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

Barbara Cervasi, Emory University
Diane G. Carnathan, Emory University
Katherine M. Sheehan, Emory University
Luca Micci, Emory University
Mirko Paiardini, Emory University
Raj Kurupati, Wistar Institute
Steven Tuyishime, Wistar Institute
Xiang Yang Zhou, Wistar Institute
Jim Else, Emory University
Sarah J. Ratcliffe, University of Pennsylvania

Only first 10 authors above; see publication for full author list.

Journal Title: Journal of Virology
Volume: Volume 87, Number 17
Publisher: American Society for Microbiology | 2013-09, Pages 9420-9430
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/JVI.01456-13
Permanent URL: http://pid.emory.edu/ark:/25593/fk7q7

Final published version: http://jvi.asm.org/content/87/17/9420

Copyright information:
© 2013, American Society for Microbiology. All Rights Reserved.
Accessed August 10, 2019 8:07 PM EDT
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.

The global spread of the human immunodeficiency virus (HIV) pandemic, currently affecting over 30 million individuals worldwide, emphasizes the urgency to develop a safe and effective HIV/AIDS vaccine. However, this effort is difficult due to several aspects of HIV biology, including (i) the extreme heterogeneity of the virus, (ii) the absence of clear correlates of immune protection against either transmission or disease progression, (iii) the ability of the virus to become immunologically silent when the infection is latent, and (iv) the fact that any vaccine-induced immune response to HIV may generate virus-specific, activated memory CD4+ T cells that are preferential targets for the virus and thus may favor its transmission (reviewed in references 1 and 2).

Due to the current absence of immunogens that can elicit HIV-specific broadly neutralizing antibodies, numerous vaccine strategies that aim at eliciting antiviral CD8+ T cell-mediated cytotoxic-T-lymphocyte (CTL) responses have been developed (reviewed in reference 3). Indeed, several lines of evidence indicate that HIV/simian immunodeficiency virus (SIV)-specific CTLs may control virus replication during HIV/SIV infection (4–11) and possibly even prevent and/or abort the infection in the setting of specific vaccination regimens (12, 13). Adenovirus (Ad) vectors present several features that made them a promising AIDS vaccine platform, particularly in terms of generating strong CTL responses to viral antigens (reviewed in reference 14). A striking feature of Ad vectors is that they persist for prolonged times at low levels and continue to produce their transgene products, resulting in persistent populations of transgene-specific “effector” CD8+ T cells in both peripheral and lymphatic tissues (15).

Several replication-defective human adenoviruses (AdHu), including AdHu serotype 5 (AdHu5), AdHu35, and AdHu26, have been tested as HIV/SIV vaccines in nonhuman primates (NHPs) and small-scale phase I/II clinical trials, indicating that these vectors are highly immunogenic and may confer significant protection against SIV replication (16–22). Unfortunately, a large-scale phase Ib clinical trial of AdHu5 as a candidate AIDS vaccine (the Merck STEP trial) failed to protect from HIV acquisition, with preexisting neutralizing antibodies to the vector reducing the level of vaccine-induced HIV-specific T cell responses and possibly increasing the risk of HIV acquisition (23). To circumvent preexisting immunity to AdHu5, as well as other serotypes, we developed chimpanzee adenoviruses (AdC) as vectors due to very low prevalence rates of neutralizing antibodies against these viruses in the human population (24). AdC vectors based on various serotypes (SAd-V23/AdC6, SAd-V24/AdC7, and SAd-V25/AdC68) are...
Immunization of Macaques with AdC-SIV

In this study, we assessed immunogenicity and protection against low-dose intrarectal SIVmac239 challenges in two groups of 10 rhesus macaques (RMs) vaccinated with a heterologous AdC prime-boost regimen using AdC7 expressing a Gag-Tat fusion protein (AdC7gagtat) for priming, followed by an AdC6gagtat boost (AdC7/6 group) and vice versa (AdC6/7 group). Ten additional RMs were used as unvaccinated controls. We observed that both AdC6/7 and AdC7/6 induced robust SIV-specific T cell responses as measured by tetramer staining and functional assays. While no significant protection from SIV transmission was observed in either group of vaccinated RMs, AdC7/6-SIV immunization was associated with a transient but significant decrease in the setpoint viral load. The observed immunogenicity and partial protection from virus replication in the absence of any envelope immunogen suggest that AdC-based vectors represent a promising platform for candidate AIDS vaccines.

**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 × 10^12. 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 adenovirus 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 10^11 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]) that was provided by Chris Miller and Koen Van Rampay 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, fluoro-phore (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, andanalyzed 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 H14A3; BD Biosciences, San Jose, CA) was added at the start of all stimulation periods. The cocktail of antibodies for surface staining included Aqua Blue Viability Dye (Invitrogen), CD8 QD705 (clone 3B5; Invitrogen), CD14 QD655 (clone TuK4; Invitrogen), CD20 QD655 (clone H147; Invitrogen), CD4 APC-Cy7 (clone OKT-4; BD Biosciences), CD95 PE-Cy5 (clone DX2; BD Biosciences), and CD28 ECD (clone CD28.2; Beckman Coulter). The cocktail for intracellular staining included CD3 Pacific Blue (clone SP34-2; BD Biosciences), gamma interferon (IFN-γ) APC (clone B27; BD Biosciences), interleukin 2 (IL-2) PE (clone rat MQ1-17H12; BD Biosciences), and tumor necrosis factor alpha (TNF-α) Alexa 700 (clone 700 mAb11; BD Biosciences).

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 to meet the assumption of normality in the null model. Differences in the multifunctionalities of the responding T cells showed only one or two functions, and no significant difference in the multifunctionalities of the vaccine-induced CD8+ T cell responses was observed between AdC6/7- and AdC7/6-immunized animals.

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 TCID50) 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, virologic, and immunologic 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 TCID50) 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 using multiparametric flow cytometry. Both AdC6/7-SIV- and AdC7/6-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-
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.
that was not statistically significant. Of note, one of the control RM 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 controls (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 after virus acquisition 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 RMs 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 mm3 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 differences 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 experiencing a breakthrough SIV infection exhibited a rapid and substantial decline in their CD4$^+$ T cells in the rectal-biopsy specimens (Fig. 6G and H), with the cells showing a trend toward
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 responses prior to challenges (measured as a fraction of total CD8$^+$ T cells by tetramer staining) and the risk of acquiring SIV 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 virions 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 RM's, 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-
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$^+$ CCR5$^+$ and CD4$^+$ Ki-67$^+$ 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.
2 to 4. In spite of the deletion of the E1 domain, which encodes crucial transcriptional regulators, Ad vectors persist in vivo with some limited production of its viral antigens (15). AdC7, unlike AdC6 vectors, could produce E3 polypeptides that block apoptosis through TNF-α, Fas, or TRAIL, which in turn could affect the duration of antigen presentation (33).

In this study, we attempted to determine whether any specific aspects of the cellular immune response induced by the AdC7-based vectors were associated with protection from SIV acquisition or from SIV viremia after breakthrough infection (data not shown). However, this extensive analysis did not identify any specific correlation. Furthermore, no correlation was observed between the risk of SIV acquisition and the level of potential CD4+ target cells for the virus in mucosal tissues. This apparent lack of obvious immune correlates of protection is in fact not surprising and likely reflects the extreme complexity of the interaction between SIV and the various immunological effects elicited by AdC7-based vectors (or any vectors) in terms of SIV-specific cellular immune responses, activation of innate immune pathways, and changes in the overall mucosal microenvironment. An ongoing analysis of the transcriptional profile in the blood of a subset of RMs that showed increased susceptibility or, conversely, increased resistance to SIV acquisition may help us identify molecular correlates of protection from SIV acquisition conferred by the tested AdC7-based candidate AIDS vaccines.

We believe that the results of the current study of AdC7-based candidate AIDS vaccines are of interest, as they provide novel information on the immunogenicity of these vectors and their potential effect in protecting from both SIV acquisition and virus replication after breakthrough infection. At the same time, we also acknowledge that the observed protection from virus replication disappeared between 4 and 6 months postinfection, and no significant protection from virus transmission was observed in this study. This lack of persistent protection from virus replication despite robust immunogenicity was also observed when RMs were immunized with AdHu5 vectors and challenged with SIVmac239 (34). To improve the efficacy of an SIV-expressing AdC7/6 immunization regimen, we are currently planning a number of follow-up studies that involve (i) the addition to the protocol of Env immunogens (i.e., AdC7-based vectors expressing SIV-Env, as well as those expressing the SIV Gag/Tat fusion protein) that will elicit Env-specific humoral immune responses; (ii) specific modifications of the vector that may improve its immunogenicity, such as the use of mosaic transgene products (35); and (iii) the inclusion of mucosal adjuvants that may enhance the antiviral innate immune response. Hopefully, these studies will better define the clinical potential of AdC7-based immunogens as candidate AIDS vaccines.

ACKNOWLEDGMENTS

This work was supported by NIH grant IPCAVD U19 AI074078-01 (to H.C.J,E. and G.S.). In addition, it was supported by NIH/NCCR P51RR000165 and is currently supported by the Office of Research Infrastructure Programs grant OD P51OD011132 to the Yerkes National Primate Research Center. Steven Tuylisime is the recipient of an HHMI Gilliam Fellowship.

We thank the Emory Center for AIDS Research (CFAR) Virology core for their technical support.

REFERENCES


