Low-Dose Penile SIVmac251 Exposure of Rhesus Macaques Infected with Adenovirus Type 5 (Ad5) and Then Immunized with a Replication-Defective Ad5-Based SIV *gag/pol/nef* Vaccine Recapitulates the Results of the Phase IIb Step Trial of a Similar HIV-1 Vaccine

Huma Qureshi,^{a,b} Zhong-Min Ma,^{a,b} Ying Huang,^c Gregory Hodge,^b Michael A. Thomas,^d Janet DiPasquale,^d Veronique DeSilva,^{a,b} Linda Fritts,^{a,b} Andrew J. Bett,^e Danilo R. Casimiro,^e John W. Shiver,^e Marjorie Robert-Guroff,^d Michael N. Robertson,^e Michael B. McChesney,^b Peter B. Gilbert,^c and Christopher J. Miller^{a,b}

Center for Comparative Medicine^a and California National Primate Research Center, University of California, Davis, California, USA^b; SCHARP, Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA^c; Vaccine Branch, NCI, NIH, Bethesda, Maryland, USA^d; and Merck Research Laboratories, West Point, Pennsylvania, USA^e

The Step Trial showed that the MRKAd5 HIV-1 subtype B Gag/Pol/Nef vaccine did not protect men from HIV infection or reduce setpoint plasma viral RNA (vRNA) levels but, unexpectedly, it did modestly enhance susceptibility to HIV infection in adenovirus type 5 (Ad5)-seropositive, uncircumcised men. As part of the process to understand the results of the Step Trial, we designed a study to determine whether rhesus macaques chronically infected with a host-range mutant Ad5 (Ad5hr) and then immunized with a replication defective Ad5 SIVmac239 Gag/Pol/Nef vaccine were more resistant or susceptible to SIV infection than unimmunized rhesus macaques challenged with a series of escalating dose penile exposures to SIVmac 251. The Ad5 SIV vaccine induced CD8⁺ T cell responses in 70% of the monkeys, which is similar to the proportion of humans that responded to the vaccine in the Step Trial. However, the vaccine did not protect vaccinated animals from penile SIV challenge. At the lowest SIV exposure dose (10³ 50% tissue culture infective doses), 2 of 9 Ad5-seropositive animals immunized with the Ad5 SIV vaccine became infected compared to 0 of 34 animals infected in the other animal groups (naive animals, Ad5-seropositive animals immunized with the empty Ad5 vector, Ad5-seronegative animals immunized with the Ad5 SIV vaccine, and Ad5-seronegative animals immunized with the empty Ad5 vector). Penile exposure to more concentrated virus inocula produced similar rates of infection in all animal groups. Although setpoint viral loads were unaffected in Step vaccinees, the Ad5 SIV-immunized animals had significantly lower acute-phase plasma vRNA levels compared to unimmunized animals. Thus, the results of the nonhuman primate (NHP) study described here recapitulate the lack of protection against HIV acquisition seen in the Step Trial and suggest a greater risk of infection in the Ad5-seropositive animals immunized with the Ad5 SIV vaccine. Further studies are necessary to confirm the enhancement of virus acquisition and to discern associated mechanisms.

In September 2007, a double-blind phase IIb Step Trial of the Merck adenovirus 5 (Ad5) HIV-1 subtype B Gag/Pol/Nef vaccine was halted when interim analysis showed that the vaccine appeared to be no better than a placebo in preventing HIV infection (2, 30). The Step Trial enrolled 3,000 volunteers at 34 sites in North America, the Caribbean, South America, and Australia, where HIV-1 subtype B is predominant. The vaccine elicited CD8⁺ HIV-specific T cells in 73% of vaccinees, and a similar proportion of infected and uninfected vaccinees had these responses (2, 30). Unexpectedly, there were an increased number of HIV-1 infections in Ad5-seropositive male vaccine recipients (2). Although there was no overall effect of vaccination on setpoint plasma viral RNA (vRNA) in HIV-infected Step vaccinees (2), additional analyses found that in a subset of the immunized Step Trial participants with specific major histocompatibility complex class I (MHC-I) alleles there was sieving of HIV genotypes and a trend toward lower setpoint plasma vRNA levels (12).

Before additional clinical efficacy trials of vector-based HIV vaccines are undertaken, it would be prudent to understand the immunologic and virologic mechanisms that explain these results. We describe here the development of an animal model that will allow these studies to be conducted and present the results of an initial attempt to recapitulate the key findings of the Step Trial in

an animal model. SIVmac251 infection of rhesus macaques (RM) recapitulates most features of HIV pathogenesis, including infection of CD4⁺ CCR5⁺ target cells (T cells, macrophages, and dendritic cells), unrelenting virus replication that leads eventually to AIDS, chronic aberrant immune activation, and systemic infections that are generally founded by one to a few virus variants after rectal, vaginal, or penile SIV exposure (8, 9, 14, 17, 20, 24, 27, 31–33, 35, 42, 44, 45). Thus, the RM/SIV model can be used to study the complex host-virus interactions in HIV infection. The goal of the present study was to determine whether RM chronically infected with a host range mutant adenovirus 5 (Ad5hr) and then immunized with an Ad5 SIVmac239 Gag/Pol/Nef vaccine were more resistant or susceptible to a series of repeated penile SIVmac 251 exposures than unimmunized RM.

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Address correspondence to Christopher J. Miller, cjmiller@ucdavis.edu.

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MATERIALS AND METHODS

Animals. All animals used in the present study were adult male RM (*Macaca mulatta*) housed at the California National Primate Research Center in accordance with the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care International Standards and with the recommendations in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (NIH). The Institutional Animal Use and Care Committee of the University of California, Davis, approved these experiments (protocol 11479). For blood collection, animals were anesthetized with 10 mg of ketamine hydrochloride (Park-Davis)/kg or 0.7 mg of tiletamine HCl and zolazepam (Telazol; Fort Dodge Animal Health, Fort Dodge, IA)/kg injected intramuscularly. All efforts were made to minimize suffering. Details of animal welfare and steps taken to ameliorate suffering were in accordance with the recommendations of the Weatherall report, "The Use of Nonhuman Primates in Research" (44a). Animals were housed in an air-conditioned facility with an ambient temperature of 21 to 25°C, a relative humidity of 40 to 60%, and a 12-h light/dark cycle. Animals were individually housed in suspended stainless steel wire-bottom cages and provided with a commercial primate diet. Fresh fruit was provided once daily, and water was freely available. Animals were assigned to experimental groups to distribute the TRIM5α genotype, MHC-I genotype, and country of origin as evenly as possible (Table 1).

Study design, Ad5hr inoculation and replication-incompetent Ad5 SIV vaccine. Eighteen monkeys were infected with Ad5hr, fully competent for replication in monkey cells (19), by three sequential mucosal inoculations in order to induce natural immunity to the vector prior to immunization with the Ad5 SIVmac239 *gag/pol/nef* vaccine. At 20 weeks prior to immunization with the Ad5 SIVmac239 vaccine, Ad5hr was administered both orally and nasally at a dose of 1.5×10^9 PFU (Fig. 1), and at 12 and 8 weeks prior to immunization with the Ad5 SIVmac239 vaccine, 1.5×10^9 PFU of Ad5hr was administered intratracheally (Fig. 1). One of the eighteen animals was euthanized for medical reasons unrelated to the study prior to the third Ad5hr inoculation. Eight weeks after the third Ad5hr inoculation (Fig. 1), nine of the Ad5hr-infected macaques were immunized with 10^{10} particles of each individual rAd5 SIV *gag/pol/nef* vector Ad5 SIVmac239 *Gag/Pol/Nef* (4, 40). This group is designated as the Ad5 Vx-SIV group. The other eight Ad5hr-infected macaques were inoculated with 10^{10} particles of the empty replication-incompetent MRKAd5 vector, and this group is designated as the Ad5 Vx-empty group. In addition, nine macaques designated as the Vx-SIV group were never exposed to Ad5hr, but they were immunized with the Ad5 SIV vaccine, and another nine macaques, never exposed to Ad5hr, were inoculated with the empty replication-incompetent Ad5 vector and were designated the Vx-empty group. At 4 and 24 weeks after the primary immunization, the animals were boosted with the same vector construct and then 1 week later they were challenged by penile exposure to SIV (Fig. 1). The interval between the last immunization and when Step Trial participants were HIV exposed is unknown, but sexual activity was uninterrupted by the vaccine protocol, thus to model HIV exposure soon after the complete vaccine regimen was completed and when vaccine-induced immunity and the potential for protection were greatest, SIV exposures began 1 week after the last immunization. Thus, animals would be expected to have strong SIV-specific and Ad5-specific immunity at the time the SIV exposures were begun. Eight animals never exposed to Ad5hr, the Ad5 vector, or SIV antigens served as controls (naive control group) (Fig. 1). The study endpoints were SIV infection and plasma vRNA levels after challenge.

Penile SIVmac251 exposure/challenge. A cell-free stock of SIVmac251 (UCD-6/04) was produced in *Staphylococcus enterotoxin A*-stimulated rhesus monkey peripheral blood mononuclear cells (PBMC) (42) and used for these studies. This SIVmac251 stock (UCD-6/04) contains ca. 10^9 vRNA copies/ml and 10^5 50% tissue culture infection doses (TCID₅₀)/ml when titered on CEMX174 cells. On the day of challenge, serial 10-fold dilutions of the SIV stock were made in phosphate-

buffered saline; a 2-ml portion of the virus inoculum was used for all of the penile exposures in the present study. For the penile SIVmac251 challenge inoculation, we modified our previously published procedure (27). Briefly, a small cup was fashioned from a closed end of a disposable 15-ml conical centrifuge tube, and 2 ml of virus inoculum was placed into the cup. The penis was extended, the foreskin was retracted, and the glans and shaft were inserted into the inoculum and held there for 5 min. Then, the penis was allowed to retract into the foreskin and the animal was placed into its cage in dorsal recumbency. An additional 250 μ l of virus inoculum was then dropped into the space between the foreskin and the glans, and the animals were allowed to recover from anesthesia. Animals in the present study could be exposed 10 times to an inoculum of 10^3 TCID₅₀, 10 times to an inoculum of 10^4 TCID₅₀, and twice in 1 day to an inoculum of 10^5 TCID₅₀ (Fig. 1). Although not all animals were challenged on the same day, the study was scheduled so that Ad5 Vx-SIV and Ad5 SIV-empty animals were challenged on the same days and Vx-SIV and SIV-empty animals were challenged on the same days. The SIV inoculation series for a particular animal was stopped when vRNA levels steadily increased in two consecutive plasma samples.

Detection and quantitation of Ad5hr DNA in nasal secretions. For several days following infection, nasal secretions were collected and stored at -70°C . A nested PCR assay (3) that amplifies a portion of the Ad5 fiber gene was used to detect Ad5 DNA. If a sample was positive in the nested PCR assay, and then a TaqMan PCR was used to measure the number of adenovirus DNA copies in 5 μ l of sample, and all samples were tested in duplicate. For the TaqMan assay, the forward primer was 5'-TTC GAT GAT GCC GCA GTG GTC TTA CAT GCA C-3', the reverse primer was 5'-TTT CTA AAC TTG TTA TTC AGG CTG AAG TAC G-3', and the probe was 5'-FAM-CCG GGT CTG GTG CAG TTT GCC CGC as previously published (7). A standard curve made using serial 10-fold dilutions of the pCR-Ad5/Ad6 plasmid (nucleotides [nt] 18697 to 19197 of the Ad5hr exon gene cloned into TOPO2.1) was used to quantify copies in the samples. Briefly, 5- μ l preboiled (95°) samples were added to a solution containing 12.5 μ l of ABI Universal master mix, 2.0 μ l of 10 μ M Ad5/6 forward primer, 2.0 μ l of 10 μ M Ad5/6 reverse primer, 0.625 μ l of 10 μ M Ad5 probe, and 2.875 μ l of water. The default amplification protocol for the ABI Sequence Detection System 7000 was used. Samples below the detection limit (100 copies) were reported as negative. The nested PCR assay was used as a screening assay on all samples (Fig. 2A), and the TaqMan PCR assay was used to estimate copy number in samples that were positive in the nested PCR assay (Fig. 2B).

Assessment of Ad5hr antibody responses. Ad5 neutralizing antibody titers were determined as previously described (41). Plasma, heat inactivated at 56°C, was plated into 96-well flat-bottom plates (50 μ l of 4-fold dilutions) and incubated with 50 μ l of Ad5-luciferase stock (Ad5-Apt-Luc; Crucell Holland B.V., Leiden, Netherlands) at ca. 500 PFU/cell for 1 h at 37°C in 5% CO₂. A549 cells (10^4 in 100 μ l in F-12 Kaighn's medium containing 10% fetal bovine serum, 50 μ g of gentamicin/ml, and 2 mM glutamine) were added to each well. Positive controls consisting of virus plus cells with no plasma, and negative controls consisting of cells plus medium with no virus or plasma, were included in each plate. After incubation at 37°C in 5% CO₂ for 24 h, 100 μ l of medium was removed from each well and replaced with 100 μ l of BriteLite solution (BriteLite Plus 100 kit; Perkin-Elmer), mixed, and incubated for 2 min. Luciferase activity was detected by transferring 200 μ l of each cell-substrate mixture to a 96-well black flat-bottom plate and measuring the luminescence within 15 min using a Victor² 1420 multilabel counter (Perkin-Elmer). Neutralizing titers were defined as the plasma dilutions resulting in 50% reduction in luciferase activity relative to that of preinfection samples diluted 1:20.

SIV RNA isolation and cDNA synthesis. Plasma samples were thawed at room temperature, and RNA was isolated from 0.5 ml of plasma using a QIAamp Ultrasens viral kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol and eluted in 50 μ l. The vRNA was reverse transcribed into cDNA using SuperScript III reagents (Invitrogen, Carlsbad,

TABLE 1 Animals, immunization groups, and outcome of challenge

Immunization group	Animal no.	Animal group	Animal origin ^a	TRIM5 α genotype ^b	MHC-I allele(s) ^c	Peak plasma vRNA ^d
Immunized	33776	Ad5 Vx-SIV	1/8 Chinese	TFP/TFP		5.77
	35380	Ad5 Vx-SIV	Indian	Q/TFP	A02, A11	5.9
	35584	Ad5 Vx-SIV	1/4 Chinese	Q/TFP	B01	–
	35629	Ad5 Vx-SIV	Indian	Q/TFP		–
	36224	Ad5 Vx-SIV	Indian	TFP/TFP	A01	–
	36321	Ad5 Vx-SIV	1/8 Chinese	Q/TFP	A01	6.63
	36338	Ad5 Vx-SIV	Indian	TFP/TFP	B01	6.8
	36658	Ad5 Vx-SIV	1/4 Chinese	Q/TFP	A01	6.84
Unimmunized	36982	Ad5 Vx-SIV	Indian	Q/TFP	A02	–
	35987	Ad5 Vx-empty	Indian	Q/CypA		7.32
	36016	Ad5 Vx-empty	Indian	TFP/TFP	A01, B01	7.91
	36173	Ad5 Vx-empty	Indian	TFP/TFP	A01, A02	–
	36380	Ad5 Vx-empty	Indian	TFP/TFP	A11, B08	–
	36523	Ad5 Vx-empty	Indian	TFP/TFP		–
	36637	Ad5 Vx-empty	Indian	Q/Q	A02	6.61
	36961	Ad5 Vx-empty	Indian	Q/TFP	A08	8.1
Immunized	36965	Ad5 Vx-empty	Indian	Q/TFP	B03, B04	7.43
	35919	Vx-SIV	Indian	TFP/TFP	A01, A08	6.79
	35939	Vx-SIV	1/4 Chinese	Q/TFP	A02, B08	5.26
	36264	Vx-SIV	Indian	Q/TFP	A02, B08	–
	36272	Vx-SIV	Indian	Q/Q	B01	5.9
	36303	Vx-SIV	1/4 Chinese	TFP/TFP	B01	6.54
	36323	Vx-SIV	Indian	TFP/TFP		7.38
	36609	Vx-SIV	Indian	TFP/CypA		5.61
Unimmunized	36629	Vx-SIV	Indian	Q/TFP	A02	6.01
	37332	Vx-SIV	Indian	TFP/TFP	A02	–
	34310	Vx-empty	Indian	TFP/TFP	A02, B01, B17	6.93
	34976	Vx-empty	Indian	Q/TFP		7.44
	36270	Vx-empty	Indian	Q/Q	A02, B01	6.52
	36344	Vx-empty	Indian	TFP/TFP	A08	–
	36411	Vx-empty	Indian	Q/TFP	A01	7.21
	36520	Vx-empty	Indian	Q/TFP	B17	–
Unimmunized	36643	Vx-empty	Indian	Q/TFP	A11	6.44
	36784	Vx-empty	1/8 Chinese	Q/TFP	B08	7.19
	36859	Vx-empty	1/4 Chinese	TFP/TFP	A02	–
	36254	Naive control	Indian	TFP/CypA		7.44
	36503	Naive control	Indian	Q/TFP		–
	37302	Naive control	Indian	Q/TFP		–
	35581	Naive control	Indian	TFP/TFP		5.64
	36199	Naive control	Indian	TFP/TFP		7.93
Unimmunized	36368	Naive control	Indian	TFP/TFP		7.92
	36589	Naive control	1/8 Chinese	Q/CypA		7.61
	37079	Naive control	1/8 Chinese	TFP/TFP		5.47

^a The country of origin for the parental stock of the animal. The designation “Indian” indicates the animal’s parents were imported from India. For Indian $\times$ Chinese hybrid animals, the fraction of Chinese parentage is noted.

^b Trim5 α alleles that are associated with effects on SIV replication (18). There are three alleles listed, and the allele on each chromosome is indicated: TFP, Q and CypA. The TFP and CypA alleles are associated with lower plasma vRNA levels in SIV-infected RM (18, 23).

^c MHC-I alleles A01, B17, and B08 are associated with lower plasma vRNA levels in SIV-infected RM (26, 34).

^d Expressed as the log₁₀ vRNA copies/ml plasma. A dash indicates the animal was not infected.

CA) with 2 μ l of 50 μ M dT₂₃ VN, 2 μ l of 10 mM deoxynucleoside triphosphates, and 22 μ l of viral RNA. This mixture was heated to 65°C for 5 min, followed by incubation on ice for 2 min. A master mix of the following was then added: 8 μ l of 5 $\times$ first-strand buffer, 2 μ l of 0.1 M dithiothreitol, 2 μ l of RNase OUT recombinant RNase inhibitor (40 U/ μ l), and 2 μ l of SuperScript III RT (200 U/ μ l), followed by incubation at 25°C for 5 min, 50°C for 60 min, and 70°C for 15 min, followed in turn by the addition of 1 μ l of *Escherichia coli* RNase H (5U/ μ l) and additional incubation at 37°C for 20 min. cDNA was stored at –20 to –80°C until amplification.

Quantitative SIV RNA analysis. A well-described reverse transcription-PCR was used to detect and quantify SIVgag RNA levels in plasma samples (14, 43). The copy number of SIVgag was calculated based on standard curves for a SIVgag plasmid spanning a concentration range from 0.1 to 10⁸ copies. The limit of quantitation for the assay is 125 copies/ml based on plasmid spiking experiments with negative plasma. Although samples with 1 copy/ml were consistently detected in a well, 125 copies/ml were needed to place the point on the linear portion of the assay standard curve. Thus, a sample that was negative was graphed as

FIG 1 Overview of the experimental design. The designations used for each animal group are indicated in the left margin, and the dose of SIV used for each penile exposure is indicated by the colored hash marks.

having one copy/ml. The results were analyzed with SDS 7900 system software version 2.3 (Applied Biosystems). The results for each sample are reported as log₁₀ vRNA copies per ml of plasma RNA.

MHC-I haplotyping and TRIM 5α genotyping. MHC-I haplotyping was performed by the Rhesus Macaque MHC Typing Core, University of

Wisconsin Hospital and Clinics, as described previously (25). Trim5α polymorphisms were determined by amplifying and sequencing TRIM5α genes from genomic DNA using a described procedure (18). Based on the nucleotides at positions 339 to 349 of the B30.2/SPRY region, animals were classified into three categories: Trim5^{TFP}, Trim5^Q, or Trim5^{CYP} (Table 1). The Trim5^{TFP} and Trim5^{CYP} alleles have been reported to modestly suppress SIVmac251 replication in infected animals (18, 23).

Intracellular staining for cytokine production by SIV-specific T cells. For intracellular staining of PBMC, cryopreserved samples were thawed and rested overnight at 37°C in a 5% CO₂ atmosphere in RPMI 1640 medium (Gibco/Invitrogen, Inc.) containing 10% fetal calf serum. The next day, the cells were adjusted to 10⁶/100 μl and incubated with anti-CD28 and anti-CD49d antibodies (1 μg/ml, final concentration; BD Biosciences) as costimulatory molecules, in a total volume of 200 μl of RPMI 1640–10% fetal bovine serum. Samples were stimulated with two pools of peptides spanning SIV gag (including a pool of peptides that span p27), three pools of peptides spanning SIV pol, and one pool of peptides spanning SIV nef. All peptides were 20mers that overlapped by 10 amino acid residues (ProImmune, Inc.) that were stored at 5 μg/ml in dimethyl sulfoxide (DMSO). Negative control cultures contained costimulatory molecules and DMSO, and a positive control culture was stimulated with staphylococcal enterotoxin B (0.2 μg/ml; Sigma-Aldrich). During stimulation, the cells were incubated for 6 h at 37°C in the presence of brefeldin A (Sigma-Aldrich). After stimulation, the cells were washed and then incubated with anti-CD3-Pacific Blue, anti-CD8-APC-Cy7, and anti-CD4-peridinin chlorophyll protein-Cy5.5 (Clone L200) for 20 min and

FIG 2 Virus shedding after Ad5hr inoculation. (A) Number of animals with detectable Ad5hr DNA in nasal secretions after each Ad5hr inoculation based on the nested PCR assay. (B) Level of Ad5hr DNA in nasal secretions after the first Ad5hr inoculation, 20 weeks before the first immunization with the replication defective Ad5 SIV vaccine based on the TaqMan PCR assay.

then washed again and fixed with 1% paraformaldehyde. Fixed samples were permeabilized (0.5% saponin), incubated with anti-gamma interferon (anti-IFN- γ)-APC clone B27, anti-tumor necrosis factor alpha (TNF- α)-PE-Cy7 clone MAb11, and anti-interleukin-2 (anti-IL-2)-PE clone MQ1-17H12 for 20 min at room temperature, and then washed with permeabilizing buffer and fixed with 1% paraformaldehyde. All monoclonal antibodies were from Pharmingen/Becton Dickinson, San Diego, CA. The data were acquired using a FACSaria flow cytometer (Becton Dickinson) and analyzed using FlowJo software (Treestar, Inc.) and a Macintosh G5 computer (Apple, Inc.) At least 100,000 events in the forward scatter/side scatter lymphocyte gate were acquired from all cultures. The background level of cytokine staining varied between the samples and the peptide pools. For reporting the single cytokine positive CD8⁺ T cell responses, a sample was considered positive if the frequency cytokine⁺ cells was >0.04 after subtracting the DMSO control. The cutoff value of 0.04% was based on the background responses in unimmunized animals.

Statistical methods. To define the penile exposure that resulted in infection, we assumed that a vRNA⁺ plasma sample detected within 5 days of a penile exposure was due a transmission event that resulted from the inoculation the previous week. The peak viral load was defined as the highest log₁₀ vRNA level in any plasma sample collected over the 7-week postinfection observation period. All of the analyses described below were conducted separately for each of three dosage groups (10³ TCID₅₀; 10³ TCID₅₀ and 10⁴ TCID₅₀ combined; and 10³ TCID₅₀, 10⁴ TCID₅₀, and 10⁵ TCID₅₀ combined). Kaplan-Meier estimates of the survival function were generated for each arm or group of arms of interest, and a log-rank test was used to compare the survival functions between animal groups. Box plots of plasma vRNA levels in the infected animals were generated and a Wilcoxon rank-sum test (WRS) was used to test equality between animal groups. Finally, a discrete time survival model (15, 16) was used to model the relative probability of infection per penile SIV challenge of one group versus another. All *P* values are two-sided, and *P* values of <0.05 are considered significant.

RESULTS

Virology of Ad5hr infection in RM. Using procedures previously described (36), 18 male RM (Table 1, Fig. 1) were inoculated orally and intranasally with 1.5×10^9 infectious particles of Ad5hr404 (19) 20 weeks before immunization with the Ad5 SIVmac239 Gag/Pol/Nef vaccine or the Ad5 empty vector. At weeks -12 and -8, all 18 animals were reinoculated intratracheally with the same dose of Ad5hr404 (Fig. 1). Using a sensitive nested PCR assay, Ad5hr DNA was detected in the nasal secretions of 17 of 18 RM in the first 48 h after the Ad5hr inoculation at week -20, but by week -12 no RM was shedding Ad5hr (Fig. 2A). After reinoculation at weeks -12 and -8, Ad5hr DNA was detected in the nasal secretions of only two or three animals. We also measured the levels of Ad5 DNA present in nasal secretion using a less sensitive TaqMan PCR assay that could only quantify Ad5 DNA in 75% of samples that were positive in the nested PCR assay. Ad5hr DNA levels in secretions were highest on day 1 after inoculation (median of 556 Ad5 DNA copies/ μ l secretions) and declined by 50% (median of 223 Ad5 DNA copies/ μ l secretions) on day 2 (Fig. 2B). On day 7 after inoculation (week -19), Ad5 DNA levels in the few animals still shedding virus were unchanged (median of 214 Ad5 DNA copies/ μ l secretions). By day 14 after inoculation (week -18), Ad5hr DNA could be measured at low levels (35 Ad5 DNA copies/ μ l secretions) in one of six animals that were still positive by nested PCR.

Ad5hr neutralizing antibody responses. One week after Ad5hr inoculation (week -19), 8 of 18 animals had Ad5-specific serum neutralizing antibody responses with titers in the range of

FIG 3 Ad5 neutralizing antibody titers after Ad5hr inoculation. Note that after the second Ad5hr inoculation, all animals had neutralizing titers to Ad5, and at 6 weeks prior to immunization 17 of 18 animals had titers >1:200.

128 to 2,048. All 18 animals were positive for Ad5hr neutralizing antibodies 2 weeks after the second Ad5hr infection (week -10) with titers in the range of 32 to >5,120 (Fig. 3). After the third Ad5hr inoculation, all macaques exhibited neutralizing antibody titers in the range of 128 to >5,120. The Step Trial included 1,500 participants with low (≤ 200) Ad5 antibody titers at enrollment and 1,500 participants with high Ad5 titers of >200 (2). Based on their Ad5-specific antibody titers, all but one of the Ad5hr-infected RM in the present study would fall into the cohort of Step Trial participants with high Ad5 antibody titers.

Breadth of Ad5 SIV vaccine induced CD8⁺ T cell responses. Of the 18 animals immunized with the Ad5 SIV vaccine (Ad5 Vx-SIV and Vx-SIV), 11 (61%) made CD8⁺ T cell responses to Gag, 10 (55%) developed CD8⁺ T cell responses to Pol, and 6 (33%) made CD8⁺ T cell responses to Nef at some point after the first vaccination (Table 2). Animals infected with Ad5hr before immunization with the Ad5 SIV vaccine (Ad5 Vx-SIV) made CD8⁺ T cell responses to only two of the three vaccine antigens, i.e., Gag and Pol (Table 2), while most of the Ad5-seronegative Vx-SIV animals responded to all three antigens. While the proportion of animals in the two groups responding to Gag was similar, Pol-specific responses were detected in only three of nine (33%) Ad5 Vx-SIV animals compared to seven of nine (77%) of the Vx-SIV animals (Table 2). Finally, Nef-specific CD8⁺ T cell responses were not detected in the Ad5 Vx-SIV animals but were common (6 of 9 [67%]) in the Vx-SIV animals (Table 2). The proportion of RM and humans making CD8⁺ T cell responses to the vaccine antigens after immunization with the MRKAd5 vaccine was similar, but the decreased breadth of immune responses attributed to preexisting Ad5 immunity seemed more marked in RM (Table 2).

Strength and cytokine profile of Ad5 SIV vaccine-induced CD8⁺ T cell responses. Four weeks after the first immunization, Gag-specific CD8⁺ T cell responses were detected in only 4 of 18 immunized macaques (Ad5 Vx-SIV and Vx-SIV), and the mean frequency of the Gag specific IFN- γ ⁺ CD8⁺ T cells in responders was 0.095% of total CD8⁺ T cells (range, 0.05 to 0.14%; Fig. 4). Four weeks after the second immunization 6 of 18 RM had Gag-specific CD8⁺ T cell responses, and the mean frequency of Gag-specific IFN- γ ⁺ CD8⁺ T cells was 0.12% of total CD8⁺ T cells (range, 0.04 to 0.4%). By the day of the first penile SIV exposure (1 week after the third immunization), 7 of 18 vaccinated macaques had Gag-specific CD8⁺ T cell responses, with Gag-specific IFN- γ ⁺ CD8⁺ T cells comprising

TABLE 2 Summary of CD8⁺ T cell responses to the Ad5 SIV *gag/pol/nef* vaccine

Animal no.	Group	Gag ^{a,b}	Pol ^a	Nef ^a
33776	Ad5 Vx-SIV	–	–	–
35380	Ad5 Vx-SIV	–	++	–
35584	Ad5 Vx-SIV	++ ^c	–	–
35629	Ad5 Vx-SIV	++ ^d	–	–
36224	Ad5 Vx-SIV	++	–	–
36321	Ad5 Vx-SIV	–	+	–
36338	Ad5 Vx-SIV	–	–	–
36658	Ad5 Vx-SIV	+++ ^e	+	–
36982	Ad5 Vx-SIV	+	–	–
Total (%)		5/9 (55)	3/9 (33)	0/9
% Step vaccinees ^f		70	40	60
35919	Vx-SIV	++	–	–
35939	Vx-SIV	–	–	–
36264	Vx-SIV	–	++	–
36272	Vx-SIV	+	+	+++
36303	Vx-SIV	++	++	++
36323	Vx-SIV	+	++	++
36609	Vx-SIV	++	+++	+++
36629	Vx-SIV	–	+++	++
37332	Vx-SIV	++	+++	+++
Total (%)		6/9 (67)	7/9 (78)	6/9 (67)
% Step vaccinees ^f		73	59	68

^a Peptide pools covering Gag, Pol, and Nef were used to test PBMC collected at week 4 ($n = 18$), week 8 ($n = 17$), and week 24 ($n = 9$) after the first immunization.

^b Gag p27 was tested at week 25 ($n = 18$).

^c One cytokine was made upon antigen stimulation.

^d Two cytokines were made upon antigen stimulation.

^e Three cytokines were made upon antigen stimulation.

^f Note that responses in the humans immunized with the HIV version of the vaccine were analyzed by ELISPOT, and the results in RM immunized with the SIV version of the vaccine are based on flow cytometry assays (38).

0.16% (range, 0.09 to 0.27%) of all CD8⁺ T cells in responding animals. The SIV Gag- and Pol-specific T cells in the Ad5 Vx-SIV animals were either IFN- γ ⁺ alone or IFN- γ ⁺/IL-2⁺. In contrast, the Gag, Pol, and Nef CD8⁺ T cells in the Vx-SIV animals were predominantly positive for 2 or 3 cytokines (IFN- γ ⁺/TNF- α ⁺ or IFN- γ ⁺/IL-2⁺/TNF- α ⁺).

Effect of immunization on susceptibility to penile SIVmac251 exposure. Although the timing of HIV exposure relative to immunization in the Step Trial is not known, the animals in the present study were challenged with cell-free SIVmac251 applied to the foreskin and glans of the penis 1 week after the last immunization (Fig. 1). Animals were exposed in this manner to 10^3 TCID₅₀ of SIVmac251 once a week for 10 weeks, then once a week for 10 weeks to 10^4 TCID₅₀ of SIVmac251, and finally twice in 1 day to 10^5 TCID₅₀ of SIVmac251. Exposures were stopped when there were two consecutive plasma samples with rising vRNA levels. At the end of the 22 SIVmac251 penile exposures, 6 of 8 (75%) of the naive control animals were SIV infected, and among the animals with preexisting Ad5 immunity, 5 of 8 (63%) of the Ad5 Vx-empty vector-immunized animals and 5 of 9 (56%) of Ad5 Vx-SIV immunized animals were SIV infected (Fig. 5). In the animals that were not infected with Ad5 prior to immunization, 6 of 9 (67%) of the Vx-empty vector-immunized animals and 7 of 9 (78%) of the Vx-SIV-immunized animals were SIV infected (Fig. 5). Note that although one of the Vx-empty animals (animal 36344) had multiple episodes of transient viremia, the inoculation

series continued uninterrupted, and a systemic infection never became established in this animal (Fig. 5).

For a more rigorous analysis of susceptibility to infection, Kaplan-Meier estimates of the survival function were generated for each animal group at each of the three challenge doses (Fig. 6). Although only 2 animals were infected after exposure to a dose of 10^3 TCID₅₀, both of them were Ad5 Vx-SIV animals. Moreover, we used a log rank test to compare composites of the groups. Of particular interest were comparisons between the composite groups (i) immunized versus unimmunized animals with previous Ad5 immunity (Ad5 Vx-SIV versus Ad5 Vx-empty); (ii) immunized (Ad5 Vx-SIV + Vx-SIV) versus unimmunized animals (Ad5 Vx-empty + Vx-empty + control); and (iii) immunized animals with preexisting Ad5 immunity compared to all others. *P* values for those comparisons are presented in Table 3. At the lowest exposure dose (10^3 TCID₅₀), a significant difference in risk of infection was detected between the immunized animals with preexisting Ad5 immunity (Ad5 Vx-SIV) compared to all of the other animal groups ($P = 0.004$ based on a log-rank test, Table 3). We also used a discrete time survival model (15, 16), assuming a leaky vaccine effect to test vaccine efficacy in one composite of the groups versus another. Using this model, among animals with preexisting Ad5 immunity, the estimated relative risk of infection for immunized versus unimmunized animals was >1 either at the lowest exposure dose (10^3 TCID₅₀) or when combining dose levels of 10^3 and 10^4 TCID₅₀ (Table 3). A likelihood-ratio test based on the leaky model was conducted to compare the per-exposure infection probability between the composite groups at the lowest exposure dose: the relative risk of infection approached statistical significance in immunized animals compared to unimmunized animals ($P = 0.056$), and the relative risk was significantly higher in immunized animals with preexisting Ad5 immunity compared to all of the other animals ($P = 0.010$). Note that when infections at the low and middle exposure doses (10^3 and 10^4 TCID₅₀) are combined, the relative risk of the Ad5 Vx-empty group was lower than the Vx-empty group ($P = 0.05$, based on a log-rank test) but not from the unimmunized control group ($P = 0.31$, based on a log-rank test).

The TFP and CypA Trim5 α alleles are associated with lower plasma vRNA levels in RM infected with the SIVsm family of viruses (SIVsmE660 and SIVsmE543), but there is minimal effect on infections with the SIVmac family of viruses (18, 23). The TFP

FIG 4 Frequency of vaccine-induced IFN- γ -secreting SIV Gag-specific CD8⁺ T cells in immunized animals. PBMC samples collected at 4 and 8 weeks after the first immunization were stimulated with two peptide pools covering the p55 Gag precursor, while the samples collected at 25 weeks were stimulated only with a gag p24 peptide pool.

FIG 5 Plasma SIV RNA levels after penile exposure. (A) Naive control animals; (B) animals infected with Ad5hr then immunized with the Ad5 SIV vaccine; (C) animals infected with Ad5hr then immunized with the empty Ad5 vector; (D) animals immunized with the Ad5 SIV vaccine; (E) animals immunized with the empty Ad5 vector. The color of the arrows under the x-axis indicates the SIV dose used for each inoculation and, in animals that became infected, the color of the symbol or line indicates the dose of SIV that resulted in the systemic infection. The dotted black line in panel E indicates a period of transient viremia in one animal that never developed a typical systemic infection and thus was not classified as infected. The horizontal black line indicates the cutoff for accurately quantifying vRNA in plasma.

FIG 6 Effect of immunization on SIV acquisition after penile exposure. Kaplan-Meier estimates of the survival function were generated for each animal group or for composites of the groups at each of the three challenge doses. (A) After 10 penile SIV exposures to 10^3 TCID₅₀ of SIV, 2 of 9 Ad5 SIV-immunized animals were infected, while none of the 34 animals in the other four groups were infected. Equal proportions of animals in all five groups were infected after 10 penile SIV exposures to 10^4 TCID₅₀ of SIV (B) and 2 penile SIV exposures (separated by 4 h) to 10^5 TCID₅₀ of SIV (C).

and CypA Trim5 α alleles restrict SIV replication by binding to the virus capsid protein to prevent uncoating and block replication, but the sequence specificity of the Trim5 α -capsid interaction explains why the effect is limited to SIVsm viruses (18). However, it was recently reported that monkeys homozygous for the restrictive Trim5 α alleles resist becoming infected after penile inoculation with SIVsmE660 (46), and thus we sought to determine whether the susceptibility of the animals in the present study to penile SIVmac251 inoculation was related to their TRIM5a genotype. Both of the Ad5 Vx-SIV animals that became infected with exposure to 10^3 TCID₅₀ of SIVmac251 were homozygous for the TFP allele (Table 1) that is associated with resistance to infection with after penile SIVsm E660 inoculation. In fact, if group assignment is not considered, 11 of 18 TFP/TFP animals and 3 of 3 TFP/CypA animals became infected after penile SIV inoculation compared to 11 of 18 TFP/Q animals, 3 of 3 Q/Q animals, and 1 of 1 Q/CypA animals (Table 1). Thus, in the present study there was no difference in the rate of SIV acquisition between animals with the nonpermissive TFP and CypA alleles and animals with the permissive Q allele.

Effect of immunization on SIVmac251 replication after transmission. As shown in Fig. 5, there was no evidence that prior infection with Ad5 altered SIV replication in the Ad5 Vx-SIV group. Thus, the Ad5 Vx-SIV and Vx-SIV animals are hereafter grouped together and referred to as the “immunized animals,”

while the Ad5 VX-empty and VX-empty animals and naive control animals are “unimmunized animals.” The peak plasma vRNA level in immunized animals was $\sim 1 \log_{10}$ lower compared to the unimmunized animals (Fig. 7B). In addition, at 6 weeks postinfection, the plasma vRNA level in immunized animals was at least $1 \log_{10}$ lower than in unimmunized animals ($P < 0.05$, WRS test, data not shown). Finally, the vRNA area under the curve (AUC) through 7 weeks postinfection from immunized animals was 15% lower compared to the unimmunized animals ($P < 0.01$, WRS test, data not shown). Thus, the immunized animals that became infected had significantly lower plasma vRNA levels than unimmunized animals in the first few weeks after infection. Of note, the 2 Ad5 Vx-SIV animals that became infected at a dose of 10^3 TCID₅₀ had relatively low plasma vRNA levels from 3 to 7 weeks postinfection compared to unimmunized animals (Fig. 8), suggesting that there was a vaccine effect after infection in these two animals despite the fact that they became infected at the lowest SIV dose.

DISCUSSION

Several versions of the Merck Ad5-based vaccine have been tested in nonhuman primates (NHP), but preclinical studies of this vaccine have never been conducted in Ad5-seropositive animals that were then SIV challenged by penile inoculation. The present study was designed to determine whether a repli-

TABLE 3 Relative risk estimates for animal groups based on the indicated comparisons analyzed under a discrete time survival model assuming a leaky vaccine effect

SIV dose	P_0^a	P_1^b	RR ^c	P^d
Ad5 Vx-SIV (II) vs. Ad5 Vx-empty (I)				
10 ³ TCID ₅₀	0	0.024	∞	0.15
≤10 ⁴ TCID ₅₀	0.007	0.027	4.46	0.15
≤10 ⁵ TCID ₅₀	0.039	0.033	1.12	0.77
Ad5 Vx-SIV + Vx-SIV (II) vs. Ad5 Vx-empty + Vx-empty + control				
10 ³ TCID ₅₀	0	0.011	∞	0.08
≤10 ⁴ TCID ₅₀	0.021	0.030	1.40	0.40
≤10 ⁵ TCID ₅₀	0.035	0.039	1.10	0.69
Ad5 Vx-SIV (II) vs. Vx-SIV + Ad5 Vx-empty + Vx-empty + control (I)				
10 ³ TCID ₅₀	0	0.024	∞	0.004
≤10 ⁴ TCID ₅₀	0.024	0.027	1.14	0.80
≤10 ⁵ TCID ₅₀	0.037	0.033	0.88	0.71

^a Averaged per exposure infection probability for group I in the paired composite groups, over different challenge dose levels, under the leaky model assuming homogeneous per exposure infection probability within the group for a given challenge level and independence between the probability of infection and the number of prior challenges

^b Averaged per exposure infection probability for group II in the paired composite groups, over different challenge dose levels, using the same model and assumptions as in footnote a.

^c Relative risk (RR) ratio of averaged per exposure infection probability for group II versus that for group I using the same model and assumptions as in footnotes a and b.

^d That is, the *P* value based on the log-rank test comparing the two composite groups.

cation defective Ad5-based SIV *gag/pol/nef* vaccine that is very similar to the vaccine used in the Step Trial would alter susceptibility to infection after penile SIV exposure. HIV infection rates in the Step Trial were the same in Ad5-seronegative vaccine and placebo recipients, but infection rates were more than twice as high in Ad5-seropositive vaccinees compared to placebo recipients (2). As in the Step Trial, there was no evidence of protection from SIV infection in immunized animals, and there was an indication that the vaccine may have enhanced the susceptibility of animals that were Ad5 seropositive at the time they were immunized with the Ad5 SIV vaccine. As in the animals in the present study, the elevated risk of HIV infection seen in Ad5-seropositive men was absent in Ad5-seronegative men receiving the vaccine (2). Importantly, Ad5-seropositive animals immunized with the empty Ad5 vector were not susceptible to the low-dose SIV challenge, suggesting that SIV-specific immunity was responsible for the enhanced susceptibility. However, because this conclusion is based on only two of nine animals becoming infected at the lowest challenge dose, a larger study is needed to confirm the results and to define the mechanisms behind any enhanced infectivity.

The conclusion that the Merck Ad5 HIV-1 subtype B Gag/Pol/ Nef vaccine may have enhanced HIV acquisition in the Step Trial was based on the finding that 49 of the 914 male vaccine recipients were infected with HIV versus 33 of the 922 male placebo recipients (2). Thus, there was a difference of 16 infections among the approximately 1,800 men (0.89%) in the two groups in the trial (2). The conclusion of the present study that an SIV vaccine enhanced SIV acquisition is based on 2 infected animals out of 18

immunized animals and 0 of 25 unimmunized animals infected, a difference of 2 infections among the 47 NHP (4.2%). Thus, results in humans and NHP suggest that an Ad5 vector-based AIDS vaccine marginally increased virus acquisition, making it less likely that the findings in these studies are statistical artifacts. Further studies are warranted to determine whether a similar risk exists in women and to characterize the mechanisms underlying increased susceptibility to HIV infection in people vaccinated with Ad5 vector-based HIV vaccines.

Despite the small number of animals in the present study, it is possible to propose some preliminary hypotheses to explain the host-virus interactions responsible for the study outcome. At the low and middle virus doses, the infection risk of the Ad5 Vx-empty group was lower than the Vx-empty group, thus there was no evidence of enhanced susceptibility to penile SIV exposure in the Ad5-seropositive NHP immunized with the empty Ad5 vector. These results lead us to hypothesize that vaccine-induced vector-specific immune responses did not contribute to altered susceptibility in the immunized NHP. Rather, it seems that vaccine-induced SIV-specific immune responses are responsible for the enhanced susceptibility in Ad5-seropositive, immunized NHP. Further, as with the Step Trial, this effect was not seen in the immunized animals that were Ad5 seronegative at immunization. Although the presence of SIV-specific CD4⁺ T cells in mucosal tissues was not examined here, the hypothesis that vaccine-elicited SIV-specific T cells contribute to enhanced susceptibility is consistent with the fact that HIV and SIV preferentially infect virus specific CD4⁺ T cells (1, 10, 28). The hypothesis that Ad5-specific immunity does not explain the results of the Step Trial is supported by the results of a recent case-control study that found no evidence that Ad5 seropositivity increases susceptibility to HIV infection (6).

Given the dual role of CD4⁺ T cells as orchestrators of the immune responses to HIV vaccines and preferred targets for HIV infection, the balance of CD4⁺ versus CD8⁺ T cell and B cell responses induced by candidate AIDS vaccines may be critical in determining vaccine efficacy (13). Unfortunately, a similar empty vector control group was not included in the Step Trial, so the role of Ad5-specific and HIV-specific T cells in determining the Step Trial results is not known.

There was no significant effect of vaccination on setpoint plasma vRNA levels in the Step Trial (2). With the caveat that such analysis can be vulnerable to bias, a *post hoc* analysis of 87 participants who became infected during the trial found that

FIG 7 Effect of immunization on peak plasma SIV RNA levels after penile challenge. (A) Peak plasma vRNA levels of immunized animals that became infected were significantly lower compared to the unimmunized, Ad5 empty vector animals that became infected. (B) Peak plasma vRNA levels of SIV-immunized animals that became infected were significantly lower than all unimmunized animals (Ad5 empty vector animals and naive control animals combined) that became infected. WRS, Wilcoxon rank sum test.

FIG 8 Plasma SIV RNA levels were relatively low in the two Ad5 Vx-SIV animals that became infected at the lowest virus dose. (A) Plasma vRNA levels of individual animals that became infected. Red lines indicate two Ad5 Vx-SIV-infected animals after exposure to 10^3 TCID₅₀ of SIVmac251. Black lines indicate six unimmunized animals (control) that became infected after exposure to 10^4 or 10^5 TCID₅₀ of SIVmac. Blue lines indicate 10 immunized animals (Ad5 Vx-SIV and Vx-SIV) that became infected after exposure to 10^4 or 10^5 TCID₅₀ of SIVmac. (B) The mean plasma vRNA levels of groups of animals that became infected. The red line indicates mean ($\pm$ the standard error of the mean) plasma vRNA level of the two Ad5 + Vx-SIV animals infected after exposure to 10^3 TCID₅₀ of SIVmac. The black line indicates the mean plasma vRNA level of the six unimmunized animals (control) that became infected after exposure to 10^4 or 10^5 TCID₅₀ of SIVmac251. The blue line indicates the mean plasma vRNA level of the 10 immunized animals (Ad5 Vx-SIV, Vx-SIV) that became infected after exposure to 10^4 or 10^5 TCID₅₀ of SIVmac.

immunized individuals with specific MHC-I alleles had lower viral loads than placebo recipients (12). Further, HIV-1 genome sequences from newly infected vaccine recipients were genetically more distant from the vaccine Gag/Nef protein sequences than the HIV-1 sequences from infected placebo recipients, providing evidence of selective pressure on HIV founder populations from vaccine-induced T cell responses in the Step Trial (39). Thus, the Step data suggest that the Ad5 HIV-1 subtype B vaccine had a limited effect on virus replication and evolution in some immunized individuals with specific MHC-I alleles. In contrast, we found that the RM immunized with the replication defective Ad5-based SIV *gag/pol/nef* vaccine had significantly lower plasma vRNA levels in acute infection (peak and 6 weeks postinfection) than did the unimmunized animals. The animals in the present study were not maintained after 7 weeks postinfection, so it is not possible to determine whether the vaccine effect extended into the set-point phase of infection, but it seems likely that control of replication would have steadily waned as cytotoxic-T-lymphocyte escape variants emerged. While lower set point plasma vRNA levels in Step Trial were limited to individuals with specific MHC-I alleles (12), in the present study the control of SIV replication in the acute phase of infection extended to all immunized animals without regard to MHC-I haplotype.

The seemingly conflicting findings of enhanced susceptibility to infection in some vaccinated animals but postinfection control of virus replication in all immunized animals suggests that either (i) the same vaccine elicited T cell responses that enhanced virus transmission also may have contributed to control of virus replication after infection or (ii) vaccine-elicited T cell responses enhanced virus transmission in some vaccinated animals but contributed to control of virus replication after infection in others. In fact, compared to unimmunized animals (Ad5 Vx-empty, Vx-empty, and control), the two Ad5 Vx-SIV animals that became infected at a dose of 10^3 TCID₅₀ had relatively low plasma vRNA levels from 3 to 7 weeks postinfection (Fig. 8), providing some support for the first hypothesis, although further studies are needed to determine the relative importance of the two possibilities.

The Merck Ad5 HIV-1 subtype B vaccine elicited CD8⁺ T cell responses in 73% of the recipients, but there were no obvious differences between the breadth, magnitude, or polyfunctional profile of HIV-specific T-cell responses of the infected and non-infected Step Study vaccine recipients (30). However, it was recently shown that the number of HIV-Gag specific interferon γ secreting T cells in the Step Trial vaccine recipients can be linked to polymorphisms located in specific HLA-B alleles that are associated with natural immune control of HIV replication (11), providing an immune mechanism that can explain the sieving of HIV-1 founder genotypes (39). In addition, it is clear that Ad5-specific antibodies blunted the vaccine-induced HIV-specific immune responses of Step Trial participants (30) and altered the quality of the HIV-specific T cell immunity induced by the Merck Ad5 HIV-1 subtype B vaccine (37). In the present NHP study, approximately 70% of immunized animals made CD8⁺ T cell responses to at least one vaccine antigen at some point after immunization. However, preexisting Ad5-specific immunity decreased the number of animals responding to SIV Pol and Nef peptides, and most Gag CD8⁺ T cell responses were comprised of monofunctional IFN- γ -secreting cells.

Ad5 vector-based vaccines are highly effective against a chimeric simian-human immunodeficiency virus 89.6P (SHIV89.6P) challenge (21, 22, 40), leading to the conclusion that NHP models using SHIVs are not adequate for preclinical evaluation of candidate HIV vaccines, especially those designed to elicit effector T cell responses (33). In fact, subsequent NHP studies showed that although Ad5-SIV vaccines are ineffective against a high-dose SIVmac239 challenge, the immunized RM that became infected controlled viral replication better than naive animals (4, 5, 29). The NHP model described here carefully mimicked the design of the Step Trial and demonstrates that NHP studies using challenge viruses and routes of exposure designed to mimic the details and complexity of HIV sexual transmission can reflect the results of human clinical trials of AIDS vaccines. NHP models of HIV transmission provide the opportunity to understand the complex interactions between host immune responses to the vaccine vector, the expressed antigen, and HIV susceptibility. The results of the

Step Trial and the current report suggest that some candidate AIDS vaccines have the potential to enhance HIV acquisition.

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