine efficacy should guide the evaluation of AIDS vaccines in humans.

The protection afforded by vaccines can be mediated by humoral or cellular immune responses. Because a strategy to elicit broadly neutralizing antibodies to HIV-1 has not yet been developed, HIV-1 vaccine candidates now entering advanced phase clinical testing are designed to induce potent cellular immunity (1). It is hoped that such vaccines will generate populations of virus-specific T lymphocytes that rapidly expand following infection. By this means, viral spread might be contained, leading to reduced chronic viral replication and prolonged disease-free survival. The expectation that such vaccines will confer clinical benefit comes from the association of lower set-point plasma viremia with prolonged survival in HIV-1-infected humans (2). In some nonhuman primate models, particularly those using the chimeric simian-human immunodeficiency viruses (SHIVs), vaccines that elicit potent cellular immune responses have resulted in reduced viremia and preserved CD4⁺ T lymphocytes after viral challenge (3–5). However, the biology of these SHIVs differs from the usual transmitted primary HIV-1 isolates (6). It is possible that the pathogenic simian immunodeficiency (SIV) model may provide a more predictive and rigorous test of vaccine efficacy. In some SIV challenge models, vaccine-induced reductions in viremia and progression to AIDS have been shown (7–9), although in the case of the commonly used SIVmac239/251 strain, only transient reductions in viremia were observed. Because chronic set-point viremia was presumed to

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predict long-term survival (10), these SIV challenge studies were terminated before the deaths of the experimental animals, and it was suggested that T cell-based vaccines may not confer clinical benefit to humans (11,12).

We evaluated the clinical benefit afforded by an SIV-specific cellular immune response elicited in rhesus monkeys through a plasmid DNA prime/recombinant E1-deleted, E3-inactivated adenovirus serotype 5 (rAd) boost vaccination regimen (13–16). The monkeys selected for study did not express the MHC class I allele *Mamu-A*01* because of its association with particularly efficient control of SIV/SHIV replication (17,18). Six monkeys in each of five experimental groups received different vaccination regimens (Fig. 1) (16). The monkeys were monitored prospectively using pooled peptide Elispot and intracellular cytokine staining assays as measures of cell-mediated immunity, and for neutralizing antibody responses (13–15). Monkeys were then challenged by intravenous route with SIVmac251 and monitored for viral replication and changes in CD4⁺ T lymphocyte subsets (16,19).

The vaccination regimens elicited high-frequency SIV-specific cellular immune responses, with greater responses detected in the monkeys that received the DNA prime/rAd boost than those that received only rAd immunizations (Fig. 1). After virus challenge, there were no statistically significant differences in any parameters of clinical outcome between the various groups of experimentally vaccinated monkeys. Therefore, all 24 experimentally vaccinated monkeys were subsequently treated as a single group and compared to the 6 sham-vaccinated control monkeys.

Plasma SIV RNA levels were significantly lower in the experimentally vaccinated monkeys compared with the controls at the peak of viral replication, with this difference persisting through day 112 after virus challenge (Fig. 2, A and B). Thereafter, no significant difference was observed in plasma viral RNA levels between the experimentally vaccinated and control monkeys. Although the prediction would be that comparable set-point viral load measurements in the vaccinated and control monkeys would be associated with comparable survival, we elected to follow the monkeys for another 750 days. In fact, as late as day 850 after virus challenge, the vaccinated monkeys maintained a statistically significant survival advantage over the control monkeys (Fig. 3A) (16). Therefore, the set-point plasma viral RNA levels did not predict the relative duration of survival in these vaccinated monkeys.

We next evaluated whether other measurements of viral replication or immune function might serve as predictors of survival. A quantitative measure of viral replication during the first 126 days after infection was determined for each animal by integrating the plasma SIV viral RNA levels between days 3 and 126. The vaccinated monkeys had significantly lower values than the controls (Fig. 3B), which suggests that differences in total viral replication during this early phase of infection are associated with differences in long-term survival.

There is increasing evidence that the loss of memory CD4⁺ T lymphocytes during acute infection is important in AIDS pathogenesis (20,21). To examine this phenomenon in the present study, peripheral blood memory CD4⁺ T lymphocytes were assessed at a single time point, and their association with clinical outcome in the cohort of SIV-infected monkeys was evaluated. We first assessed these values on day 126 after challenge (Fig. 4), a time point at which plasma virus RNA levels were comparable in the vaccinated and control monkeys (Fig. 4A). Although the proportions of naïve and effector memory CD4⁺ T lymphocyte populations were comparable in the peripheral blood of the vaccinated and control monkeys, central memory CD4⁺ T lymphocyte populations were larger in the vaccinated group (Fig. 4, B to D, and fig. S1). Similar immunologic data were obtained on day 112 after challenge (fig. S2). Moreover, when animals were divided into terciles based on magnitudes of absolute peripheral blood CD4⁺ T lymphocyte counts on day 126 after challenge, no differences in survival were

seen (Fig. 4E). Yet, when a similar analysis was done dividing monkeys into terciles based on central memory CD4⁺ T lymphocyte counts, a clear survival advantage was apparent for the monkeys with the highest counts (Fig. 4F). Similar results were seen in an evaluation of data generated on day 112 after challenge (fig. S3). These data sets also revealed an association between the survival of infected animals and the preservation of central memory CD4⁺ T lymphocytes after infection in a Cox proportional-hazards model ($P = 0.05$, likelihood ratio test). No association was observed in these monkeys between central memory CD8⁺ T lymphocyte counts and survival (fig. S4). Thus, although set-point plasma viral RNA levels were indistinguishable between the vaccinated and control monkeys, a single determination of the peripheral blood central memory CD4⁺ T lymphocyte count was associated with increased survival in these animals.

We then assessed the ramifications of this preservation of total central memory CD4⁺ T lymphocytes in the vaccinated, challenged monkeys on the SIV-specific immune response. On day 203 after challenge, the total central memory CD4⁺ T lymphocytes remained better preserved in the vaccinated than in the control monkeys, and the SIV Gag-specific CD4⁺ and CD8⁺ T lymphocyte IFN- γ , TNF- α , and IL-2 responses were greater in the vaccinated than in the control monkeys (figs. S5 and S6). Interestingly, however, no difference was observed in the magnitude of the SIV neutralizing antibody responses in the vaccinated and control monkeys as late as 126 days after challenge (fig. S7). Therefore, the preservation of total central memory CD4⁺ T lymphocytes in the vaccinated monkeys was associated with preserved virus-specific T lymphocyte responses.

Finally, we evaluated the contribution of vaccine-elicited immunity to survival in this cohort of monkeys. Dividing the 16 monkeys that received Gag immunogens into halves based on the magnitude of peak vaccine-elicited SIV Gag-specific total T lymphocyte responses measured by IFN- γ Elispot assay (Fig. 4G) and IFN- γ CD4⁺ T lymphocyte responses measured by ICS assay (Fig. 4H), a survival advantage was apparent for the monkeys with the highest frequency vaccine-elicited cellular immune responses. The Elispot data also revealed an association between vaccine-elicited cellular immune responses and survival in a Cox proportional-hazards model ($P = 0.015$, likelihood ratio test). Therefore, the magnitude of the immune responses generated by the vaccine was associated with survival after virus infection.

These findings suggest that vaccine protection against high levels of viral replication during only the first weeks following an AIDS virus infection may provide sufficient protection against central memory CD4⁺ T cell loss to confer a survival advantage to infected individuals. Moreover, current models of large human HIV-1 vaccine efficacy trials propose the use of set-point viral load and total CD4⁺ T lymphocyte count as surrogate markers for a beneficial vaccine effect (22,23). It has been presumed that a lower set-point viral load or a higher set-point CD4⁺ T lymphocyte count after infection will portend a better AIDS-free survival. The results of the present study indicate that set-point viral load and total CD4⁺ T lymphocyte count may not have predictive value in this setting. Rather, the quantitation of central memory CD4⁺ T cells in a vaccine trial several months after infection may be an important immune correlate of long-term protection and predict the efficacy of an HIV-1 vaccine.

Most important, this cohort of vaccinated monkeys followed for 850 days after challenge with the highly pathogenic SIVmac251 provides a distinctive data set for exploring the mechanisms underlying the vaccine-associated survival. The demonstration of an association between the magnitude of the vaccine-elicited immune responses and the duration of survival after challenge provides a framework for understanding the immune protection conferred by cellular immune-based vaccines. Moreover, the prolonged survival conferred by a vaccine that stimulates T cell immunity provides support for pursuing clinical efficacy trials of such HIV-1 vaccines, even if they do not induce sterilizing immunity.

References and Notes

1. Letvin NL. *Annu. Rev. Med* 2005;56:213. [PubMed: 15660510]
2. Mellors JW, et al. *Science* 1996;272:1167. [PubMed: 8638160]
3. Barouch DH, et al. *Science* 2000;290:486. [PubMed: 11039923]
4. Amara RR, et al. *Science* 2001;292:69. [PubMed: 11393868]
5. Shiver JW, et al. *Nature* 2002;415:331. [PubMed: 11797011]
6. Nishimura Y, et al. *Proc. Natl. Acad. Sci. U.S.A* 2004;101:12324. [PubMed: 15297611]
7. Ourmanov I, et al. *J. Virol* 2000;74:2740. [PubMed: 10684290]
8. Polacino P, et al. *J. Virol* 1999;73:618. [PubMed: 9847367]
9. Polacino PS, et al. *J. Virol* 1999;73:8201. [PubMed: 10482571]
10. Lifson JD, et al. *J. Virol* 1997;71:9508. [PubMed: 9371613]
11. Casimiro DR, et al. *J. Virol* 2005;79:15547. [PubMed: 16306625]
12. Vogel TU, et al. *J. Virol* 2003;77:13348. [PubMed: 14645590]
13. Letvin NL, et al. *J. Virol* 2004;78:7490. [PubMed: 15220422]
14. Santra S, et al. *J. Virol* 2005;79:6516. [PubMed: 15858035]
15. Seaman MS, et al. *J. Virol* 2005;79:2956. [PubMed: 15709015]
16. Materials and methods are available as supporting material on *Science* Online
17. Pal R, et al. *J. Virol* 2002;76:292. [PubMed: 11739694]
18. Seaman MS, et al. *J. Virol* 2005;79:4580. [PubMed: 15795244]
19. Sun Y, et al. *J. Immunol* 2005;174:4753. [PubMed: 15814700]
20. Mattapallil JJ, et al. *Nature* 2005;434:1093. [PubMed: 15793563]
21. Veazey RS, et al. *J. Virol* 2000;74:57. [PubMed: 10590091]
22. Gilbert PB, et al. *J. Infect. Dis* 2005;192:974. [PubMed: 16107949]
23. Hulgán T, et al. *J. Infect. Dis* 2005;192:950. [PubMed: 16107946]
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Fig. 1.

Peak cellular immune responses elicited by vaccination. Groups of monkeys were immunized at weeks 0, 4, and 8 with plasmid DNA expressing the indicated gene products and at week 40 with recombinant adenoviruses carrying the same genes. Two weeks later, cellular immune responses were assessed in peripheral blood mononuclear cells (PBMC) by IFN- γ Elispot assay. Data are expressed as spot-forming cells (SFC) per 10⁶ PBMC.

Fig. 2.

Plasma viral RNA levels after SIVmac251 challenge. The monkeys were challenged by intravenous route with pathogenic SIVmac251, and plasma viral RNA levels were assessed prospectively. (A) Plasma viral RNA data are shown for each individual monkey, with the data from the control monkeys shown in red. (B) Data were assessed at each time point by the Wilcoxon rank sum test to determine whether vaccinated monkeys differed from the group of control monkeys in their plasma viral RNA levels. The *P* value for that comparison is shown for each data set, and the horizontal line indicates a *P* value of approximately 0.05.

Fig. 3.

Differences between control and experimentally vaccinated monkeys in survival and plasma viral RNA levels after challenge. **(A)** Kaplan-Meier survival curves for the control and experimentally vaccinated monkeys after SIVmac251 challenge. The comparison of survival in these groups of monkeys has been done using a log-rank test (16). **(B)** Comparison of area under the curve calculations for the plasma viral RNA levels in the control and experimentally vaccinated monkeys between days 3 and 126 after challenge. The comparison was analyzed using the Wilcoxon rank sum test.

Fig. 4.

Survival is associated with the preservation of peripheral blood central memory CD4⁺ T lymphocytes and vaccine-elicited cellular immune responses. (A) Plasma viral RNA levels and (B) percent of naïve (CD28⁺CD95⁻) CD4⁺ T cells, (C) effector memory (CD28⁻CD95⁺) CD4⁺ T cells, and (D) central memory (CD28⁺CD95⁺) CD4⁺ T cells were compared in the experimentally vaccinated and control monkeys on day 126 after challenge using the Wilcoxon rank sum test (16) (fig. S1). For panels (B) to (D), the percentage values are based on a denominator of total CD4⁺ T cells. Kaplan-Meier survival curves for all monkeys after challenge divided into thirds based on (E) absolute number of peripheral blood CD4⁺ T lymphocytes or (F) central memory CD4⁺ T lymphocytes. The 16 monkeys that received a Gag immunogen were divided into halves based on magnitude of vaccine-elicited peak (G) total Gag-specific IFN- γ Elispot responses or (H) CD4⁺ T lymphocyte Gag-stimulated intracellular IFN- γ expression. The comparisons of survival in these groups of monkeys were done using a log-rank test.