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The effect of early versus delayed challenge after vaccination in controlling SHIV 89.6P infection

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ABSTRACT

We sought to determine how effectively a $CD8^+$ T cell inducing vaccine controls SHIV-89.6P infection in rhesus macaques at a range of challenge times post-vaccination. To this end, twenty eight *Mamu-A*01*$^+$ rhesus macaques were given replication incompetent human serotype 5 adenovirus vector expressing SIVmac239 gag DNA and boosted 24 weeks later. Groups of 4 monkeys were then challenged with SHIV-89.6P at 1, 3, 6, 12, and 24 weeks after the boost. We compared the kinetics of viral load, $CD4^+$ and virus-specific $CD8^+$ T cells in these macaques. Measurements of $CD8^+$ T cells taken before challenge show an exponential decay between 1 and 12 weeks following vaccination ($p<0.0001$). After week 12, no further decay was observed. Twenty of 24 vaccinated animals maintained more $CD4^+$ T cells and kept their viral load at least one order of magnitude lower than the control animals throughout the chronic phase of the study. All 24 vaccinated animals survived the duration of the study. The viral and T cell kinetics over the first two weeks differed between the vaccinated groups, with more recent vaccination improving the early control of virus (p-value = 0.027). The rates of virus specific $CD8^+$ T cell expansion were greater in animals having higher viral loads at one week ($r=0.45$, $p=0.029$), suggesting that the kinetics of early viral load may have a role in virus specific $CD8^+$ T cell generation, although these early differences did not lead to different clinical outcomes within the vaccinated animals.

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Introduction

HIV infects 33 million people worldwide (UNAIDS and WHO, 2007). Even as treatment for HIV advances and reaches larger numbers of individuals, the risk of transmitting the disease remains high, particularly during acute infection, and a vaccine is the best hope to curb the spread of infection. However, vaccines based on $CD8^+$ T cell immunity have had mixed success: current results in nonhuman primate models, especially with SHIV challenge, indicate that these vaccines may attenuate the severity of infection, but do not prevent infection itself (Johnston and Fauci, 2007). We have previously demonstrated that the inability of $CD8^+$ T cells to prevent the establishment of persistent SHIV infection occurs because they do "too little too late" to control early viral growth in the first 10 days after infection (Davenport et al., 2004). This was subsequently confirmed in SIVsmE660 and SIVmac239 infection (Abdel-Motal et al., 2005; Reynolds et al., 2005).

One explanation for the inability of $CD8^+$ T cells to control early infection is that there are too few of them to efficiently detect and kill infected cells. But even very high numbers of virus-specific $CD8^+$ T cells do not lead to earlier viral control (Davenport et al., 2004, 2007; Shiver et al., 2002). Higher levels of specific $CD8^+$ T cells before infection do, however, lead to a better prognosis in chronic infection (Davenport et al., 2005). This suggests that rather than absolute cell number, some aspect of the quality or activation state of $CD8^+$ T cells may be essential for viral control.

The time period between the last vaccine boost and challenge with infectious virus may impact the state or quality of $CD8^+$ T cells. Recently activated T cells may be functionally different from cells that encountered their cognate antigen a longer time ago. For this reason, we were interested in studying how the kinetics of SHIV primary infection in recently vaccinated macaques differ from the kinetics in macaques vaccinated some time previously. How long a vaccine confers protection after administration is a crucial question that will determine the frequency a person needs to be vaccinated and may also have implications for the design of vaccines (Anderson et al., 2005).

Here we analyze the kinetics of virus and vaccine-induced specific immunity in a set of vaccinated *Mamu-A*01*$^+$ rhesus macaques

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challenged at different times (1 to 24 weeks) after the last vaccination boost. A high level of $CD8^+$ T cell immunity was induced by the vaccine soon after immunization, which decayed during the first 12 weeks post-boost and then stabilized. Thus, the level of protection afforded by a vaccination regimen may depend on either the magnitude of the response at different times since vaccination or the quality of these responses (Anderson et al., 2005).

Our quantitative analysis of viral kinetics suggests that recent vaccination better controls virus early in primary infection. We speculate that recent antigen exposure may alter the phenotype, function, or location of the responding cells, with an effect in reducing viral load. Better early control did not, however, affect the long term outcome in SHIV 89.6P infection.

Results

Kinetics of infection

Fig. 1 shows the average viral load, $CD8^+$ $p11CM^+$ T cell counts, and $CD4^+$ T cell depletion, the number of $CD4^+$ T cells/μL divided by the $CD4^+$ T cell count on the day prior to infection, for each vaccination group. Compared with the control group, all vaccinated groups have a lower peak and chronic stage viral load, retain a larger proportion of $CD4^+$ T cells, and have more antigen specific $CD8^+$ $p11CM^+$ T cells.

All but four vaccinated animals (one in group A, two in group D, and one in group F) were able to keep their chronic viral levels at least one order of magnitude lower than the control animals' viral loads, with several vaccinated animals having viral loads below the limit of detection (50 virions/μL) for the duration of the study. At approximately six months (178 days) post-infection, the distribution of the viral loads of individual animals did not differ between the vaccinated groups ($p=0.48$) (Fig. 1). Nevertheless, there was a significant positive correlation ($r=0.72$, $p=1.3\times10^{-5}$) between the peak viral load in the acute phase of infection and the viral load on day 178, stressing the importance of making vaccines that better control early infection.

Effect of time to challenge on specific $CD8^+$ T cell kinetics

The maximum number of $p11CM^+$ $CD8^+$ T cells before challenge was observed one week after vaccination and boost (median = 173 cells/μl). Subsequently, for the first 12 weeks after vaccination and boost, but prior to SHIV-89.6P challenge, the number of $p11CM^+$ $CD8^+$ T cells declined exponentially with a half-life of four weeks (Fig. 2) ($p<0.0001$). Interestingly, we could not detect a further reduction in $CD8^+$ $p11CM^+$ T cell levels between weeks 12 and 24, perhaps indicating the attainment of a steady state level (both the 12 and 24 week groups have a median of 4 cells/μL).

As noticed before (Abdel-Motal et al., 2005; Davenport et al., 2004, 2005; Reynolds et al., 2005), after SHIV/SIV challenge there is a time lag before an expansion in antigen specific $p11CM^+$ $CD8^+$ T cells can be measured. As in (Davenport et al., 2005), the length of the time lag, t_{on}, was correlated with the initial number of $p11CM^+$ $CD8^+$ T cells across vaccinated groups: the higher the number of $CD8^+$ $p11CM^+$ T cells at challenge, the longer it took for an expansion to be detected ($r=0.48$, $p=0.018$). Because the number of $p11CM^+$ $CD8^+$ T cells at challenge falls with time since vaccination, we also found that t_{on} negatively correlates with challenge time in groups B–E, the groups which show a decay of baseline $p11CM^+$ $CD8^+$ T cells from week 1 to 12 ($r=-0.53$, $p=0.033$).

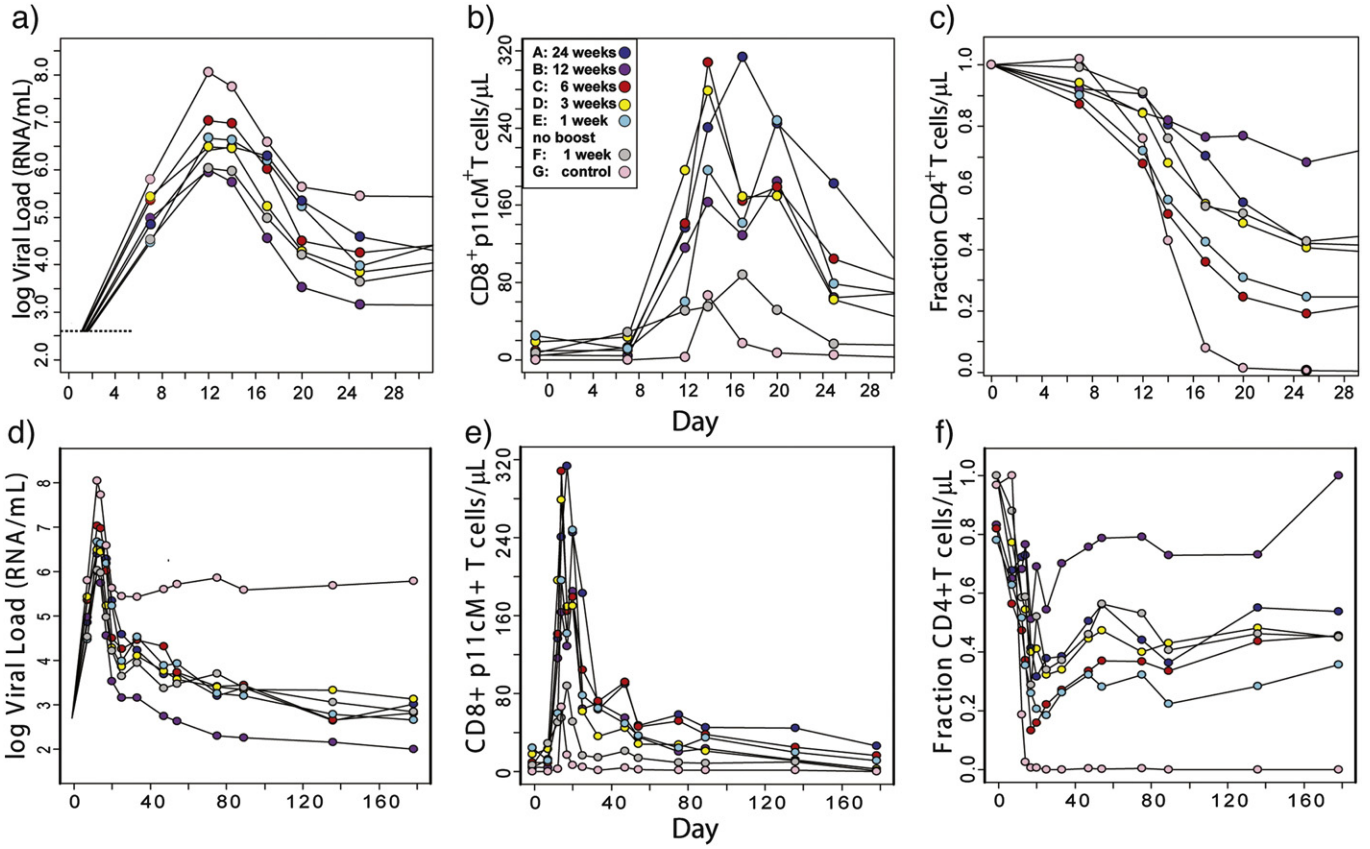

Fig. 1. Acute infection profiles differ with challenge time (profiles are averaged over the four monkeys in each group): a) and d) viral load, b) and e) $CD8^+$ $p11CM^+$ T cells/μL, and c) and f) $CD4^+$ T cell depletion, i.e., the number of $CD4^+$ T cells/μL divided by the CD4 count on the day prior to infection. The viral load at day seven is significantly lower in the more recently vaccinated animals, groups E and F.

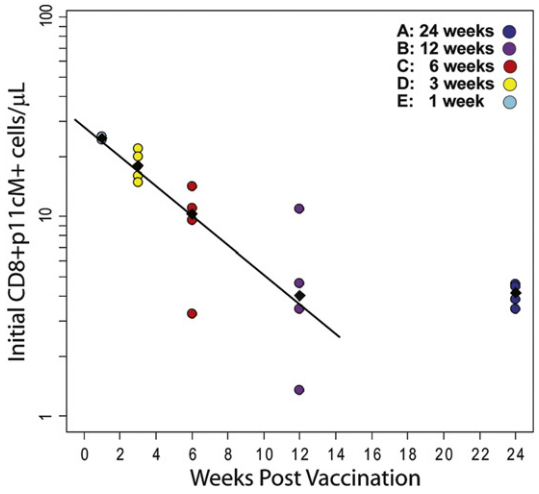

Fig. 2. Decay and plateau of CD8$^+$ p11CM$^+$ T cells after vaccination, individual animals (colored circles) and group median (black diamonds) are shown. In the boosted and vaccinated groups, the animals' antigen-specific CD8$^+$ T cells drop monotonically with time between week 1 and 12. Group A measured at 24 weeks post-boost has the same number of CD8$^+$ T cells as Group B, measured at 12 weeks.

CD4$^+$ T cell depletion

We next investigated the relationship of CD4$^+$ T cell depletion with virus and with CD8$^+$ p11CM$^+$ T cell kinetics. We found a positive correlation between the peak viral load and the amount of CD4$^+$ T cell depletion that occurred between days 1 to 25 ($r=0.88$, $p=1.0\times10^{-9}$; and $r=0.80$, $p=3.8\times10^{-6}$ considering only the vaccinated groups), consistent with our previous study (Davenport et al., 2006). To analyze the processes involved in CD4$^+$ T cell depletion, we compared the depletion by day 25 with the area under the viral load curve and with the area under the p11CM$^+$ CD8$^+$ T cell curve from day 0 to 25 (Fig. 3). These areas under the curves are indicative of the total exposure of CD4$^+$ T cells to virus and specific CD8$^+$ T cells, respectively. Interestingly, CD4$^+$ T cell depletion at day 25 was highly correlated with total virus exposure up to day 25 ($r=0.82$, $p=2.4\times10^{-6}$), but not with total exposure to p11CM$^+$ CD8$^+$ T cells through day 25 ($r=0.25$, $p=0.23$). Although this finding is suggestive of CD4$^+$ T cell depletion induced by direct and indirect viral effects, it is not definitive as other immune factors not analyzed could contribute to CD4$^+$ T cell depletion, such as CD8+ T cell responses to other epitopes, bystander effects, or antibody-mediated cellular citotoxicity.

Early vs. late challenge led to differences in early viral load levels

Because animals in groups E and F were challenged just one week after being vaccinated, it is possible that their baseline p11CM$^+$ CD8$^+$ T cells are qualitatively different from those at baseline in the other vaccinated groups. In particular we could expect that early after vaccination, the bulk of antigen-specific CD8$^+$ T cells may still be activated. If this is the case, then control of the virus in the groups challenged at one week should be similar and faster than in the other groups, since a time delay to activation would not be present. Indeed, on day 7, groups E and F have a statistically lower viral load than the vaccinated groups A–D, challenged at 24, 12, 6, and 3 weeks (p-value = 0.027; highlighted in Fig. 1a). This is observed even though the animals in group F did not receive a boost inoculation. It is important to note that there is no correlation between the initial number of p11CM$^+$ CD8$^+$ T cells and the viral load on day 7 in vaccinated animals, indicating that the initial number of p11CM$^+$ CD8$^+$ T cells is not sufficient to predict early viral control. This suggests that the functional difference of p11CM$^+$ CD8$^+$ T cells between the animals infected sooner after vaccination rather than later may influence the kinetics of virus early in infection.

Early vs. late challenge and the expansion of p11CM$^+$ CD8$^+$ T cells

At the time of infection, groups challenged early (E, F), versus late (A–D), have significantly more p11CM$^+$ CD8$^+$ T cells expressing Ki67, a marker of cell proliferation ($p=0.0001$). Nonetheless, the start time of observable antigen-specific CD8$^+$ T cell expansion after infection, t_{on}, does not statistically differ between groups E–F and groups A–D, which had lower levels of Ki67 at day 0.

We next looked for differences in the expansion rates of antigen-specific cells between groups E and F and groups A–D after expansion began, using a linear-mixed effects modeling approach (Pinheiro, 2000). We analyzed two estimates of expansion: one incorporating all points in acute infection between the minimum and the maximum p11CM$^+$CD8$^+$ T cell count, and the other including only the points showing the fastest expansion rate (see Methods). In both cases, we found that p11CM$^+$CD8$^+$ T cells expand more slowly in groups E and F, with the fastest expansion rate being statistically slower in groups E–F than in groups A–D ($p=0.004$).

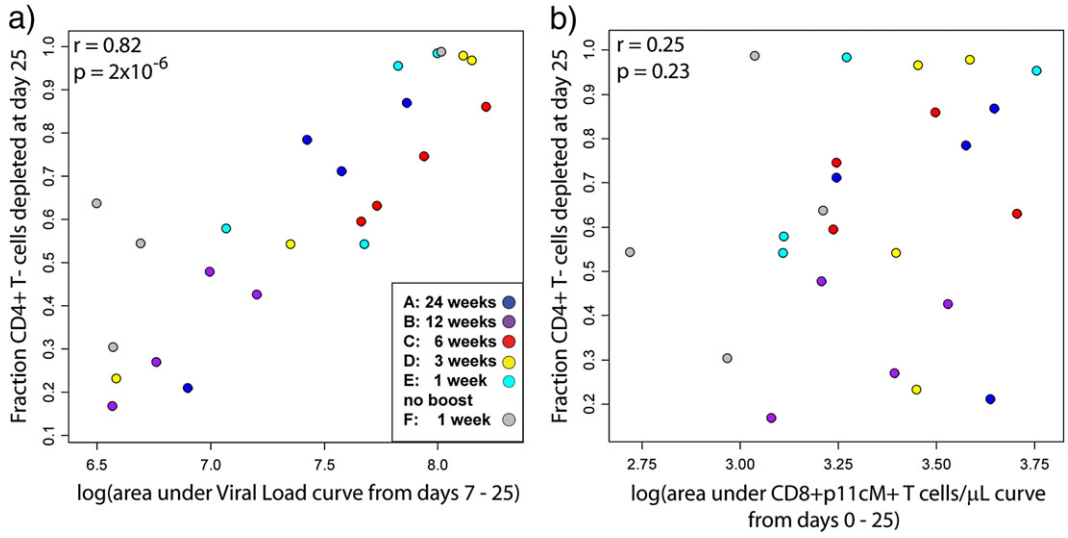

Fig. 3. What is killing CD4$^+$ T cells? (a) CD4$^+$ T cell depletion correlates much more strongly with the area under the viral load curve than (b) with the total number of specific CD8+ T cells produced, *i.e.* area under the p11CM$^+$ CD8$^+$ T cell curve.

Because antigen-specific CD8$^+$ T cells expand at different rates in the early and late challenge groups, we asked if this had any impact on viral kinetics. To examine the growth in virus, we used two linear mixed effects models for the change in viral load: one with all points from day 7 through the viral peak, and one including only the points defining the fastest expansion. Both models show that groups E and F, which have slower p11CM$^+$ CD8$^+$ T cell expansion, have faster viral load expansion after day 7 than groups A–D, with the difference in the fastest expansion rate reaching statistical significance ($p=0.003$).

Correlation between viral load and p11CM-specific CD8$^+$ T cell expansion

The results of the previous section seem to indicate that higher viral loads on day 7 in groups A–D lead to faster expansion of specific CD8$^+$ T cells, so we analyzed the correlation between these two variables in detail. As in our previous work, we found no correlation between initial p11CM$^+$ CD8$^+$ T cell number and viral load expansion rate up to day 10 (Davenport et al., 2004). However, here we asked the converse question and found that higher viral loads at day 7 correlated with faster expansion rates of p11CM$^+$ CD8$^+$ T cells from day 7 to the peak (at a median of 12 days) ($r=0.45$, $p=0.029$) in the vaccinated groups (Fig. 4a). Moreover, the viral load at day 7 has a positive correlation with the area under the p11CM$^+$ CD8$^+$ T cell count curve ($r=0.59$, $p=0.003$), which can be seen as a surrogate for total production of specific CD8$^+$ cells (Fig. 4b). That is, the viral load at day 7 is a good indicator of how many p11CM$^+$ CD8$^+$ T cells will be produced.

Conclusions

An important consideration in designing a vaccine is to know how long protection lasts. This is especially true in the case of vaccines inducing cell mediated immunity, as is the case of many vaccines being studied against HIV infection, because there is much less experience with this type of vaccine. Recent vaccination may be expected to produce higher virus specific CD8$^+$ T cell counts and may be expected to lead to better early viral control. However, dissecting the contribution of greater numbers of specific CD8$^+$ T cells from qualitative aspects of the response is difficult. Knowing the time scales of protective immunity will help inform choices such as vaccination and boosting schedules. If recent exposure to antigen activates T cells and enhances the ability of virus specific CD8$^+$ T cells to control virus early after infection, perhaps we would need to consider strategies of multiple and frequent vaccinations, which may require vectors that do not lead to immunity against later boosts of the vaccine, or even vaccines including persistent antigens.

Recent CD8$^+$ T cell vaccination trials have met no success in humans, and the use of models such as SHIV 89.6P in rhesus macaques has been questioned as being too pathogenic to aptly model HIV (Feinberg and Moore, 2002). Although SHIV89.6P may not be an ideal model of HIV vaccination, it is still much in use (Borsetti et al., 2008; Koopman et al., 2008; Patterson et al., 2008; Ridolfi et al., 2008; Sadagopal et al., 2008). Moreover, quantitative analyses of SHIV 89.6P may help us understand its behavior better and, thus it serves as a tool to probe the immune system and shed light on the relationships between virus and immune system kinetics.

An important finding of this study is that the level of immunity, as measured by the number of antigen-specific CD8$^+$ T cells at time of challenge, decays exponentially over at least the first 12 weeks post-boost, then stabilizes from 12 to 24 weeks post-boost. In spite of this, the "clinical" protection afforded by the vaccine, as measured by disease progression (*i.e.*, increases in viral load and depletion of CD4$^+$ T cells), was similar in the different groups of vaccinated monkeys, including a group that did not receive a boost (Casimiro et al., 2005).

Though the clinical outcome was similar across the vaccinated groups, we also found important differences. Those monkeys challenged earlier, at only one week after vaccination (groups E and F), showed a qualitatively different viral growth than animals challenged later (groups A–D). The former show early viral control at a level that has not been seen in settings of later challenge, even when up to 25% of total CD8$^+$ T cells were virus specific (Shiver et al., 2002). Soon after vaccination, many specific T cells are expected to still be activated by antigen, and may also be located in peripheral tissues. Consistent with this hypothesis, we found higher levels of Ki67, a proliferation antigen, in the recently vaccinated groups. However, a more complete characterization of these cells, which was not possible in this study, needs to be done, including markers of avidity, effector phenotype, activation, etc.

The lower viral load early after challenge did not lead to lower peak viral levels. In particular, in the recently vaccinated animals there was a slower expansion of the p11CM-specific CD8$^+$ T cells in the second week of infection, which was associated with faster growth of virus between day 7 and the day of peak viral load. We speculate that the virus specific CD8$^+$ T cell pool in monkeys challenged soon after vaccination contain more activated cells with less capacity for additional expansion, which are more efficient at controlling infection

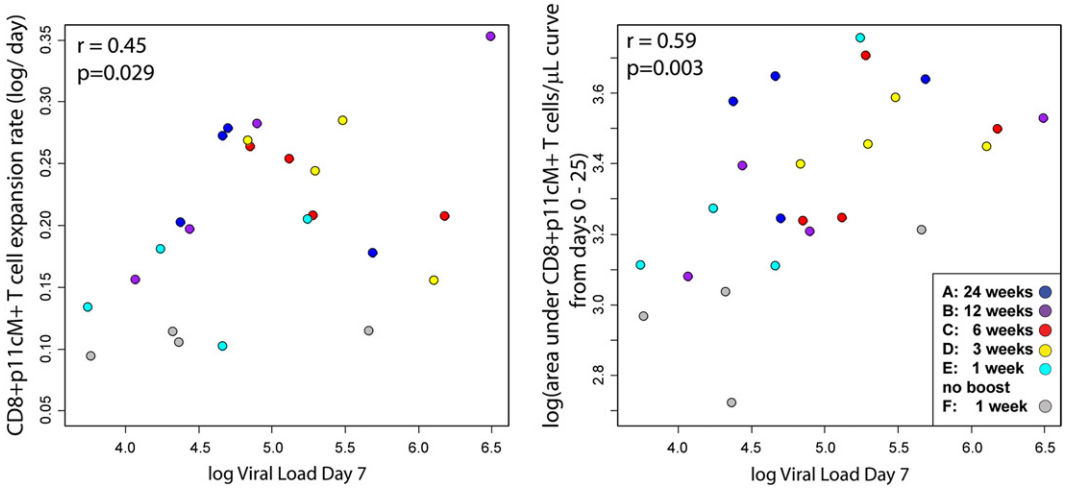

Fig. 4. Early infection influences specific CD8$^+$ T cell production: (a) A higher viral load at day 7 is correlated with a greater expansion rate of p11CM-specific CD8$^+$ T cells and (b) is also a good indicator of the total number of p11CM-specific CD8$^+$ T cells that will be produced, as measured by the area under the curve of CD8$^+$ T cells.

early on. The result of this trade-off in cellular function is that the animals in groups E and F end up having peak viral loads similar to the other vaccinated groups.

Virus-specific $CD8^+$ T cells may moderate viral growth, but viral levels may also drive virus specific $CD8^+$ T cell expansion. Overall, the expansion of these antigen-specific cells was faster and the total production of these cells was larger in the animals with higher viral loads at one week. Consistent with this, the animals in groups E and F, which had lower viral loads at one week had a slower expansion rate of $p11CM^+$ $CD8^+$ T cells.

It is remarkable that we found these correlations and differences between macaques challenged early versus late after vaccination, if we note that only one specific aspect of the immune response was studied (the kinetics of $p11CM^+$ $CD8^+$ T cells). In previous studies we have shown that subdominant epitopes also have kinetics similar to the dominant epitope (Davenport et al., 2004; De Boer et al., 2003). Still, the immune response to the virus is much more complex with many components that we did not consider, such as the response in tissues (eg. GALT), the functional activity of the CD8+ T cells, and the CD4+ specific response. In particular, it will be interesting to measure activation and proliferation state of CD4+ T cells early versus late after vaccination. Unfortunately, in this retrospective kinetic study, we did not have this information.

In summary and in spite of the caveats just mentioned, our results indicate that the delay in $CD8^+$ T cell control of virus observed in a number of studies can be partially overcome in the setting of recent antigen exposure. Animals vaccinated within a week of challenge had significantly lower early (day 7) viral loads. However, although recent exposure to antigen is associated with reduced viral load in the first week after infection, viral growth was higher in the second week, perhaps as a result of the failure of expansion or the death of an already activated T cell population. This work demonstrates that the effects of these specific $CD8^+$ T cells depend not only on their initial numbers, but also on the level of recent antigen exposure. Whether the relative control of virus then leads to slower expansion of more $p11CM^+$ $CD8^+$ T cells is something that needs more careful quantitative investigation. Continued modeling and quantitative analyses of vaccine trials will be invaluable to understanding the early dynamics and function of $CD8^+$ T cell inducing vaccines.

Methods

Animals and vaccination-challenge protocols

Twenty-eight *Mamu-A*01*$^+$ monkeys were divided into seven groups of four monkeys each. Five groups were vaccinated with a replication-incompetent human adenovirus serotype 5 (10^{11} viral particles) expressing SIVmac239 *gag* DNA and received a vaccination boost 24 weeks later (see Fig. 5 for the experimental protocol). These groups were then challenged intra-rectally with SHIV-89.6P at 24, 12, 6, 3, and 1 week following the boost (labeled A, B, C, D, and E, respectively). Group F received no boost and was challenged one week after vaccination. The control group, G, received no vaccination. $CD4^+$ T cells and viral loads were measured at irregular intervals for 10 months. MHC-tetramer reagents were used to count $CD8^+$ T cells capable of binding to the immunodominant SIV *gag* p11CM epitope presented by the *Mamu-A*01* MHC molecule. Ki67 (B56, BD Pharmingen) expression on $p11CM^+$ $CD8^+$ T cells was measured by flow cytometric methods (Appay and Rowland-Jones, 2002; Champagne et al., 2001; Oxenius et al., 2001). All vaccinated monkeys, except those in group B, were euthanized about 325 days following the challenge. The monkeys in the control group (G) succumbed to infection in a median of 178 days. Further details on animal care, vaccine and challenge stock preparation, and cellular assays are provided in (Casimiro et al., 2005; Shiver et al., 2002).

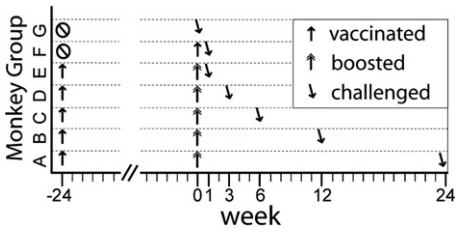

Fig. 5. Experimental protocol: 28 monkeys were divided into groups of four, labeled A–G. Groups A–E received a vaccine then a boost 24 weeks later. These groups were challenged with SHIV-89.6P at the week corresponding to the slanted arrow. Group F only received a vaccine at the same time the other groups received their boost and was challenged one week later. Group G, the control group, was infected with SHIV-89.6P and received no vaccine or boost.

Statistical analysis

We define the time of infection as time 0. Virus-specific $CD8^+$ T cell expansion rates were calculated by fitting, via linear regression, the rise of log $CD8^+$ $p11CM^+$ T cells/μL versus time. We used this method to estimate the expansion rate from the baseline to peak (occurring around day 12). The same approach was used to estimate the maximal expansion rate seen in the data: if the expansion slowed before it reached the peak, we used only the data from the first time point above the baseline to the time before the expansion slowed down. We defined the start of $CD8^+$ $p11CM^+$ T cell expansion (t_{on}) as the backward extrapolated time when the maximal expansion rate linear regression line intersects the baseline $CD8^+$ $p11CM^+$ T cell value; thus, $t_{on}=(\log_{10}(T_0)-b_{10})/g_{10}$, where T_0 is the baseline $CD8^+$ $p11CM^+$ T cell value on the day prior to infection, b_{10} is the intercept, and g_{10} is the slope found by linear regression (Davenport et al., 2005). Linear mixed effects models were used to compare $CD8^+$ $p11CM^+$ T cell and viral load expansion rates, using the *lme* function in the software package *R* (2008). Spearman's rank correlation was used to assess the significance of correlations between variables, and the Kruskall–Wallis test was used to determine whether individual variables differ significantly between the vaccination groups. Significance was assessed at the 0.05 level.

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