Immunological and Virological Analyses of Rhesus Macaques Immunized with Chimpanzee Adenoviruses Expressing the Simian Immunodeficiency Virus Gag/Tat Fusion Protein and Challenged Intrarectally with Repeated Low Doses of SIVmac

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Human adenovirus (AdHu)-based candidate AIDS vaccine can provide protection from simian immunodeficiency virus (SIV) transmission and disease progression. However, their potential use may be limited by widespread preexisting immunity to the vector. In contrast, preexisting immunity to chimpanzee adenoviruses (AdC) is relatively rare. In this study, we utilized two regimens of prime-boost immunizations with AdC serotype SAd-V23 (also called AdC6) and SAd-V24 (also called AdC7) expressing SIV Gag/Tat to test their immunogenicity and ability to protect rhesus macaques (RMs) from a repeated low-dose SIVmac239 challenge. Both AdC6 followed by AdC7 (AdC6/7) and AdC7 followed by AdC6 (AdC7/6) induced robust SIV Gag/Tat-specific T cell responses as measured by tetramer staining and functional assays. However, no significant protection from SIV transmission was observed in either AdC7/6- or AdC7/6-vaccinated RMs. Interestingly, in the RMs showing breakthrough infections, AdC7/6-SIV immunization was associated with a transient but significant (P = 0.035 at day 90 and P = 0.033 at day 120 postinfection) reduction in the setpoint viral load compared to unvaccinated controls. None of the measured immunological markers (i.e., number or functionality of SIV-specific CD8+ and CD4+ T cell responses and level of activated and/or CCR5+ CD4+ target cells) at the time of challenge correlated with protection from SIV transmission in the AdC-SIV-vaccinated RMs. The robust immunogenicity observed in all AdC-immunized RMs and the transient signal of protection from SIV replication exhibited by AdC7/6-vaccinated RMs even in the absence of any envelope immunogen suggest that AdC-based vectors may represent a promising platform for candidate AIDS vaccines.
FIG 1 Immunogenicity and protection from low-dose rectal SIVmac239 challenge by AdC6/C7-SIVgag/tat and AdC7/C6-SIVgag/tat vectors. Shown is a schematic representation of the experimental design, which involved two immunizations with AdC6-SIVgag/tat followed after 6 months by AdC7-SIVgag/tat (group 1) or AdC7-SIVgag/tat followed after 6 months by AdC6-SIVgag/tat (group 2). Each group included six MamuA*011 and 4 MamuA*011 adult Indian RMs. All RMs, as well as an additional 10 unvaccinated control animals, were challenged with repeated low-dose intrarectal SIVmac239 every 2 weeks up to 15 times. For logistical reasons, the RMs were staggered in two cohorts (A and B), with the challenge for the 1st cohort conducted 4 months after the last immunization and the challenge for the 2nd cohort conducted 5 months after the last immunization. The times of immunization, challenge, and sample collection are indicated. PB, peripheral blood.

MATERIALS AND METHODS

Animals. Thirty healthy, non-SIV-infected Indian rhesus macaques (18 MaMu-A*01 positive and 12 MaMu-A*01 negative) were used in this study. All animals were housed at the Yerkes National Primate Research Center and maintained in accordance with NIH guidelines. These studies were approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Adenovirus-based SIV vaccines. Ad vectors were based on chimpanzee serotypes SAd-V24 (also called AdC7) and SAd-V23 (also called AdC6). Vectors were generated from recombinant viral molecular clones. They had E1 deleted and hence were replication defective, and an expression cassette containing a codon-optimized sequence of a SIVmac239 Gag-Tat fusion gene under the control of the cytomegalovirus (CMV) promoter was placed into the E1 domain. Upon rescue and expansion on HEK 293 cells, the vectors were purified by CsCl gradient centrifugation. The vectors were titrated for their contents of virus particles (vps) by spectrophotometry at 260 nm using the following formula: optical density at 260 nm (OD260) × dilution × 1.1 × 1012. The content of infectious virus particles was measured by nested reverse transcriptase (RT)-PCR with transgene- or Ad (hexon)-specific primers on RNA isolated from HEK 293 cells infected for 5 to 7 days with serial dilutions of vector. Batches were tested for endotoxin using the Limulus amebocyte lysate (LAL) gel clot method and a commercial kit. Genetic integrity and identity were assessed by isolation of viral DNA. The recombinant DNA, in parallel with the original molecular clones and shuttle plasmids used for generating molecular clones, was digested with a set of restriction enzymes and analyzed by gel electrophoresis. Expression of the transgene product was confirmed by Western blot analysis of Ad vector-infected CHO cells stably transfected to express the coxsackie adeno virus receptor.

Vaccination protocol. The heterologous “prime-boost” regimen used in this study consisted of two immunizations performed at day 0 (prime) and after 24 weeks (boost), as detailed in Fig. 1. Each immunization was comprised of the AdC6 or AdC7 vector expressing SIVgag/tat injected intramuscularly (i.m.) at a dose of 1011 vps per macaque. One experimental group of 10 RMs was primed with AdC6 vectors followed by boosting with AdC7, while the other group of 10 RMs was primed with AdC7 vectors and boosted with AdC6. Ten additional unvaccinated animals were used as controls.

Viral challenge. Four to 5 months following the final immunization, all vaccinated RMs, as well as the control animals, were challenged intrarectally every 2 weeks with a repeated low dose of SIVmac239 (300 50% tissue culture infective doses [TCID50] per rectal injection) that was provided by Chris Miller and Koen Van Rampany at the California National Primate Research Center, Davis, CA.

Tissue collection and processing. Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation. Procedures for lymph node (LN) biopsy, rectal biopsies, and bronchoalveolar lavage (BAL), as well as isolation of lymphocytes from the obtained samples, were performed as previously described (26).

Immunophenotyping and flow cytometry. Multicolor flow cytometric analysis was performed on mononuclear cells isolated from blood,
lymph nodes, and mucosal tissues (rectal-biopsy specimens [RBs]) according to standard procedures using human monoclonal antibodies that were found to cross-react with RMs. Predetermined optimal concentrations of the following antibodies were used: anti-CD4 Pacific blue (clone OKT4) (from Biolegend, San Diego, CA); anti-CCR5 allophycocyanin (APC) (clone 3A9), anti-CD8 APC-Cy7 (clone SK1), anti-CD95 phycoerythrin (PE)-Cy5 (clone DX2), anti-CD3 Alexa 700 (clone SP34-2), and Ki-67 fluorescein isothiocyanate (FITC) (clone B56) (from BD Biosciences, San Diego, CA); and anti-CD28 PE-Cy7 (clone 28.2) (from eBioscience, San Diego, CA). Flow cytometric acquisition and analysis of samples was performed on at least 100,000 events on an LSRII flow cytometer driven by the DIVA software package (Becton Dickinson). Analysis of the acquired data was performed using FlowJo software (Tree Star, Inc., Ashland, OR).

**Tetramer staining.** Major histocompatibility complex (MHC) class I tetramers were prepared and conjugated to streptavidin APC fluorophore (Molecular Probes), as previously described (27). The level of SIV-specific CD8+ T cells was assessed using soluble tetrameric Mamu-A*01 MHC class I tetramers specific for SIVmac239 immunodominant epitopes Gag181-189 CM9 (CTPYDINQM) and Tat28-35 SL8 (STPESANL). Lymphocytes isolated from blood and tissues were incubated with conjugated tetramer, along with surface antibody conjugates, and analyzed for tetramer and surface marker expression using an LSRII Flow Cytometer (Becton Dickinson) equipped with fluorescence-activated cell sorter (FACS) DIVA software.

**Multifunctional assessment of SIV-specific T cell responses.** The function of SIV-specific CD8+ T cells was assessed by flow cytometry after stimulation with peptide pools of 15-mers (overlapping by 11 amino acids) spanning the SIVmac239 Gag and Tat proteins, as described in reference 6. Peptides were prepared from peptide stocks obtained from the NIH AIDS Research and Reference Reagent Program (Bethesda, MD), reconstituted in dimethyl sulfoxide (DMSO), and pooled. All peptides were used at a final concentration of 2 μg/ml. Anti-CD107a FITC (clone H4A3; BD Biosciences, San Jose, CA) was added at the start of all stimulation periods. The cocktail of antibodies for surface staining included FITC-conjugated CD3 Pe-Cy7 (clone DX2); anti-CD4 Qdot655 (clone TuK4; Invitrogen), CD14 Qdot655 (clone TuK4; Invitrogen), CD16 Qdot655 (clone TuK4; Invitrogen), and CD20 Qdot655 (clone DX2), anti-CD20 Alexa 700 (clone SP34-2; BD Biosciences), and Ki-67 fluorescein isothiocyanate (FITC) (clone B56) (from BD Biosciences, San Diego, CA); and anti-CD28 PE-Cy7 (clone 28.2) (from eBioscience, San Diego, CA). Flow cytometric acquisition and analysis of samples was performed on at least 100,000 events on an LSRII flow cytometer (Becton Dickinson) equipped with fluorescence-activated cell sorter (FACS) DIVA software.

**Plasma viral load determination.** Quantitative real-time RT-PCR assay to determine the SIVmac239 viral load was performed as previously described (28). The sensitivity of the assay is 50 copies/ml of plasma.

**Statistical analysis.** Cox models were used to test for differences in the number of challenges to infection and any association with activation responses. Differences in responses by vaccine regimens were assessed using mixed-effects models. Log transformations were applied as necessary, and normal, gamma, and negative binomial distributions were assumed as appropriate. Tukey-Kramer or Sidak adjustments for multiple comparisons were applied. Statistical analyses were conducted using SAS 9.3.

**RESULTS**

**Study design: immunization and challenge.** In this study, we assessed the immunogenicity and protection from low-dose rectal challenge with SIVmac239 conferred by two AdC-based heterologous prime-boost vaccination regimens. The study design is shown in Fig. 1, including immunization regimens, challenge, and timing of sample collections. In the first regimen (group 1), 10 RMs were immunized with AdC6 expressing SIV Gag/Tat fusion protein (AdC6-SIVgag/tat) that was given I.m. at a dose of 10^11 vps, followed after 24 weeks by a second immunization with AdC7 expressing the same SIV Gag/Tat fusion protein (AdC7-SIVgag/tat) at the same dose and by the same route. In the second regimen (group 2), 10 RMs were immunized with AdC7-SIVgag/tat, followed by AdC6-SIVgag/tat, with all vaccines given with the same schedule, dose, and route as group 1. Ten additional, unvaccinated animals were used as controls. Of note, each group of RMs included 6 MaMu-A*01-positive and 4 MaMu-A*01-negative animals. All RMs were repeatedly challenged intrarectally with a low dose (300 TCID_{50}) of SIVmac239 that was given every 2 weeks. Animals that tested positive for SIV viremia at a level greater than 1,000 copies/ml of plasma were not challenged further and instead were followed for up to 180 days to monitor the clinical, virological, and immunological course of the infection. Note that for logistical reasons, during the challenge phase of this experiment, the RMs were divided into two batches of 15 individuals each (i.e., cohort A and cohort B each included animals belonging to all three groups) that started challenge ~4 and ~5 months after the last immunization, respectively. As described below, the three AdC-vaccinated RMs that remained non-SIV infected throughout the low-dose challenge phase of this experiment were eventually challenged with a high dose of SIVmac239 (3,000 TCID_{50}) to ensure that they were not intrinsically resistant to the infection, but their viral load data postchallenge were excluded from the relevant analysis.

**AdC-based vaccines induced robust SIV-specific cellular immune responses.** The vaccine-induced SIV-specific CD8+ T cell responses were enumerated in the six Mamu-A*01+ RMs included in groups 1 and 2 by tetramer staining for CD8+ T cells specific to the well-characterized immunodominant epitopes Tat-SL8 and Gag-CM9. As shown in Fig. 2A to D, tetramer-positive CD8+ T cells were readily identifiable after the first immunization in both groups of immunized RMs, and their fraction of CD8+ T cells increased after the second vaccination for the Gag-CM9-positive cells, while the levels of Tat-SL8-positive cells remained stable after the second immunization. Consistent with previous studies (29–31), the magnitude of Gag-specific responses was consistently higher than that of Tat-specific responses. We also detected robust CD8+ T cell responses to the immunodominant Gag CM9 and Tat SL8 epitopes of SIV by tetramer staining in lymphocytes derived from LNs and RBs that were collected twice prior to SIV challenge (1 week post-second immunization, i.e., during the effector phase) and 5 weeks before challenge, i.e., during the memory phase (data not shown).

To functionally characterize the development of vaccine-induced SIV-specific T cell responses, we stimulated PBMCs from each vaccinated RM with overlapping peptide pools of 15-mers from SIVmac239 Gag and assessed the production of IFN-γ, TNF-α, and IL-2 and the expression of CD107a by multiparametric flow cytometry. Both AdC6/SIV- and AdC7/SIV-immunized RMs showed a total number of SIV-specific T cells ranging from 0.5 to 2% CD8+ T cells (Fig. 2E) and 0.2 to 1.0% CD4+ T cells (Fig. 2F), with a nonsignificant trend toward stronger CD4+ T cell responses in AdC7/6-vaccinated animals. The majority of the responding T cells showed only one or two functions, and no significant difference in the multifunctionalities of the vaccine-induced CD8+ and CD4+ T cell responses was observed between AdC6/7- and AdC7/6-immunized animals (data not shown). Collectively, these data indicate that expression of SIV
antigens from AdC6 and AdC7 vectors effectively induced robust levels of functional SIV-specific CD8+ T cells.

Effects of AdC-SIV immunizations on the levels of CD4+ CCR5+ and CD4+ Ki-67+ T cells. A potential side effect of any candidate AIDS vaccine is enhancement of the risk of HIV acquisition by increasing the levels of CD4+ CCR5+ and/or activated T cells, which act as primary targets for the infection. To examine this possibility, we measured the levels of CD4+ CCR5+ and proliferating (i.e., CD4+ Ki-67+) T cells in the blood, lymph nodes, and rectal-biopsy specimens of the AdC6/7- and AdC7/6-vaccinated animals at various time points during the immunization procedure. As shown in Fig. 3A, the level of CD4+ CCR5+ T cells showed only a transient increase in peripheral blood after the second immunization (Fig. 3A). We next examined the levels of CD4+ CCR5+ T cells at two time points after the second immunization in the lymph nodes and rectal-biopsy specimens and observed stable levels of the cells (Fig. 3B and C), although no preimmunization baseline samples were available for the tissues. We also examined the levels of CD4+ Ki-67+ T cells at the same time points and in the same tissues (Fig. 3D to F) and found that the levels of these cells remained stable in blood between baseline (i.e., prior to vaccination) and the latest time point before SIV challenge. Of note, a transient peak in CD4+ Ki-67+ T cells was observed a week after the second immunization (Fig. 3D). When the levels of CD4+ Ki-67+ T cells were examined in lymph nodes or rectal biopsy specimens at two time points after the second immu-
nization (Fig. 3E and F), we observed relatively stable levels of the cells in both tissues. Again, no preimmunization baseline samples were available for the tissues. Taken together, these data indicate that the immunization regimen used induced only a transient increase in the levels of CD4+ CCR5+ and CD4+ Ki-67+ T cells in peripheral blood.

Acquisition of SIV after repeated low-dose intrarectal challenge. In the next phase of this study, all RMs (group 1, AdC6/7; group 2, AdC7/6; and group 3, controls) were challenged biweekly up to 15 times with a low dose (300 TCID₅₀) of SIVmac239 that was given intrarectally. The number of challenges required to acquire the infection (defined as a viral load of >1,000 copies/ml of plasma) is shown in Fig. 4. When the entire cohort of RMs was examined (Fig. 4A), we observed that all controls were infected after 11 challenges and all AdC6/7-SIV-vaccinated animals were infected after 13 challenges. In contrast, 2 out of 10 animals of the AdC7/6-SIV group remained uninfected after 15 challenges, corresponding to an apparent 20% protection from SIV acquisition.

FIG 3 Levels of CCR5+ and proliferating CD4+ T cells after AdC-SIV immunization. (A to C) Average levels of CD4+ CCR5+ T cells measured as percentages of total CD3+ CD4+ T cells in peripheral blood (A), lymph nodes (B), and rectal-biopsy specimens (C) of AdC6/7- and AdC7/6-vaccinated RMs. (D to F) Average levels of CD4+ Ki-67+ T cells measured as percentages of total CD3+ CD4+ T cells in peripheral blood (D), lymph nodes (E), and rectal-biopsy specimens (F) from the same animals. The error bars indicate SD.

FIG 4 Viral acquisition after low-dose rectal SIVmac239 challenge. Shown are the numbers of challenges required for acquisition of SIV infection in AdC6/7- and AdC7/6-vaccinated RMs, as well as unvaccinated controls.
that was not statistically significant. Of note, one of the control RMs showed persistently undetectable viremia after the first positive result (i.e., ≤1,000 SIV RNA copies/ml of plasma after challenge 8) and failed to seroconvert for SIV-specific antibodies; as such, we excluded this animal from further analysis, given its uncertain infection status.

Somewhat intriguingly, when we analyzed separately the RMs that were challenged 5 months after the last immunization (i.e., four animals from group 1, four from group 2, and six controls), we observed protection from challenge of 50% (i.e., 2 out of 4 animals) of the AdC7/6-SIV group, with a likelihood of significant difference between AdC7/AdC6-SIV-vaccinated and naive animals, with a $P$ value of 0.047 (data not shown). As our study was neither designed nor powered to perform this type of subset analysis, we decided to report this observation without any further discussion. We therefore concluded that the AdC6/7-SIV and AdC7/6-SIV immunization regimens used did not result in significant protection from SIV transmission in this cohort of SIV-challenged RMs.

**AdC-SIV-vaccinated RMs show lower virus replication after breakthrough SIV infection.** Previous studies indicated that Ad-based candidate AIDS vaccines expressing SIV Gag resulted in lower levels of virus replication in SIV-infected RMs than in control animals (20–22). To determine whether the immunization regimens used in the current study exhibited the same effect, we monitored the levels of SIV viremia at multiple time points and up to day 180 postinfection, i.e., when all animals were sacrificed. As shown in **Fig. 5A**, all RMs experiencing breakthrough SIV infection showed a typical trend of virus replication characterized by a peak of viremia in the range of $10^6$ to $10^8$ copies/ml of plasma, followed by a postpeak decline that led to the chronic “setpoint” viremia in the range of $10^3$ to $10^6$ copies/ml of plasma. Importantly, the level of setpoint viremia was approximately 1 log unit lower in AdC6/7 RMs than in controls and close to 2 log units lower in AdC7/6 animals than in controls. As shown in **Fig. 5B**, the difference in viremia between AdC7/6 RM and controls was significant at days 90 and 120 postinfection ($P = 0.0355$ and $P = 0.03393$, respectively), while at later time points, the differences were no longer significant. Collectively, these results indicate that both AdC-based immunization regimens resulted in improved control of virus replication after the acute phase of infection and that this effect was more pronounced in AdC7/6-immunized RMs between days 90 and 120 postinfection.

**Dynamics of CD4$^+$ T cells in blood, lymph nodes, and rectal-biopsy specimens after breakthrough SIV infection.** We next examined the dynamics of circulating CD4$^+$ T cells, as well as lymph node- and rectal-biopsy specimen-based CD4$^+$ T cells, in the RMs included in the current study that experienced breakthrough SIV infection. As shown in **Fig. 6A** to **D**, most of the animals showed an expected decline of circulating CD4$^+$ T cells measured as either the absolute number of cells per mm$^3$ of blood or the percentage of CD3$^+$ T cells. Interestingly, no significant differences were observed between AdC6/7- or AdC7/6-immunized RMs and control.
animals throughout the study in terms of peripheral CD4^+ T cell
counts, even though at day 70 after infection, AdC6/7 RMs
showed a nonsignificant trend (P = 0.052) toward higher CD4^+ T
cell counts than control animals. Similarly, no significant differ-
ences were observed between AdC6/7- or AdC7/6-immunized
RMs and control animals in terms of CD4^+ T cell levels in the
lymph nodes (Fig. 6E and F). Finally, as expected, all RMs experi-
encing a breakthrough SIV infection exhibited a rapid and sub-
stantial decline in their CD4^+ T cells in the rectal-biopsy speci-
mens (Fig. 6G and H), with the cells showing a trend toward

FIG 6 Dynamics of CD4^+ T cells in the blood, lymph nodes, and rectal-biopsy specimens after breakthrough SIV infection. Circulating levels of CD4^+ T cells were measured as absolute counts per cubic millimeter of blood (A to C) and percentages of CD3^+ T cells (B to D) at multiple time points after breakthrough SIV infection in individual animals (A and B) and averaged within each study group (C and D). (E and F) Lymph node CD4^+ T cell levels were measured as percentages of CD3^+ T cells in individual animals (E) and averaged within each study group (F). (G and H) CD4^+ T cell levels in rectal-biopsy specimens were also measured as percentages of CD3^+ T cells in individual animals (G) and averaged within each study group (H). AdC6/7, AdC6/7-SIV-vaccinated RMs; AdC7/6, AdC7/6-SIV-vaccinated animals. The error bars represent the SD of the different study groups.
higher levels in AdC6/7-vaccinated animals than in controls ($P = 0.051$) at day 175 postinfection. Taken together, these results indicate that the transient partial control of virus replication observed in AdC7/6-SIV-immunized RMs after breakthrough infection was not associated with significantly better preservation of the CD4$^+$ T cell pool.

**Lack of a relationship between the magnitude and functionality of SIV Gag-specific T cell responses before challenge and SIV acquisition.** To investigate the relationship between the AdC6/7- or AdC7/6-induced T cell response and protection from SIV acquisition during the challenge phase of our experiment, we first performed a correlation analysis between the total levels of CD8$^+$ T cell-mediated responses prior to challenges (measured as a fraction of total CD8$^+$ T cells by tetramer staining) and the risk of acquiring breakthrough infection. As shown in Fig. 7A to C, we found no correlation between the total levels of SIV Gag-specific CD8$^+$ T cell responses in peripheral blood, lymph nodes, and rectal-biopsy specimens prior to challenge and the number of SIV challenges required to successfully infect the RMs. Similarly, no correlation was found between the level of SIV Tat-specific responses in these tissues and the risk of virus acquisition (data not shown). In addition, we found no significant association between the total levels of SIV Gag-specific T cells (as measured by intracellular cytokine staining after in vitro peptide stimulation) and the risk of acquiring SIV infection (data not shown).

**We next investigated a potential relationship between polyfunctionality of CD8$^+$ T cell responses (measured as the fraction of cells producing IFN-γ, TNF, and IL-2 and expressing CD107 in response to SIV Gag peptides) in peripheral blood and the number of SIV challenges required to transmit SIV (Fig. 7D) and observed no significant correlation. A similar lack of correlation was observed for the level and functionality of SIV Gag-specific CD4$^+$ T cell responses (data not shown). Collectively, these results show an absence of significant correlation between the magnitude or functionality of AdC-induced SIV-specific T cell-mediated responses and the risk of acquiring SIV in this model of repeated low-dose intrarectal challenge.

**Lack of a relationship between levels of target CD4$^+$ T cells before challenge and risk of SIV acquisition.** We next examined, in our cohort of AdC-vaccinated RMs, the relationship between the level of CD4$^+$ T cells that may serve as targets for SIV infection (measured as a percentage of CD4$^+$ CCR5$^+$ and CD4$^+$ Ki-67$^+$ T cells in both blood and rectal-biopsy specimens at the last time point prior to challenge) and protection from SIV acquisition. As shown in Fig. 8A to D, we plotted the percentage of CD4$^+$ CCR5$^+$ (Fig. 8A and C) and CD4$^+$ Ki-67$^+$ T cells (Fig. 8B and D) observed in blood (Fig. 8A and B) and rectal-biopsy specimens (Fig. 8C and D) prior to challenge and the number of virus exposures necessary to achieve a breakthrough SIV infection. This analysis revealed no correlation between the examined markers of CD4$^+$ “target cells” in either peripheral blood or rectal-biopsy specimens prior to challenge and the numbers of SIV challenges required to success-

**FIG 7 Relationship between levels and functionality of SIV-specific CD8$^+$ T cells and number of challenges needed to achieve SIV infection. (A to C) The levels of SIV-specific CD8$^+$ T cells in peripheral blood, lymph nodes, and rectal-biopsy specimens as measured by tetramer staining for the MamuA*01-restricted SIV Gag CM9 epitope before challenge do not predict the risk of acquiring SIV infection during the repeated intrarectal low-dose challenge. AdC6/7, AdC6/7-SIV-vaccinated RMs; AdC7/6, AdC7/6-SIV-vaccinated animals. (D) Lack of correlation between the number of functions displayed by SIV Gag-specific CD8$^+$ T cells and the number of challenges needed to acquire SIV infection. HD indicates the animals that were infected with a high dose of SIV at the end of the repeated low-dose challenge phase.**
fully infect the animals. Together with the data presented in Fig. 7, these results suggest that the risk of acquiring SIV in this model of repeated low-dose intrarectal challenge is mediated by complex factors that cannot be defined as specific features of the vaccine-induced T cell response or as a level of target cells for the virus.

DISCUSSION

Despite a massive effort by the research community, a safe and effective AIDS vaccine is not yet available. In the current study, we used the well-established preclinical model of SIV immunization and challenges in RMs to assess the immunogenicity and protection from virus acquisition of two AdC vector-based heterologous prime-boost vaccination regimens. Ten RMs were vaccinated with AdC6 followed by AdC7 (AdC6/7) expressing SIV Gag/Tat; 10 were vaccinated with AdC7 followed by AdC6 (AdC7/6), also expressing SIV Gag/Tat; and 10 unvaccinated animals were used as controls. Two key aspects of the experimental system used are the lack of an envelope immunogen and the choice of a repeated low-dose intrarectal challenge. As such, the study was designed to determine the effect of the vector-induced cellular immune responses on SIV acquisition (and SIV viremia in the event of breakthrough infection) in the absence of any Env-specific antibodies. The rationale for this approach lies in the strong cellular immunogenicity of Ad vectors (14, 15) and demonstrations that cellular immune responses can prevent/abort infection in the setting of a CMV-based vector platform (12, 13). To our knowledge, this is the first preclinical trial of this kind using AdC-based vectors ever performed.

The key results of this preclinical study are that (i) AdC6/7-SIV and AdC7/6-SIV vectors showed robust immunogenicity that was comparable to that of the leading candidate AIDS vaccine platforms in terms of the magnitude and function of the induced cellular response (17, 31); (ii) both AdC-based vectors had only minor effects on the level of potential target cells (i.e., CD4\(^+\) Ki-67\(^+\) and CD4\(^+\) CCR5\(^+\) T cells); (iii) neither immunization regimen was associated with significant protection from SIV acquisition; (iv) breakthrough infections in the AdC7/6-vaccinated animals were associated with a significant, although transient, ~2-log-unit reduction in SIV setpoint viremia that persisted for ~4 months postinfection; and (v) no obvious immunological correlate of protection from either SIV acquisition or postinfection virus replication could be identified.

In previous studies conducted in mice, we compared AdC6 prime-AdC7 boost to AdC7 prime-AdC6 boost vaccine regimens (32) and observed that the sequences induced transgene product-specific CD8\(^+\) T cell responses of comparable magnitudes, a finding consistent with the results of the current nonhuman primate study. As such, it is unlikely that differences in the magnitudes of the responses induced by the two vector regimens can explain the trend toward higher efficacy of the AdC7/6 regimen in protecting from SIV replication. AdC6 and AdC7, which belong to distinct serotypes of family E of the Adenoviridae, differ in two respects. First, AdC6 vectors induce more potent inflammatory responses than AdC7 vectors (H. C. J. Ertl, unpublished observations), and differential activation of innate immunity may influence the functionality of adaptive immune responses. Second, the two vectors carry distinct deletions in E3, a domain that is not essential for viral replication but encodes polypeptides that subvert immune responses. Specifically, AdC6 has a deletion of all E3 open reading frames (ORFs), while AdC7 carries only a partial deletion of ORFs.
REFERENCES


