Vaccination preserves CD4 memory T cells during acute simian immunodeficiency virus challenge

Joseph J. Mattapallil,[1] Daniel C. Douek,[1] Alicia Buckler-White,[2]
David Montefiori,[3] Norman L. Letvin,[4] Gary J. Nabel,[1]
and Mario Roederer[1]

[1]Vaccine Research Center and [2]Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Disease
(NIAID), National Institutes of Health (NIH), Bethesda, MD 20892
[3]Duke University Medical Center, Durham, NC 27710
[4]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215

Acute simian immunodeficiency virus (SIV)/human immunodeficiency virus infection is accompanied by a massive destruction of CD4 memory T cells across all the tissue compartments. These early events set the course toward disease progression and immuno-deficiency. Here, we demonstrate that prior vaccination reduces this destruction during acute SIV Mac$_{251}$ infection, leading to better survival and long-term outcome. Systemic vaccination with a DNA-prime recombinant adenovirus boost regimen preserved memory CD4 T cells throughout the body. The vaccine regimen induced broad CD4 and CD8 T cell responses in all tissues examined and, importantly, induced antibodies that neutralized the primary isolate of SIV used for challenge. Finally, we demonstrate that the extent of pres-ervation of the CD4 memory compartment during the acute phase provides a strong predic-tor for subsequent progression to death. Our data provide a mechanism to explain clinical observations that acute-phase viral loads predict long-term disease progression and under-score the need for interventions that protect against early destruction of CD4 memory T cells during acute infection.

CORRESPONDENCE
M. Roederer:
roederer@nih.gov

Abbreviations used: qPCR, quantitative PCR; rAd, recom-binant adenovirus; SIV, simian immunodeficiency virus; TCLA, T cell line–adapted; VL, viral load.

Recent studies (1–4) documented the highly destructive nature of acute HIV infection: a massive loss of memory CD4 T cells through-out the body, particularly at mucosal surfaces, precedes emerging antiviral responses. This assault on preexisting memory CD4 T cells was hypothesized to disable the ability of the immune system to generate secondary im-mune responses, setting the stage for immuno-deficiency. Therefore, an important goal of an HIV vaccine is to prevent or contain the early destruction of CD4 T cells, thereby pre-serving and maintaining the integrity of the immune system.

Numerous vaccine regimens have been tested in animal models that suggest partial protection based on measures of plasma viral loads (VLs) and total blood CD4 T cell counts (5–10). However, these surrogates overlook the underlying destruction of the memory CD4 T cells that occurs in all tissues. Thus, in contrast

to the commonly described partial and transient loss of total CD4 T cells during acute infection, a substantial fraction (50–80%) of memory T cells across both mucosal and peripheral tis-sues are infected and destroyed within the first 2 wk of infection (1). This destruction may be a central mechanism accounting for subsequent immune failure.

As memory CD4 T cells are critical for maintaining immune competence while serv-ing as the primary target of HIV infection, the evaluation of a potential vaccine's efficacy must include its ability to prevent infection and destruction of these cells. In addition, newly emerging HIV-specific CD4 T cells may also serve as preferential targets for infection (11, 12). Destruction of these cells could impair the generation and maintenance of antiviral CD8 T cell responses. Given the tissue-specific dif-ferences in composition of CD4 T cells, it is important that such evaluation be made across multiple tissues, such as the mucosa and LNs. Notably, the high and variable contribution of

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Table I. Tissue sampling schedule

Sham animal	Vaccine animal	Blood	Jejunum	Inguinal LN	Mesenteric LN
4939 ○	4750 ●	P, 0, 7	P, 0, 7	P, 0, 7	P, 7
4975 ○	4736 ●	P, 0, 7, 10	P, 7, 10	P, 7, 10	P, 10
4875 ○	4942 ●	P, 0, 7, 10, 14	P, 10, 14	P, 10, 14	P, 14
4931 ○	4974 ●	P, 0, 7, 10, 14, 17	P, 14, 17	P, 14, 17	P, 17
5510 ○	4844 ●	P, 0, 7–14, 17, 21	P, 17, 21	P, 17, 21	P, 21
4755 ○	4934 ●	P, 0, 7–14, 17, 21, 28	P, 21, 28	P, 21, 28	P, 28

Listed are the days after infection (P = 1 mo before infection) at which samples were collected from each animal in the group. For four animals, 1 mL blood was collected daily between days 7 and 14 after infection. All other collections were 5 mL blood. The last collection date was a necropsy. All animals sustained only a single survival surgery.

naive CD4 T cells, which are uninvolved in acute infection, significantly skews the interpretation of HIV (and simian immunodeficiency virus [SIV])–associated changes during acute disease by masking the extent of the ongoing destruction.

We demonstrated previously that acute SIV infection is accompanied by a massive infection and destruction of memory CD4 T cells (1). Here, we sought to determine whether prior vaccination could preserve memory CD4 T cells and whether such preservation is associated with better long-term outcome and survival. We evaluated the effects of a DNA-prime recombinant adenovirus (rAd) boost vaccine regimen on cell-associated VL and memory CD4 T cell dynamics in peripheral and mucosal tissues. We show that prior vaccination induced plasma-neutralizing antibody as well as T cell immune responses in various tissues that significantly reduced the level of infection and destruction of the memory CD4 T cells after intravenous challenge. Importantly, although vaccine–induced SIV–specific CD4 T cells were preferentially infected, this was not associated with loss of protection. In fact, the vaccine–induced protection of memory CD4 T cells was associated with a better long-term outcome and survival in infected animals.

RESULTS
Vaccination tempers viral dynamics

12 rhesus macaques were vaccinated with a regimen consisting of a DNA prime followed by rAd boost. Six animals received a sham vaccine, and the other six received plasmids and rAd separately encoding SIVMac239 envelope and an SIVMac239 gag-pol fusion protein (13). Three shots of DNA were given at 1-mo intervals, and one shot of rAd was given 3 mo after the last DNA immunization. Animals were challenged with SIV Mac$_{251}$ 2 mo after the rAd immunization. Animals were killed at different time points after challenge. Biopsies and necropsies provided serial tissue specimens (Table I).

As published previously (5–10), vaccination significantly suppressed peak plasma VLs in most animals by ∼1 log, with an apparently faster resolution compared with sham (Fig. 1 a). Because plasma VL reflects actively replicating virus, these results suggest that vaccination-induced immune responses reduced the number of cells that were infected and/or more rapidly destroyed the infected CD4 T cells. Notably, the protection was variable, with one animal (no. 4934; closed green circles) exhibiting peak plasma VLs at levels comparable to

unvaccinated animals. Nonetheless, some protection was evident in this animal, as it appeared to resolve plasma VLs faster and to a lower level than the sham-vaccinated animals.

In sham-vaccinated animals, as shown previously (1), the cell-associated VL reveals a massive infection (and subsequent destruction) of memory CD4 T cells in blood and jejunum (Fig. 1 b). We showed previously (1) that longitudinal analysis of cell-associated VL dynamics (SIV-gag copies/memory CD4 T cell) is a predictor of the number of cells infected and destroyed during acute infection. Notably, studies in humans demonstrated that levels of HIV DNA in PBMCs soon after infection were predictive of disease progression independently of HIV-1 RNA levels and CD4 T cell counts (14). In the animals, vaccination significantly reduced the peak infection of memory CD4 T cells by ∼75% (Fig. 1 b) and significantly tempered the destruction occurring during acute infection.

Vaccination preserves memory CD4 T cells in multiple tissues

The tempered rate of infection and loss of CD4 memory T cells in vaccinated animals suggested that the dynamics of memory T cell loss measured phenotypically would be dramatically different compared with sham-vaccinated animals. Naive and memory T cells were identified on the basis of expression of CD45RA and CD95 as shown in Fig. S1 (available at http://www.jem.org/cgi/content/full/jem.20060657/DC1); naive T cells are CD95$^-$CD45RA$^+$ and memory T cells are all other cells. Fig. 2 a illustrates that the ratio of naive to memory cells in all three lymphoid tissues (there are essentially no naive cells in the jejunum) increases in sham-vaccinated animals due to the destruction of memory cells. In contrast, the minor decrease in the naive/memory ratio observed in vaccinated animals is consistent with the preservation of CD4 memory T cells (after the very early expansion of CD4 memory cells consistently observed after challenge [1]).

By normalizing the representation of CD4 memory T cells within each animal to its prechallenge time point, the preservation afforded by vaccination is seen in all tissues (Fig. 2 b). In comparison to sham-treated animals, vaccinated animals showed a significantly lower loss of CD4 memory cells. The preservation was greatest in the peripheral lymphoid tissues. In jejunal (mucosa) tissues, ∼25% of memory CD4 T cells were preserved. Nonetheless, preservation of this fraction of memory CD4 T cells in the mucosa could have
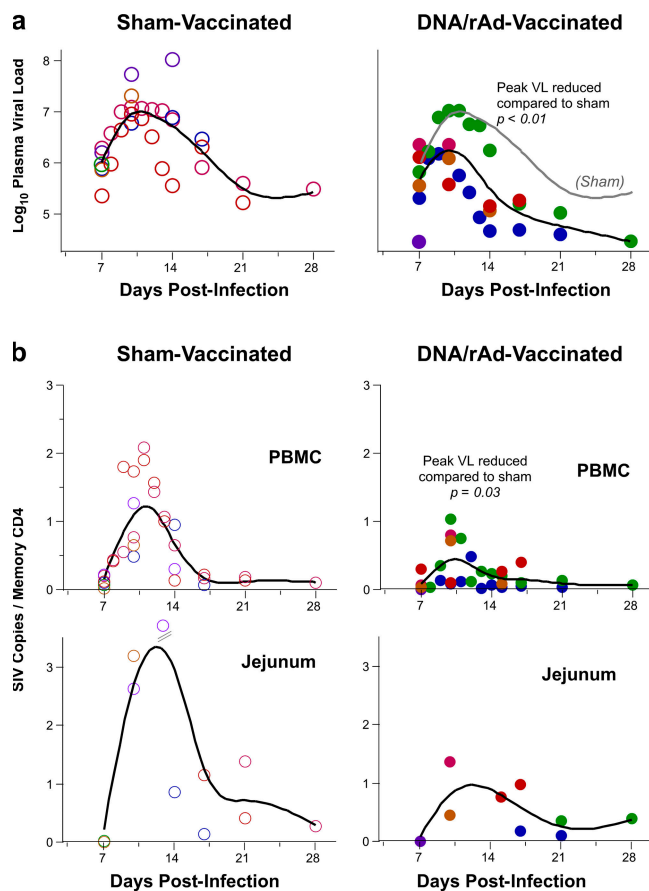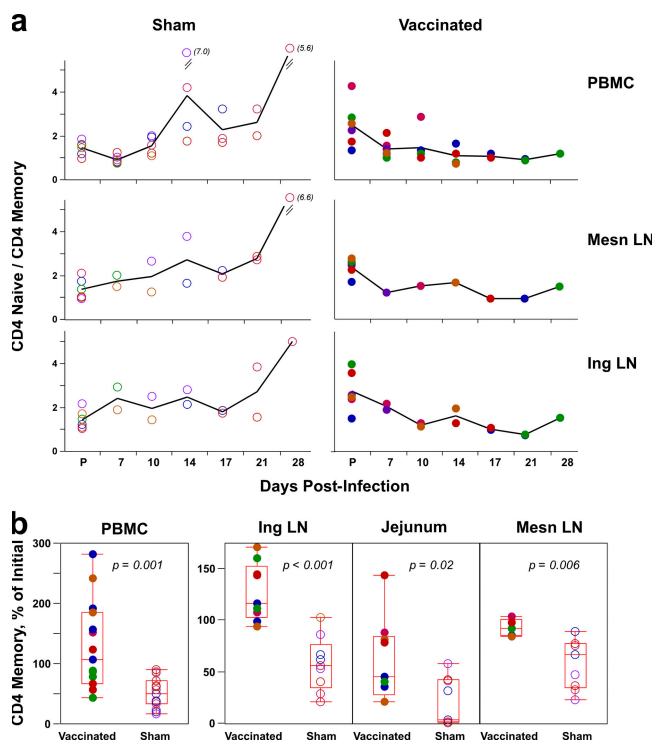
Figure 1. Viral dynamics in vaccinated macaques challenged with SIV. 12 animals were vaccinated with a DNA-prime rAd boost regimen. Six received empty vectors (left panels, open circles), and six received SIV gene–encoding vectors (right panels, closed circles). In all figures and Table I, each animal is identified by the same unique color and symbol. (a) Plasma VL. Sham-vaccinated animals followed a typical course, reaching peak plasma VLs of 10^7 copies per ml. With one exception (animal no. 4934, green circles), vaccinated animals reached peak VL a few days earlier and at nearly 1 log lower level. The lines represent fits to the geometric mean VLs, and the sham fit is reproduced in light gray on the right panel for comparison. (b) Cell-associated VL. The number of SIV copies per 10^5 memory CD4 cells was determined by qPCR on purified, sorted total memory CD4 subsets from blood (top panels) or jejunal biopsies (bottom panels). The number of memory CD4 cells infected at any time point was nearly 10-fold lower in vaccinated animals, indicating that far fewer cells were infected and thereby destroyed.

a dramatic benefit immunologically compared with the well-documented complete destruction of jejunal CD4 memory cells in unvaccinated animals (1, 2, 4, 15). Collectively, these findings demonstrate that systemic vaccination with DNA/rAd can significantly protect against the complete destruction of CD4 memory cells during the acute phase.

Vaccination induced both humoral and cellular immune responses

Numerous studies have shown that neutralizing antibodies and/or T cell responses were critical for controlling viral in-


Figure 2. CD4 T cell dynamics in vaccinated animals challenged with SIV. (a) The naive/memory ratio is shown for peripheral tissues (jejunal samples have essentially no naive T cells) before (P) and after viral challenge. In sham-vaccinated animals, a substantial preferential destruction of memory T cells proceeds in all tissues. In contrast, vaccinated animals show a dramatically different course of dynamics, with a relatively high preservation of memory T cells. (b) CD4 memory loss was computed as the change in representation within T cells compared with the prechallenge time point for each animal. The cross-sectional data for all animals measured on days 10–28 is shown for all four tissues. Statistically significant preservation of CD4 memory T cells was seen throughout the animals.

fection (16–26). We quantified both the neutralizing antibody and effector T cell responses induced by the vaccine.

As shown in Fig. 3 a, vaccination induced SIV-specific neutralizing antibody responses in all animals. In fact, the vaccine induced antibodies capable of neutralizing the relatively neutralization-resistant (27, 28), primary isolate challenge. This level of neutralizing activity is only rarely found after natural infection in unvaccinated animals (unpublished data). Importantly, the secondary neutralizing antibody response against the challenge virus was evident within 7–10 d after challenge in all vaccinated animals.

Interestingly, after challenge, the sham-treated animals achieved higher titers of neutralizing antibodies against the T cell line–adapted (TCLA) strain of SIV compared with the vaccinated animals. This may be a reflection of the lower total antigen load in the vaccinated animals, a consequence of the preexisting immune responses. Despite these higher responses, virus was still better resolved in the vaccinated animals, highlighting the potential importance of an ability to
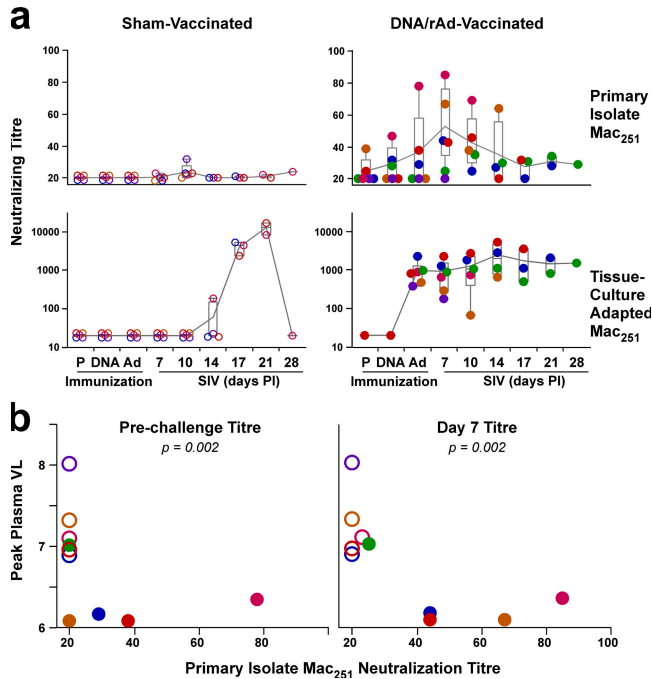
Figure 3. Humoral responses in vaccinated animals challenged with SIV. The plasma levels of neutralizing antibodies were quantified at various times before, during, and after the immunization and SIV challenge. (a) Shown are the neutralizing titres to the primary SIVMac$_{251}$ isolate (top) or to the more-easily neutralized laboratory-adapted strain (bottom). P, preimmunization titres. (b) The peak plasma VL is shown against the neutralization titre against primary SIVMac$_{251}$ measured at day 0 (prechallenge) or day 7 after challenge (significance was determined by two-sided paired *t* tests). The two animals that were killed at day 7 and did not reach peak VL are excluded. Note that although prechallenge neutralizing titres for animals 4942 (brown) and 4844 (blue) were low, they were boosted at day 7, indicating that they were present but undetectable at time of challenge.

neutralize the primary SIVmac251 challenge strain versus the TCLA strain for containment of viral replication once established. Indeed, prechallenge and early secondary neutralization responses to the challenge strain negatively associate with the peak VL achieved (Fig. 3 b). Of note, vaccinated animal number 4934, which achieved the highest peak VL, showed the lowest neutralizing antibody response against the challenge virus as well as the highest loss of memory CD4 T cells among all of the vaccinated animals.

The vaccine regimen also induced SIV-specific CD4 (Fig. 4 a) and CD8 (Fig. 4 b) T cell responses in both mucosal and peripheral tissues, measured 4 wk before challenge. Responses included expression of IFN-γ, IL-2, and TNF-α (Fig. S1). These responses were maintained until the day of challenge. After challenge, a significant expansion of SIV-specific CD4 (Fig. 4 c) and CD8 (Fig. 4 d) T cells occurred. This rapid anamnestic response represented an expansion of effector T cells preferentially producing IFN-γ (Fig. 4 e and Fig. S2, which is available at http://www.jem.org/cgi/content/full/jem.20060657/DC1). The T cell response in vaccinated ani-

mals waned after day 14 as antigen load dropped. We did not detect any significant qualitative differences between the anamnestic responses and the de novo responses; however, the de novo responses never reached the same magnitude after infection and peaked several days later compared with vaccinated animals. Notably, previous studies demonstrated that de novo–generated T cell responses arose only after the destruction of CD4 T cells in unvaccinated animals (3).

Previous studies have shown that systemic immunizations using recombinant vectors could generate mucosal immune responses (29–31). Consistent with this, both CD4 and CD8 T cell responses were induced in the jejunum (Fig. S1). This may contribute to the protection observed at mucosa. The quantitatively lower levels of antigen-specific T cells we observed in the jejunum, however, may have contributed to the relatively lower protection of memory CD4 T cells in that tissue.

Preferential infection of SIV-specific cells
Douek et al. (11) demonstrated that HIV-specific memory CD4 T cells are preferentially targeted by HIV, carrying approximately two- to fivefold more virus than total memory cells. This raised the concern that vaccine-induced virus-specific T cells may serve to accelerate infection. Indeed, a vaccine that induced solely CD4 T cell responses seemed to augment disease course (12). To address this issue in our study, we sorted SIV-specific CD4$^+$ IFN-γ$^+$ or TNF-α$^+$ cells, or total CD4 memory T cells, and quantified the infection rate using a quantitative PCR (qPCR) assay for SIV-gag. This assay was performed after challenge on vaccinated animals to quantify the infection of vaccine-induced CD4 T cells and on sham controls to quantify the infection of de novo–generated CD4 T cells.

Our analysis confirmed that vaccine-induced SIV-specific CD4 T cells may serve as preferential targets for viral infection (Fig. 4 f). We observed a trend toward a twofold greater cell-associated VL in SIV-specific CD4 T cells compared with total CD4 memory. In sham-vaccinated animals, the same rate of preferential infection was found for de novo–generated CD4 T cells as observed for the vaccine-induced CD4 T cells at later time points. However, at the earliest time point (day 7) there was a significantly lower rate of infection in the de novo–generated SIV-specific CD4 T cells. We hypothesize that the SIV-specific CD4 T cells from this time point are highly proliferating cells just arising from the naive CD4 pool. Because naive T cells are resistant to viral infection, these cells are just acquiring susceptibility. By day 10, SIV-specific CD4 T cells in sham-treated animals were as preferentially infected as those from vaccinated animals.

Extent of CD4 memory T cell loss predicts rate of progression
Memory CD4 T cells play a central, initiating role in generating secondary immune responses against previously encountered pathogens by providing help to both B and CD8 T cells. Therefore, we had hypothesized that the degree of
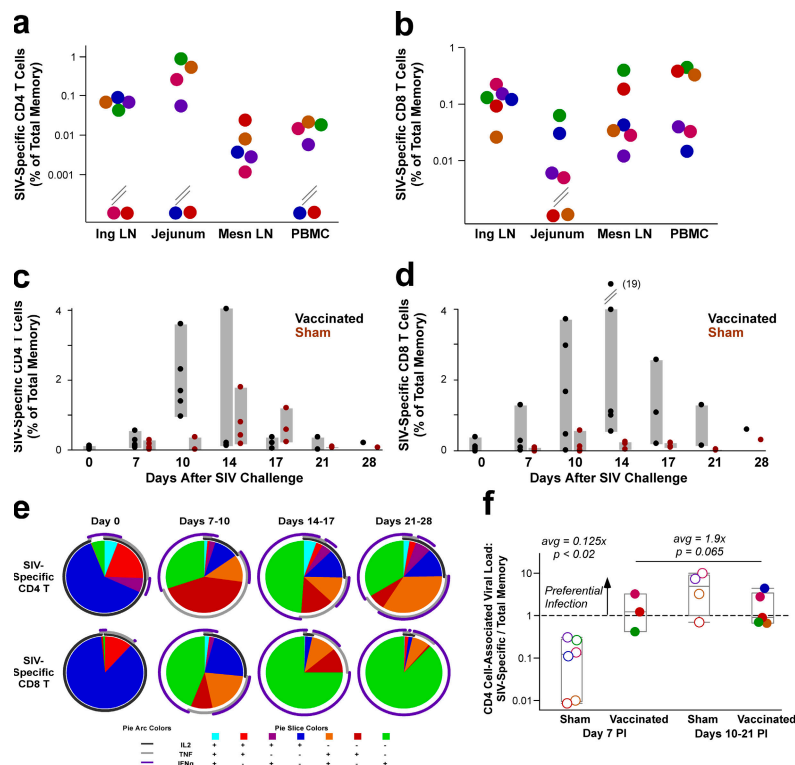
Figure 4. SIV–specific T cell responses in vaccinated animals challenged with SIV. T cells isolated from various tissues before SIV challenge were stimulated with overlapping peptide pools from the SIV gag and envelope proteins. The fraction of CD4 (a) or CD8 (b) T cells making IFN-γ, TNF-α, and/or IL-2 is shown after the full immunization regimen, 1 mo before SIV infection. After challenge, SIV-specific CD4 (c) and CD8 (d) T cells expand in all animals in peripheral blood (and other tissues; not depicted). The expansion was greater and peaked earlier for the vaccinated animals. (e) The quality of the T cell response is shown during the challenge phase for vaccinated animals. The prechallenge responses were typical of memory T cells (dominated by IL-2 and TNF).

After challenge, dramatic expansion of effector T cells producing IFN-γ was observed (see Fig. 2 for the detailed analysis of the quality of the responses for all animals). (f) SIV-specific CD4 memory T cells and total CD4 memory T cells were sorted from PBMC specimens at different time points from animals with sufficient SIV-specific responses to isolate at least several hundred cells. SIV gag DNA was quantified by qPCR. For each specimen, the ratio of the gag DNA signals in SIV-specific CD4 T cells to total memory CD4 T cells is shown. Values >1 indicate preferential infection of SIV-specific CD4 T cells, and values <1 indicate resistance to infection. Shown are the p-values for tests that the distributions have a mean different than 1.

destruction of this compartment during acute SIV/HIV infection may predict the rapidity of onset of immunodeficiency and AIDS during the chronic stage of disease (1). A corollary of this hypothesis is that vaccination before challenge, by preserving memory CD4 T cells, would lead to a better disease outcome.

We quantified the dynamics and cell-associated VLs of peripheral blood memory CD4 T cells during the first 4 wk of infection for animals in a larger vaccine study. Details of this study are reported elsewhere (32). In brief, 30 animals were vaccinated with six different regimens (including a sham vaccination) that incorporated different combinations of DNA priming and rAd boosting. These animals have been followed for more than 3 yr after intravenous SIVmac251 challenge. We measured the CD4 memory T cell numbers and associated VLs at days 7, 14, and 28 after challenge using cyropreserved specimens.

We correlated the degree of memory T cell loss (measured phenotypically) against survival. As shown in Fig. 5 a,

animals that lived longer had significantly greater preservation of CD4 memory T cells during the acute phase. By grouping the animals into terciles based on the extent of CD4 memory destruction during the acute phase, significantly different survival curves are observed (Fig. 5 b). This predictive power is not seen for peak VL (Fig. 5 c) nor for total CD4 T cell loss (Fig. 5 d). Furthermore, the significance was seen whether the analysis was performed on all animals or only the subset receiving immunogenic vaccines. A proportional hazards analysis of the three continuous variables (CD4 memory T cell destruction, peak VL, and/or total CD4 T cell destruction) revealed that only the memory T cell destruction had statistically significant power to predict survival (combined model, P = 0.02, risk ratio = 1.06 [range, 1.01–1.12] per memory CD4 T cell destroyed per μl of blood). These data show that the extent of memory CD4 T cell destruction during the acute phase of disease is a potentially critical surrogate marker for predicting subsequent disease progression.
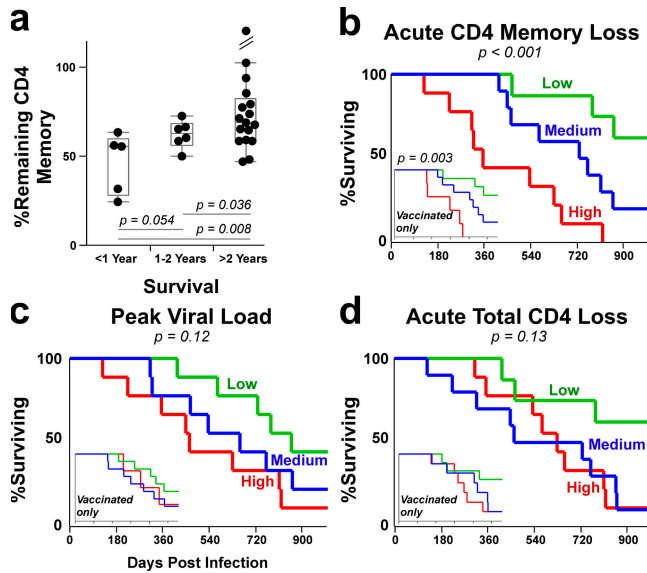
Figure 5. CD4 memory T cell destruction during acute SIV challenge predicts survival. 30 macaques (24 receiving different vaccine regimens and 6 receiving sham vaccines) were challenged with SIV Mac$_{251}$ and followed for more than 2 yr. (a) The preservation of CD4 T cells after acute challenge (i.e., fraction of CD4 memory cells remaining at day 14 compared with day 7) is shown for animals that survived less than 1 yr, 1–2 yr, or more than 2 yr. (b) Kaplan-Meier survival curves for animals grouped into terciles based on the extent of CD4 memory T cell destruction during the acute phase. Animals with the best preservation of CD4 memory (green) survived much longer than those with greater destruction. The analysis was still highly significant when excluding the sham-vaccinated animals (inset). There was no significant power of the peak VL (c) or the loss of total CD4 T cells (d) to predict survival for either the total group or for only vaccinated animals.

DISCUSSION

HIV infection causes a biphasic destruction of CD4 T cells. A massive loss of memory CD4 T cells mediated by direct viral infection occurs during the early acute stages, followed by slow, progressive loss of CD4 T cells mediated by numerous mechanisms during the chronic stage (33, 34). After this initial destruction during the acute phase is likely a homeostatically driven expansion of remaining memory CD4 T cells—an expansion that cannot replace antigen-specific responses lost during the acute phase. Given the critical role of preexisting memory CD4 T cells in generating secondary immune responses, the loss of these antigen-specific responses during acute infection sets the stage of onset of immunodeficiency. Our studies show that animals sustaining lower peak plasma VLs also exhibit lower levels of memory CD4 T cell loss. Thus, clinical observations that high peak plasma VLs are associated with more rapid progression in HIV-infected humans can be explained by the concomitant greater destruction of the CD4 memory compartment during the acute stage. In addition, our findings underscore the necessity for HIV vaccines to ameliorate the virus-mediated destruction occurring in the first 2–4 wk after infection.

We evaluated the ability of a systemic DNA–prime rAd boost vaccine regimen to induce adaptive immune responses that could alter disease progression. DNA priming was performed three times at 1-mo intervals, with the rAd boost 3 mo later. Challenge with SIV Mac251 was initiated 2 mo after the rAd immunization. As has been observed for this regimen in the SHIV challenge model (13, 16), vaccination was partially protective in that the viral dynamics were tempered, with peak plasma VLs in most animals being ∼10-fold lower than sham-treated controls after SIV challenge. Although this reduction (from 10^7 to 10^6 viral copies/ml of blood) may seem inconsequential, it was in fact accompanied by a strong reduction in the number of CD4 memory T cells that were infected and destroyed. Indeed, vaccination has a dramatic impact on this compartment. Whereas sham-treated animals lost 50–80% of their memory CD4 T cells, vaccinated animals lost only 5–20%, indicating that a large majority of pre-existing CD4 memory responses were preserved by the vaccine regimen. Early preservation of these memory CD4 T cells has a significant impact on the outcome of disease course. Loss of fewer memory CD4 T cells was directly associated with better long-term survival.

Importantly, the systemic vaccination elicited mucosal T cell responses and, after challenge, provided protection against the catastrophic loss of CD4 T cells in the gut. The importance of this site to the pathogenesis of SIV/HIV has become clear in the last few years (1, 2, 4, 15, 35–38). By far, the mucosa accounts for the greatest loss of CD4 T cells from the body. Thus, by preserving even 25% of the cells at this site, vaccination should dramatically improve mucosal immunity and health of the infected host.

Several factors could have played a role in the lower level of preservation observed in mucosal tissues, including the high dose of highly pathogenic SIVmac251, intravenous route of infection, and the disorganized nature of the effector mucosal compartment sampled in this study. Unlike the peripheral tissues, CD4 T cell targets are loosely distributed in the jejunal mucosa and could serve as readily available targets for the rapidly disseminating virus after intravenous challenge. These cells, once infected, are rapidly destroyed either by preexisting immune responses or virally mediated lysis. It is possible that a low dose of challenge by the mucosal route may lead to higher levels of memory CD4 T cell preservation in the mucosal tissues, as shown recently by Miller et al. (39). Additionally, it is possible that mucosal immunization may generate better and stronger mucosal immune responses that could more effectively prevent the loss of mucosal CD4 T cells. Nonetheless, it is important to appreciate that even a 25% preservation of memory cells, compared with the near-total destruction in unvaccinated animals, could have significant long-term benefits for maintaining the integrity of the mucosal immune system, as has been demonstrated by Picker et al. (40) These studies showed that animals that failed to repopulate mucosal tissues progressed faster compared with animals that partially repopulated the mucosal tissues.

Preservation of mucosal CD4 T cells has important implications for disease outcome and the onset of immunodeficiency. Mucosal tissues harbor most of the T cells in the body and are home to numerous pathogens. Loss of preexisting memory CD4 T cells severely compromises the ability of the mucosal immune system to maintain protective responses leading to the onset of numerous secondary infections. In addition, rapid infection and destruction of emerging HIV-specific CD4 T cell responses may severely compromise the generation and maintenance of subsequent HIV-specific CD8 T cell responses, leading to a failure to control viral infection. By maintaining the integrity of the mucosal immune system, vaccination can blunt the devastating effects of HIV and ensure better long-term outcome.

The vaccine induced both humoral and cellular immune responses. Although both have been implicated in the control of virus after challenge, the relatively small size of our study did not reveal a clear correlate of protection for either of these arms of the immune response. Nonetheless, our data suggest that the neutralization titres against the primary challenge isolate may associate with the control of peak plasma VL (and peak cell-associated VL). Notably, animal number 4934 had essentially undetectable neutralizing titres against primary SIVmac251 before challenge that did not boost by day 7 and sustained the highest VL amongst vaccinated animals—in the same range as the sham-treated animals. This animal had good neutralizing titres against the TCLA virus as well as systemic CD4- and CD8-specific responses. The T cell responses may have contributed to the eventual better control of viremia in this animal compared with sham-treated animals. These results are in concert with studies showing that administration of neutralizing antibodies attenuates pathogenicity in the SIV or SHIV models (6, 9, 41). Furthermore, our results complement recent studies (42) using CD8 depletion in SIVmac239Δ3-vaccinated macaques, suggesting that both neutralizing antibodies and CD8 T cells contribute to the containment of plasma viremia.

The generation of SIV/HIV-specific CD4 responses by a vaccine posed a conundrum; namely, although these responses are associated with better viral control by CD8 T cells, the cells provide a readily available substrate for viral replication. We found a similar level of preferential infection of SIV-specific CD4 cells in both vaccinated and unvaccinated animals as Douek et al. (11) found for chronically HIV-infected humans. Using a vaccine that induced principally CD4 responses, Staprans et al. (12) found that challenge led to exacerbated disease. In contrast, our vaccine regimen, which induces broad CD4, CD8, and humoral responses, resulted in a significantly tempered pathogenesis. Thus, we conclude that the trend toward preferential infection of vaccine-induced CD4 T cells does not bode poorly for infected subjects.

In summary, we demonstrate that cell-associated VLs and evaluation of memory CD4 T cell dynamics in multiple tissues serve as powerful correlates for measuring vaccine-induced protection. We show that a DNA-prime rAd boost vaccination dramatically reduces the level of infection and destruction of preexisting memory CD4 T cells during the early explosive phase of acute infection. Preservation of these cells will significantly aid in warding off immunodeficiency and the establishment of secondary infections. Finally, preservation of memory CD4 T cells was associated with better long-term outcome and survival. Thus, in the absence of sterilizing immunity, effective vaccination significantly blunts the destructive effects of early infection and plays an important role in maintaining the integrity of the immune system.

MATERIALS AND METHODS

Animals, infection, and samples. 42 colony-bred healthy rhesus macaques (Macaca mulatta) housed at Bioqual Inc. were used in this study. Animals were housed in accordance with American Association for Accreditation of Laboratory Animal Care guidelines and were seronegative for SIV, SRV, and STLV-1. Animals were infected with 100 animal infectious doses of uncloned pathogenic SIVmac251 intravenously. All animal studies were approved by the Vaccine Research Center (NIH) Institutional Animal Care and Use Committee.

Vaccination. Animals were grouped into vaccine ($n = 6$) and sham groups ($n = 6$). Animals in the vaccine group received 4.5 mg of DNA encoding SIVmac239 envelope and 4.5 mg of DNA encoding an SIVmac239 gag-pol fusion protein at 0, 4, and 8 wk (13, 16). DNA was administered into the quadriceps muscles using a gene gun. These animals were boosted with 10^{11} particles of rAd-5 encoding SIVmac239 envelope and 10^{11} particles of rAd-5 encoding SIVmac239 gag-pol administered intramuscularly at 24 wk. Sham controls received empty DNA vectors (0, 4, and 8 wk) and empty rAd5 vectors (20 wk). For Fig. 5, data were aggregated from 30 animals followed for more than 2 yr after challenge. Animals were vaccinated using a similar schedule as described above. There were five groups of six animals each: sham; rAd5 encoding SIVmac239 gag-pol fusion protein (no DNA prime); DNA encoding SIVmac239 envelope followed by rAd5 encoding envelope; DNA encoding SIVmac239 gag-pol followed by rAd5 encoding gag-pol; and DNA encoding SIVmac239 gag-pol and DNA encoding envelope followed by rAd5 encoding gag-pol and rAd5 encoding envelope. DNA was manufactured by Althea.

Tissue sampling. PBMCs were isolated by density gradient centrifugation. Cells were isolated from jejunum and LN biopsy and necropsy samples as described previously (15). Plasma viral RNA levels were determined by real-time PCR (ABI Prism 7700 sequence detection system; Applied Biosystems) using reverse-transcribed viral RNA as templates as described previously (432).

Antibodies and flow cytometry. All antibodies were purchased from BD Biosciences, either conjugated or unconjugated, and derivatized in our laboratory. All reagents were validated and titrated using rhesus macaque PBMCs. For phenotypic analysis, freshly isolated cells were labeled simultaneously with the following combinations of antibodies: CD3-Cy7APC, CD8-Cy5.5-PE, CD4-cascade blue, CD45RA-TRPE, CD95-APC, CCR5-PE, and CD11a-Cy7PE. SIV-envelope– and SIV-gag–specific responses were determined using overlapping peptides as described previously (3, 44, 45). Control cultures were set up for each sample without SIV peptides. After stimulation, cells were labeled with cell surface markers (CD3, CD4, CD8, CD45RA, and CD95) and ethidium monoazide (to discriminate live from dead cells [46]). After fixing (Fix/Perm kit; BD Biosciences), cells were permeabilized and labeled with IL-2-PE, IFN-γ-FITC, and TNF-α-Cy7PE (BD Biosciences). Labeled cells were fixed with 0.5% paraformaldehyde and analyzed using an LSR II or a modified Becton Dickinson DiVa.

Cell-associated VLs in sorted subsets of naive and memory CD4 T cells (discriminated on basis of CD45RA and CD95 expression [47]) were determined using a qPCR. Preferential infection of SIV-envelope– and

SIV–gag–specific CD4 T cells was determined by measuring SIV-gag DNA in total IFN-γ^+ and/or TNF-α^+ cells sorted from peripheral blood samples after stimulation with overlapping peptide pools.

qPCR assay for SIV-gag DNA. T cell–associated viral DNA was measured by a qPCR assay for SIV *gag* using a PerkinElmer ABI 7700 instrument using SIV *gag* primers and probe as described previously (11, 23).

SIV neutralization assays. Neutralizing titres were determined in a luciferase reporter gene assay performed in 5.25.EGFP.Luc.M7 cells as described previously (47). Neutralizing antibody titres are the serum dilution at which relative luminescence units were reduced by 50% compared with virus infection in the absence of test serum. The primary stock of SIVmac251 was prepared in human PBMCs. The TCLA stock of this virus was prepared in H9 cells. All serum samples were heat inactivated at 56°C for 1 h before assay. All assays were performed blinded to the identity of the animal or time points.

Data analysis. Flow cytometric data was analyzed using FlowJo version 6.1 (Tree Star, Inc). Statistical analyses and graphical presentations were computed with JMP version 5.1.2 (SAS Institute) using nonparametric Wilcoxon rank tests for distributions or Wilcoxon Chi-square for the Kaplan-Meier survival analysis.

Online supplemental material. Fig. S1 illustrates the data analysis strategy used to identify memory CD4 and CD8 T cells using the multicolor immunophenotyping panel. In addition, this figure shows examples of cytokine (TNF-α, IFN-γ, and IL-2) expression patterns for stimulated and control cell samples from jejunum, inguinal LNs, mesenteric LNs, and PBMCs from a single animal. Fig. S2 shows the individual data points for all animals at various time points after challenge to show the representation of each possible combination of the three cytokines for SIV-specific CD4 and CD8 T cells. These data values were averaged to generate the pie charts shown in Fig. 4. Figs. S1 and S2 are available at http://www.jem.org/cgi/content/full/jem.20060657/DC1.

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