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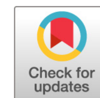
VACCINES AND ANTIVIRAL AGENTS



Combination Adenovirus and Protein Vaccines Prevent Infection or Reduce Viral Burden after Heterologous Clade C Simian-Human Immunodeficiency Virus Mucosal Challenge

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ABSTRACT HIV vaccine development is focused on designing immunogens and delivery methods that elicit protective immunity. We evaluated a combination of adenovirus (Ad) vectors expressing HIV 1086.C (clade C) envelope glycoprotein (Env), SIV Gag p55, and human pegivirus GBV-C E2 glycoprotein. We compared replicating simian (SAd7) with nonreplicating human (Ad4) adenovirus-vectored vaccines paired with recombinant proteins in a novel prime-boost regimen in rhesus macaques, with the goal of eliciting protective immunity against SHIV challenge. In both vaccine groups, plasma and buccal Env-specific IgG, tier 1 heterologous neutralizing antibodies, and antibody-dependent cell-mediated viral inhibition were readily generated. High Env-specific T cell responses elicited in all vaccinees were significantly greater than responses targeting Gag. After three intrarectal exposures to heterologous tier 1 clade C SHIV, all 10 sham-vaccinated controls were infected, whereas 4/10 SAd7- and 3/10 Ad4-vaccinated macaques remained uninfected or maintained tightly controlled plasma viremia. Time to infection was significantly delayed in SAd7-vaccinated macaques compared to the controls. Cell-associated and plasma virus levels were significantly lower in each group of vaccinated macaques compared to controls; the lowest plasma viral burden was found in animals vaccinated with the SAd7 vectors, suggesting superior immunity conferred by the replicating simian vectors. Furthermore, higher V1V2-specific binding antibody titers correlated with viral control in the SAd7 vaccine group. Thus, recombinant Ad plus protein vaccines generated humoral and cellular immunity that was effective in either protecting from SHIV acquisition or significantly reducing viremia in animals that became infected,

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consequently supporting additional development of replicating Ad vectors as HIV vaccines.

IMPORTANCE There is a well-acknowledged need for an effective AIDS vaccine that protects against HIV infection and limits *in vivo* viral replication and associated pathogenesis. Although replicating virus vectors have been advanced as HIV vaccine platforms, there have not been any direct comparisons of the replicating to the non-replicating format. The present study directly compared the replicating SAd7 to non-replicating Ad4 vectors in macaques and demonstrated that in the SAd7 vaccine group, the time to infection was significantly delayed compared to the control group, and V1V2 Env-specific binding antibodies correlated with viral outcomes. Viral control was significantly enhanced in vaccinated macaques compared to controls, and in infected SAd7-vaccinated macaques compared to Ad4-vaccinated macaques, suggesting that this vector may have conferred more effective immunity. Because blocking infection is so difficult with current vaccines, development of a vaccine that can limit viremia if infection occurs would be valuable. These data support further development of replicating adenovirus vectors.

KEYWORDS HIV vaccine, adenovirus vector, V1V2-specific antibody, SHIV challenge, correlate of protection, rhesus macaque, simian human immunodeficiency virus, adenovirus, neutralizing antibody, vaccine

During the last 30 years, a large number of replicating vaccine viral vectors have been tested in preclinical studies as HIV vaccine concepts with various degrees of efficacy, including vesicular stomatitis virus, Venezuelan equine encephalitis virus, vaccinia virus, Sendai virus, Newcastle disease virus, attenuated rabies virus, 17D yellow fever virus, influenza virus, simian varicella virus, measles virus, mumps virus, herpes simplex virus, cytomegalovirus (CMV), and adenovirus (Ad) (1). Circulating antibodies to adenoviruses in humans has led to a search for "rare" adenovirus serotypes such as Ad26 or Ad35, where preexisting immunity is unlikely, thereby increasing the likelihood of vaccine efficacy. Indeed, Ad5 is highly prevalent worldwide (60 to 70% in Europe and the United States, and up to 98% in Africa and Asia) (2) and high Ad5 preexisting neutralizing titers in noncircumcised volunteers were linked with a higher risk of infection in the STEP vaccine trial that used a replication-deficient Ad5 vector (3, 4). Thus, rare human adenovirus serotypes or nonhuman adenoviruses have the potential to be developed into vaccine vectors. The goal of the present study was to test two such vectors—human adenovirus 4 (Ad4) and simian adenovirus 7 (SAd7)—as HIV vaccines for protection against mucosal SHIV challenge. Ad4 possesses a number of unique advantages, including (i) an extremely well-established safety record in humans for the parent virus, (ii) proof of safety and immunogenicity for an analogous recombinant orally administered replicating Ad4 avian influenza vector vaccine which completed a phase 1 study (5), and (iii) preexisting immunity to Ad4 is found in only 35% of US Army trainees (6). In contrast, although Ad26- or Ad35-vectored HIV vaccines have recently shown 50% protection in a preclinical study in macaques (7), safety data in humans is limited compared to Ad4 wild-type virus, or Ad4 replicating vectors.

The potential advantage of using a replicating, as opposed to a nonreplicating vector, has recently been highlighted by vaccine studies using a replication-competent rhesus CMV (rhCMV) vector expressing SIV proteins. The rhCMV-vector approach generated strong T-cell immunity, which persisted in vaccinated macaques for years and led to exquisite control of viremia in about 50% of animals challenged with SIV (8). Since SAd7 replicates in macaques and Ad4 will not replicate in nonhuman primates (NHPs) (9), we compared these two vectors in macaques to begin to explore the role of replicating and nonreplicating vectors in eliciting lentivirus immunity. We used HIV clade C 1086.C Env to induce mucosal and systemic antibodies, including neutralizing antibodies, and SIV Gag p55 to elicit T-cell responses. In addition, we incorporated human pegivirus GB virus C (GBV-C) E2 glycoprotein in order to take advantage of the reported beneficial modulating effects of GBV-C infection on HIV outcomes. Persistent

infection with GBV-C is associated with prolonged survival in HIV-1-infected individuals (10), and acquisition of GBV-C infection in infants has been associated with reduced mother-to-child transmission of HIV (11). This interaction appears related to structural mimicry between the GBV-C E2 protein and HIV-1 particles, in particular HIV Env, since E2 protein inhibits HIV entry into cells, and sera from rabbits immunized with the E2 protein neutralize diverse HIV pseudoviruses (12–14). In addition, the presence of anti-GBV-C E2 antibodies is also associated with prolonged survival in two epidemiologic studies (15, 16). Therefore, we included GBV-C E2 glycoprotein as an additional immunogen that has HIV protective activity distinct from the Env antigen, thus offering an opportunity to enhance antibody responses and potentially protective immunity.

There are no validated models for HIV vaccine studies, since there have not yet been highly successful clinical vaccine trials. Analyses of the RV144 trial that showed ~30% efficacy found that reduced risk of acquisition was correlated positively with Envelope antibodies directed to the V2 region (17–19). In the challenge phase of this experiment, we chose to test a tier 1 clade C SHIV that has been tested in other vaccine settings in order to maximize the chances that we could measure virus blocking or other antiviral effects of the vaccine. The postchallenge results obtained show that this vaccine induces antibodies against V2 that are correlated with tight virus control or prevention.

RESULTS

Construction and characterization of SAd7 and Ad4 vectors. SAd7 and Ad4 viral vectors, expressing either clade C HIV Env 1086.C glycoprotein, SIVmac239 p55 Gag, or GBV-C E2 glycoprotein (Fig. 1A and B) were generated as described in Materials and Methods. Ad4Env has the majority of the E3 genes deleted, while SAd7Env has an intact viral genome with no Ad gene deletions. Of interest, native SAd7 virus has a severely truncated E3 region that encodes only the E3 12.5 K protein (20). Little is known regarding receptor usage of SAd7 virus, but Ad4 is thought to use the Coxsackie and Adenovirus Receptor (CAR). The effects of different viral receptor usage on Ad immunogenicity is unknown. Restriction digestion, PCR, and sequencing confirmed that the viral genome and transgene insertion cassettes were intact, the correct size, and without deletions or mutations (data not shown). HIV Env, SIV Gag, and GBV-C E2 protein expression was evaluated by Western blot analysis after infection of HEK293 cells with the Ad4 or SAd7 vectors and correct expression products were detected (Fig. 1C). In addition, for both the Ad4 and the SAd7 vectors, Env glycoproteins expressed on the surface of recombinant Ad-infected cells were recognized by a panel of Env-specific broadly neutralizing antibodies (NAbs) demonstrating correct conformation (21).

Route and dose-finding studies. In the route and dose-finding studies, macaques were inoculated with a combination of three recombinant adenovirus constructs in two types of vectors, either human adenovirus 4 (Ad4) or simian adenovirus 7 (SAd7). These vector-construct combinations were tested using two routes of administration, via either intranasal (i.n.) or intramuscular (i.m.) delivery. The SAd7 combinations were tested at escalating doses of 10^9 to 10^{11} viral particles (vp), and the Ad4 combinations were tested only at the highest dose of 10^{11} vp, since Ad4 does not replicate in macaques. There was one macaque per inoculation route and per dose tested. Inoculation was followed by weekly blood draws for 1 month to evaluate the elicited humoral and cellular immune responses. As seen in Fig. 1D, 1086.C Env-specific binding antibodies were detected 2 weeks postinoculation, reached a maximum at either 2 or 3 weeks postinoculation, and plateaued until the end of the observation period at day 28. The SAd7 vectors delivered i.m. elicited higher Env titers than when delivered i.n. In contrast, the Ad4 vectors delivered i.n. elicited higher Env antibody titers than when delivered i.m. Cellular immune responses were assessed in an enzyme-linked immunospot (ELISPOT) assay by measuring gamma interferon (IFN-γ) secretion from peripheral blood mononuclear cells (PBMCs) at 2 and 3 weeks postinoculation (Fig. 1E). Similar to the humoral responses, the Ad4 vectors delivered i.n. elicited stronger T cell responses than the Ad4 vectors delivered i.m. Analogous to the humoral responses, strong T cell responses were only elicited by the highest SAd7 doses tested. However,

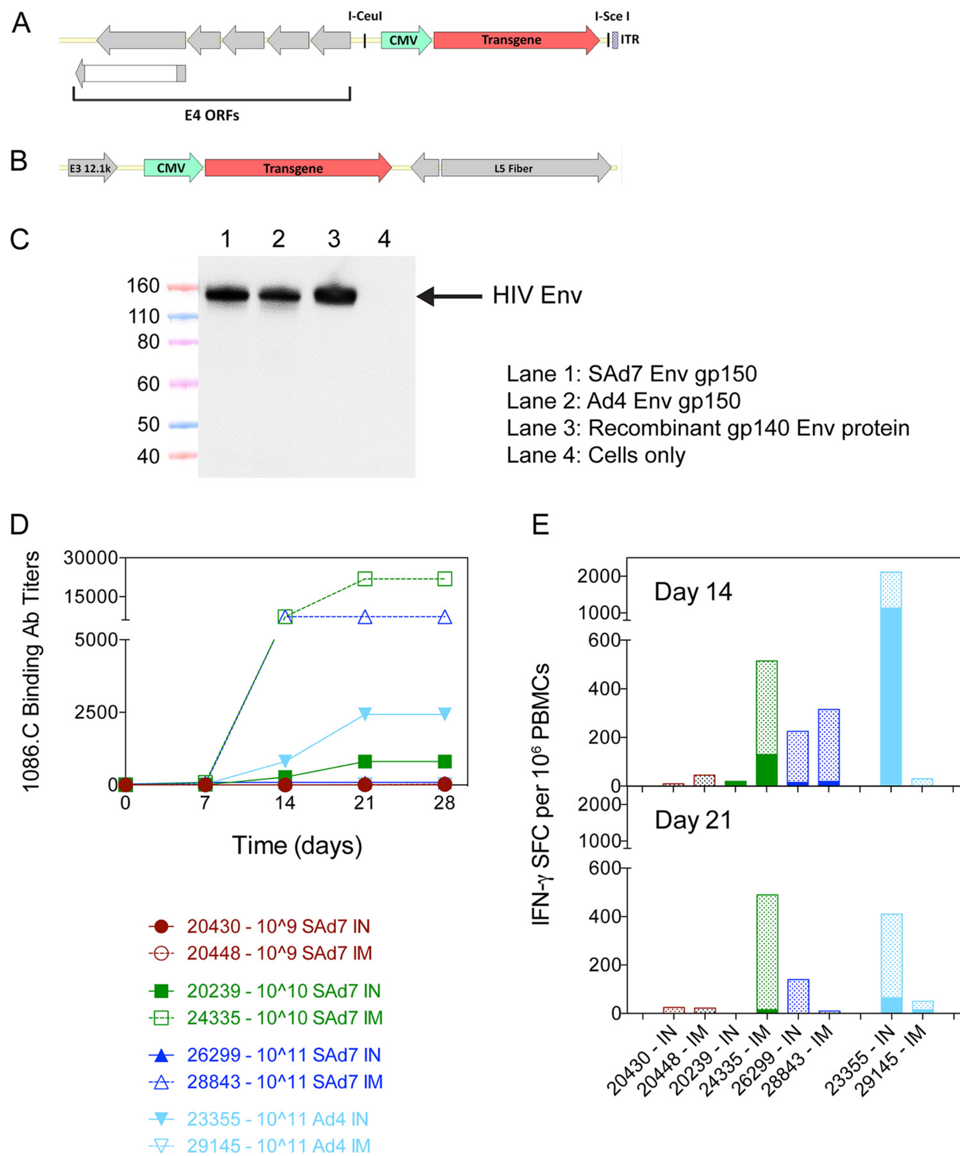

FIG 1 Ad constructs, Env protein expression, and dose-finding and route studies. (A and B) Schematic of the region of SAd7 with the expression cassette containing either HIV clade C 1086.C Env, SIVmac239 Gag, or GBV-C E2 driven by the CMV immediate-early promoter inserted between E4 and the right inverted terminal repeat (ITR) (A) and Ad4 with most of the E3 genes (except 12.1k) replaced with the expression cassette (B). (C) Transgene expression was assessed by infecting HEK293 cells with SAd7 Env gp150 or Ad4 Env gp150 virus and performing a Western blot analysis of lysates collected 48 h postinfection. (D) Humoral responses in dose-finding studies. Plasma samples were tested longitudinally for binding antibodies to 1086.C gp140 trimeric protein by ELISA. (E) Cellular responses in dose-finding studies. IFN-γ ELISPOT assays were performed using SIVmac239 Gag (solid bars) and Env (dotted bars) peptide overlapping pools at days 14 and 21 after adenovirus inoculation.

both i.n. and i.m. delivery routes elicited responses. Env-specific responses appeared somewhat stronger than the Gag-specific responses but could not be evaluated due to sample size. Based on these results, for the main study described below we elected to immunize all macaques with the combination i.m. and i.n. inoculation routes.

Experimental design for the immunogenicity and challenge study. The immunogenicity and challenge study (Fig. 2) had two vaccine groups (receiving either SAd7- or Ad4-vectored constructs) and control groups receiving empty vectors (either SAd7 wild type [WT] or Ad4 WT). The compositions of the groups are indicated in Table 1, and groups were balanced for the presence of the following major histocompatibility complex (MHC) class I alleles: Mamu-A1*01, -B*08, and -B*17. All NHPs were also

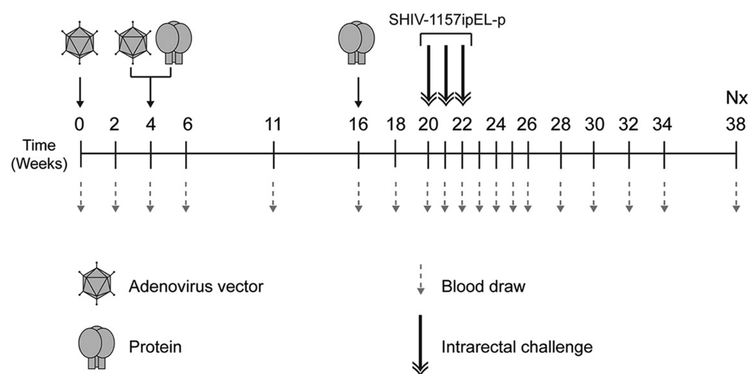

FIG 2 Vaccination scheme. The vaccine groups ($n = 10$/group) received HIV clade C 1086.C Env, SIVmac239 Gag, and GBV-C E2 as simian adenovirus 7 (SAd7)- or human adenovirus 4 (Ad4)-vectored constructs. The sham group received empty SAd7 vectors ($n = 5$) or empty Ad4 vectors ($n = 5$). NHPs were inoculated i.n. (0.5×10^{11} vp/ml) and via i.m. (1×10^{11} vp/ml) with SAd or Ad vectors at weeks 0 and 4. These NHPs also received i.m. 1086.C Env gp140 and GBV-C E2 protein boosts (25 μg each) at weeks 4 and 16. After a month rest period, the macaques were weekly challenged i.r. with 8,000 $TCID_{50}$ tier 1 clade C SHIV-1157ipEL-p until all controls were infected.

screened for SAd7 and Ad4 seropositivity, and the four seropositive animals (Table 1) were placed in control groups to avoid any anti-vector preexisting immunity. Since no differences were measured between the SAd7-WT- and Ad4-WT-treated macaques in any of the assays performed here, all control animals were combined in a single sham group for analysis purposes. In order to maximize the immune responses, macaques were immunized via a prime-boost approach (Fig. 2). Specifically, NHPs were primed i.m. and i.n. with Ad vector combinations at week 0, while at week 4 they received the Ad vectors (i.n. and i.m.) and a multiprotein boost i.m. The Ad vector combinations were codelivered i.n. and i.m. with each Ad vector delivered at doses of 0.5×10^{11} vp i.n. and 10^{11} vp i.m. The protein boosts were composed of 25 μg of HIV Env gp140 and 25 μg of GBV-C E2 glycoproteins delivered i.m.—at a different site from the Ad vaccines—in the presence of Adjuplex adjuvant. Finally, at week 16, NHPs were boosted i.m. with protein only using the combination listed above. At 4 weeks after the last protein boost, NHPs were weekly challenged intrarectally (i.r.) with 8,000 50% tissue culture infective doses ($TCID_{50}$) of clade C tier 1 SHIV-1157ipEL-p (22, 23) until all controls were infected. The macaques were then monitored for 16 to 18 weeks.

Humoral responses. Antibody responses elicited by the two vaccine strategies were monitored longitudinally by assessing binding antibody titers to 1086.C Env and to GBV-C during the immunogenicity phase (Fig. 3). In the two vaccine groups, 1086.C Env-binding antibodies were elicited by the first Ad inoculation and were boosted by subsequent immunizations (Fig. 3A). There was no statistically significant difference between the two Ad vaccine strategies. The empty vectors delivered to the sham group did not induce binding antibodies (data not shown) prior to challenge. Overall, the GBV-C binding antibody titers (Fig. 3A) were lower than the Env-binding antibody titers. Confounding the analysis was the high background anti-GBV-C response at time zero. The second immunization significantly improved the GBV-C antibody responses in the SAd7 vaccine group ($P < 0.0001$) but not in the Ad4 vaccine group. The third immunization did not boost further the GBV-C binding titers in any macaques.

The Env V1V2-specific binding antibody responses were also assayed longitudinally in the vaccine groups (Fig. 3B). V1V2 recombinant constructs (Avi-Tag polypeptides) from four different clades were tested, including the cognate Env vaccine 1086.C. Higher V1V2 antibodies specific for clade C 1086.C and clade E AE244 were measured after just one Ad inoculation versus V1V2 antibodies specific for clade B JRFL and clade F 14/00/4, which required an additional Env protein immunization. For all four constructs tested, the binding antibody titers were boosted by subsequent immunizations. There were no statistically significant differences between the two vaccine groups

TABLE 1 Class I MHC alleles and Ad serostatus

Treatment and NHP	Allele (Mamu-A*01, -B*08, -B*17)	Serostatus Ad4	Serostatus SAd7
SAd7 vaccine			
28907	B*08	–	–
29800	B*17	–	–
30153		–	–
30580	A*01	–	–
30960	A*01	–	–
30971		–	–
31104		–	–
31216	B*17	–	–
31381		–	–
31468		–	–
Ad4 vaccine			
29930	B*08	–	–
30233	A*01	–	–
30274	B*17	–	–
30548		–	–
30802	B*17	–	–
30807		–	–
30825	A*01	–	–
31007		–	–
31010		–	–
31012		–	–
SAd7 sham			
29984		+	–
30033	B*17	+	–
30391		–	–
30992		–	–
31227	B*08	+	–
Ad4 sham			
28923	B*08	–	–
30222		–	–
30556	A*01	–	+
30850		–	–
30982		–	–

when considering the same tested antigen, but within each vaccine group there were significantly different responses depending on the antigen tested. Overall, the V1V2 binding antibody titers were significantly higher for 1086.C than for JRFL and 14/00/4 for both vaccine groups (SAd7, $P < 0.0001$ and $P = 0.0004$; Ad4, $P = 0.0004$ and $P = 0.0032$, respectively). In addition, the V1V2 binding antibody titers were also significantly higher for AE244 than for JRFL for both vaccine groups (SAd7, $P = 0.0011$; Ad4, $P = 0.0315$). In contrast, only the SAd7 vaccine elicited differential responses between AE244 and 14/00/4, with significantly higher binding titers against AE244 ($P = 0.0298$).

Buccal secretion samples harvested 2 weeks after the second and third immunizations were tested for the presence of Env-specific IgG and IgA mucosal antibodies (Fig. 3C). 1086.C-specific IgG binding antibodies were detected in buccal secretions 2 weeks after two immunizations and were significantly higher in the SAd7 vaccine group than in the Ad4 vaccine group. These mucosal IgG titers were boosted by the last immunization. In contrast, only one NHP in the SAd7 vaccine group was positive for buccal IgA antibody titers (Fig. 3C). Rectal swabs tested for IgG and IgA secretions at weeks 6 and 18 did not have detectable antibodies above the background (data not shown).

NAbs in plasma directed against tier 1 SF162 pseudovirus were monitored longitudinally (Fig. 4A). NAb titers were elicited after the first Ad inoculation in both vaccine groups, and titers were boosted by subsequent immunizations, peaking at week 18, 2 weeks after the last protein boost. There was no significant difference between the two vaccine groups. In addition to SF162, neutralization of several tier 1 viruses produced

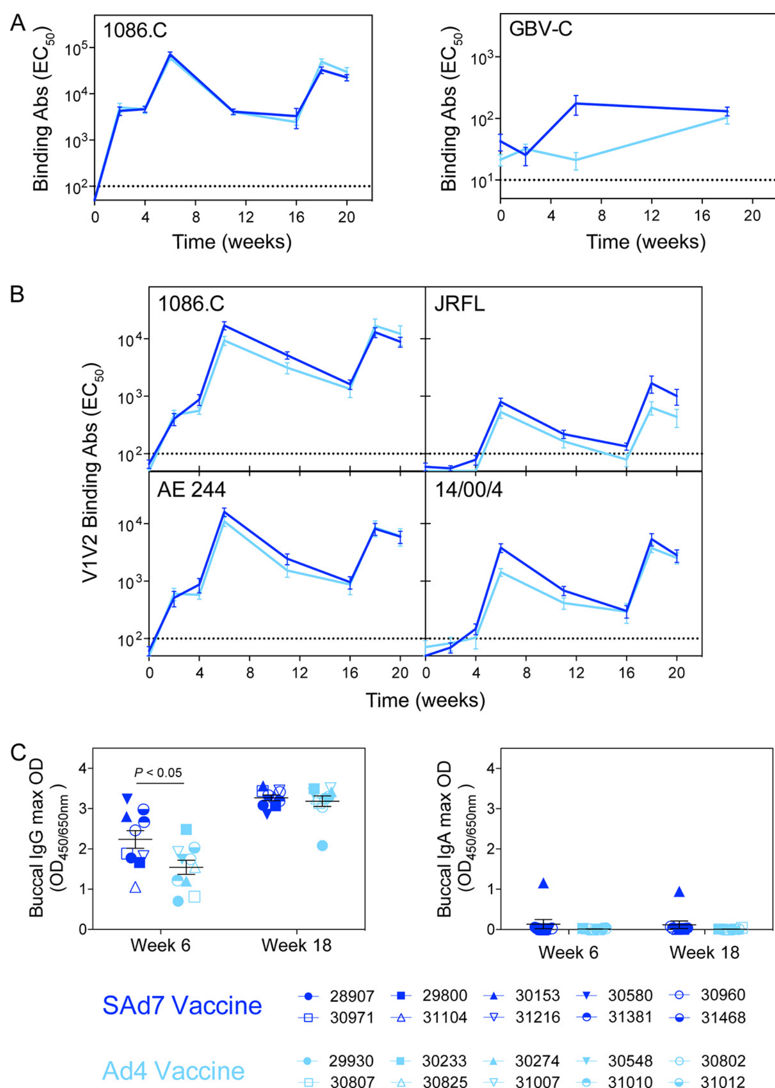

FIG 3 Binding antibody titers during immunogenicity phase. Plasma samples from vaccinated macaques were tested longitudinally by ELISA for binding antibodies to 1086.C gp140 trimeric protein and GBV-C E2 glycoprotein (A), and V1V2 constructs from four different clades (B): clade C 1086.C Env, clade B JRFL Env, clade E AE 244 Env, and clade F 14/00/4 Env. (C) Buccal secretions were tested by ELISA after the second and third immunizations (weeks 6 and 18, respectively) for IgG and IgA binding antibodies to 1086.C Env. The SAd7 group is shown in dark blue, and the Ad4 group is shown in light blue. Data are presented as group means ± the standard errors (SE) in panels A and B. NHPs are identified by individual symbols in panel C.

in 293T cells was assessed (Fig. 4B) after the combination Ad inoculation/protein immunization (week 6) and after the last protein boost (weeks 18 and 20). Neutralization titers were boosted between the second and third immunization and peaked 2 weeks after the last protein boost (week 18) against N-Luc.1157ipEL and SHIV-BaL ($P <$ 0.05 versus week 20) for both vaccine groups. Neutralization titers were maintained ($P >$ 0.05 [week 20 versus week 18]) for Q461d1 (both vaccine groups) and for MW965 (Ad4 vaccine only). For both vaccine groups, neutralization of MW965 was overall significantly higher than Q461d1 and N-Luc.1157ipEL (SAd7, $P <$ 0.0001 and $P =$ 0.0008; Ad4, $P <$ 0.0001 and $P =$ 0.0015, respectively) and neutralization of SHIV-BaL was overall higher than Q461d1 (SAd7, $P =$ 0.0407; Ad4, $P =$ 0.0045). In addition, neutralization of MW965 was overall higher than SHIV-BaL for the SAd7 vaccine only ($P =$ 0.0496). There was no neutralization of clade C tier 1 ZM109 or of the tested tier 2 viruses in the vaccinees (data not shown), and no heterologous tier 1 or tier 2

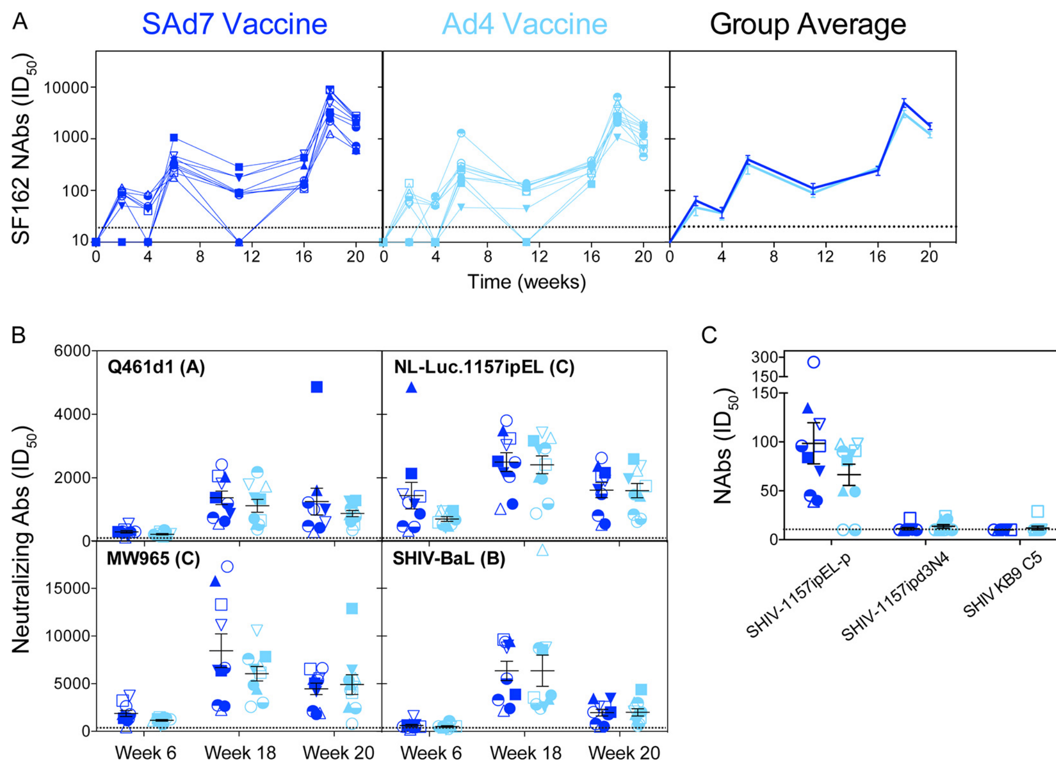

FIG 4 Heterologous NAbs. (A) Longitudinal plasma samples from vaccinated macaques were tested for neutralization of SF162 by TZM-bl assay. Individual data and group means ± the SE are displayed. (B) Plasma samples from vaccinated macaques were tested for neutralization of clade A Q461d1, clade B SHIV-BaL, clade C MW965, and NL-Luc.1157ipEL after the second immunization (week 6) and third immunization (weeks 18 and 20) in a TZM-bl assay. (C) Week 18 plasma samples from vaccinated macaques were tested for neutralization of clade C tier 1 SHIV-1157ipEL-p, tier 2 SHIV-1157ipd3N4 (45), and tier 2.5 SHIV KB9 C5 in a TZM-bl assay. Viruses were produced in 293 T cells (A and B) or human PBMCs (C). Neutralization data are expressed as the ID_{50}, i.e., the plasma dilution that neutralized 50% of the infecting virus. Group colors and animal symbols are the same as for Fig. 3.

neutralization was measured in the sham group (data not shown) prior to challenge. Finally, no autologous neutralization of the cognate tier 2 pseudovirus 1086.C was detected 2 weeks after the second and third immunizations or at the time of first SHIV challenge (data not shown).

Neutralization of SHIV strains produced in human PBMCs was also assessed 2 weeks after the last protein boost (Fig. 4C). These virus preparations are more difficult to neutralize than those produced in 293T cells, and this pattern can be seen when comparing the neutralization of the SHIV-1157ipEL-p swarm to the clonal N-Luc.1157ipEL in Fig. 4B. Only this clade C tier 1 SHIV was neutralized, and there was no significant difference between the vaccine groups. There was no neutralization of the clade C tier 2 and tier 2.5 SHIVs tested (Fig. 4C and data not shown).

ADCVI responses. In addition to neutralization, antibodies mediate other antiviral functions through their interaction with Fc receptors (24, 25). Two weeks after the last protein boost, antibody-dependent cell-mediated viral inhibition (ADCVI) was assessed. As shown in Fig. 5, both vaccine groups elicited ADCVI responses against SHIV-1157ipEL-p that were significantly higher than the sham group (SAd7 versus sham, $P < 0.001$; Ad4 versus sham, $P < 0.01$) but were not different from each other ($P > 0.05$).

T cell responses. To evaluate T cell responses, IFN-γ ELISPOT assays were performed on PBMCs 2 weeks after each inoculation (Fig. 6). Measurable Env- and Gag-specific T cell responses were detected after the first Ad inoculation. Env-specific T cell responses were boosted by the second immunization, reaching exceptionally high levels, and IFN-γ responses were maintained after the third immunization. In contrast, the Gag-specific responses were not boosted by the subsequent inoculation/immunizations and were significantly weaker than the Env-specific responses ($P < 0.0001$).

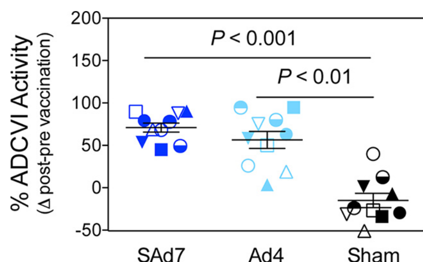

FIG 5 ADCVI responses after three immunizations. Week 18 plasma samples from macaques were tested for ADCVI activity against clade C SHIV-1157ipEL-p by determining the reduction of p27 between the negative control (target cells and effector cells alone) and the samples. Data are expressed as the ADCVI activity (%) after subtracting the preimmune values (week 0) from the week 18 values. Group colors and animal symbols are the same as for Fig. 3 for the vaccine groups, and sham macaque data are indicated in black.

T_{FH} responses specific for each immunogen (Env, Gag, and GBV-C E2) were also evaluated after the second and third immunizations (data not shown). Regardless of the vector and the vaccine insert, T_{FH} responses were low after the second immunization and did not get boosted by the third immunization. Gag-specific T_{FH} responses were not observed after the last protein boost which did not include any Gag immunogen (data not shown).

Protection from viral acquisition after SHIV challenge and viral burden measures. Macaques were challenged i.r. on a weekly basis with 8,000 $TCID_{50}$ units (by TZM-bl assay) of tier 1 clade C SHIV-1157ipEL-p until all control NHPs were infected. None of the SAd7-vaccinated macaques and 2 of 10 Ad4-vaccinated macaques were infected after the first i.r. challenge, whereas 7 of 10 controls were. After three i.r. repeated exposures to heterologous tier 1 clade C SHIV, all 10 sham-vaccinated controls were infected, whereas 4 of 10 SAd7- and 3 of 10 Ad4-vaccinated macaques remained

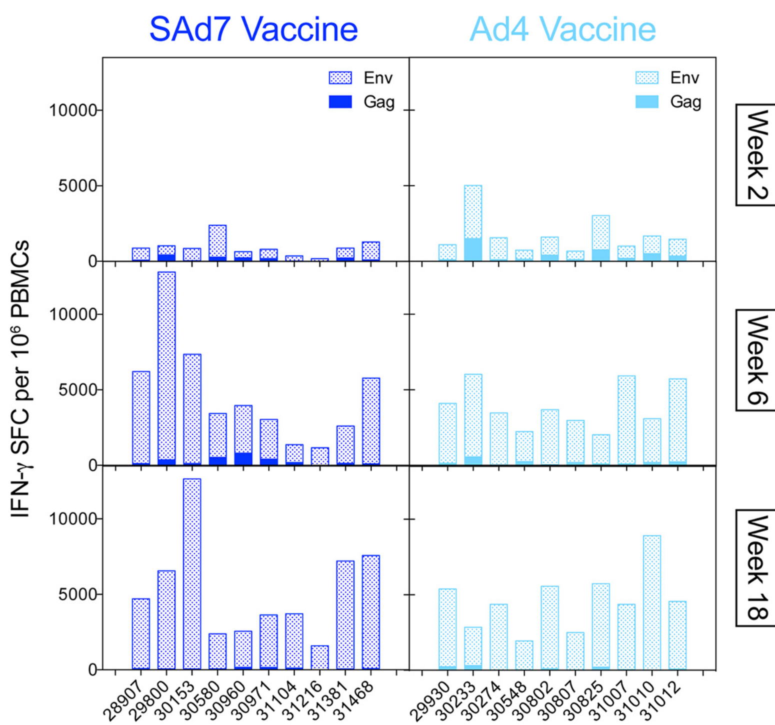

FIG 6 Powerful Env-specific IFN-γ T cell responses after two Ad vector immunizations. Two weeks after each immunization, macaque PBMCs were tested for IFN-γ production by ELISPOT assay after stimulation with pools of overlapping peptides for clade C consensus Env or SIVmac239 Gag. Data are expressed as background-subtracted SFCs per million PBMCs.

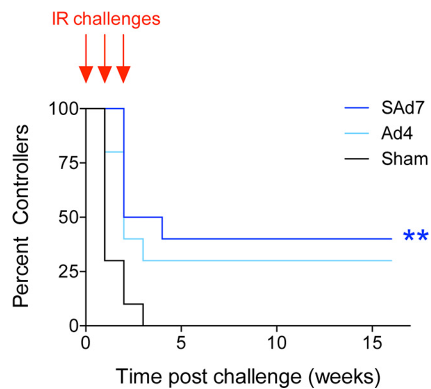

FIG 7 Significantly longer time to infection for SAd7 vaccine group than for the sham group. A modified Kaplan-Meier curve shows the percent controllers in each experimental group over the observation period (time postchallenge in weeks) based on plasma viral RNA loads. **, $P = 0.0044$ (SAd7 versus sham).

uninfected or maintained tightly controlled plasma viremia (Fig. 7); the infection rate was not different between vaccine groups. The time required for a productive infection was significantly greater in the SAd7 vaccine group than in the sham group ($P = 0.0044$); the rate of infection among animals given the Ad4 vaccine was also less than that in the sham group, although the difference was not significant ($P = 0.0564$).

All macaques were monitored up for up to 18 weeks for CD4 counts (data not shown) and the longitudinal evaluation of the plasma viral load in individual macaques and as group averages (Fig. 8A). There were no significant changes in $CD4^+$ T cells in any of the groups. Three vaccinated macaques maintained undetectable viral loads for the duration of the observation period (SAd7 30960, Ad4 30274, and Ad4 30548), and four vaccinated macaques had a single viral blip of 250 viral copies/ml or less early in infection (SAd7 30153, SAd7 31216, SAd7 31381, and Ad4 30807), thus demonstrating sterilizing immunity or tight viral control in 35% of all vaccinees. None of the macaques in the sham group had tight control of viremia. Two of these seven NHPs carried the Mamu-B*17 allele, and one of them carried the Mamu-A*01 allele (26); however, two sham-treated macaques also carried these protective alleles. Thus, we cannot attribute this level of virus control to the genetic makeup of these seven macaques. Notably, none of these protected/controlling animals had Env anamnestic responses (data not shown), and none had detectable integrated DNA in PBMCs (data not shown), a finding consistent with the plasma viremia data. In addition, they did not develop T cell responses against Vif, an antigen present only in the challenge virus and not in the vaccine (data not shown).

The plasma viral burden in the vaccinated NHPs was significantly lower than in the control animals (Fig. 8A) when measured longitudinally ($P < 0.0001$ SAd7 versus sham; $P = 0.0005$ Ad4 versus sham) or by area under the curve ($P < 0.0001$ SAd7 versus sham; $P < 0.01$ Ad4 versus sham) (Fig. 8B). In addition, the viral burden measured longitudinally was also significantly lower in the persistently infected vaccinated macaques than in the infected control animals ($P = 0.0001$, SAd7 versus sham; $P = 0.0194$, Ad4 versus sham). Importantly, the viral burden (AUC) in infected vaccinated macaques was significantly lower in the infected SAd7-immunized NHPs compared to the infected Ad4-immunized NHPs (Fig. 8C, $P = 0.035$ [Mann-Whitney test]). Furthermore, peak cell-associated viral loads were also significantly lower in vaccinated macaques compared to control animals (Fig. 8D, $P < 0.0001$, SAd7 versus sham; $P < 0.01$, Ad4 versus sham). Peak plasma viral loads were significantly lower in vaccinated macaques compared to control animals (Fig. 8E, $P < 0.0001$, SAd7 versus sham; $P < 0.01$, Ad4 versus sham) and the controllers had the lowest peak plasma viral load burden.

Immune correlates of protection and control. This study was not designed or powered to assess correlates of protection. Nonetheless, we performed an exploratory analysis and assessed humoral and cellular parameters at peak response and at time of

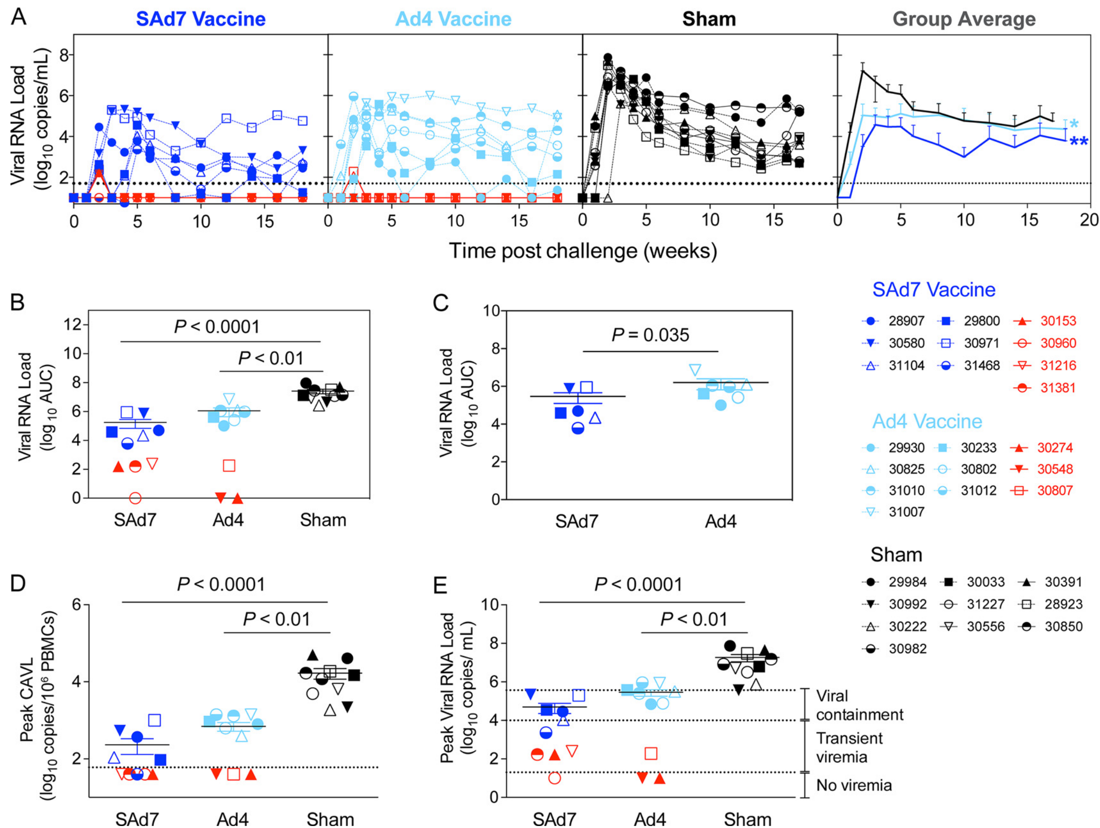

FIG 8 Viral loads. (A) Longitudinal plasma viral RNA loads. Viral RNA loads (log10 RNA copies/ml) were determined longitudinally by real-time reverse transcription-PCR. Group colors and animal symbols are the same as for Fig. 3 for the vaccine groups, and data for sham macaques are indicated in black. *, $P = 0.0005$ (Ad4 versus sham); **, $P < 0.0001$ (SAd7 versus sham). (B) Significantly lower plasma viral burden in vaccinated macaques. Plasma viral RNA loads are shown as the area under the curve (AUC); data for NHPs with no viremia or a single viral blip are indicated in red. (C) Significantly lower plasma viral burden in infected SAd7-vaccinated macaques than in infected Ad4-vaccinated macaques. (D and E) Significantly lower peak cell-associated and plasma viral RNA loads in vaccinated macaques (D and E, respectively). Data for NHPs with no viremia or a single viral blip are indicated in red.

challenge. V1V2-specific binding antibodies were associated (Spearman's test) with the outcome as measured by the time to infection, viral burden, peak plasma, and cell-associated viral loads in the SAd7 group but not in the Ad4 group (Table 2). There was a positive correlation between the time to infection and the binding antibody titers specific for all four tested V1V2 constructs (1086.C, JRFL, AE 244, and 14/00/4) at both time points except for week 20 1086.C ($P = 0.0794$). There was an inverse relationship between the peak cell-associated viral load and all V1V2-specific binding antibody titers tested at both time points (Table 2). The overall viral burden (measured as the AUC) inversely correlated with week 18 V1V2 Ab titers of all four Envs and with week 20 clade B JRFL V1V2 antibody titers ($P = 0.0108$). Peak plasma viremia inversely correlated with clade B JRFL (week 18 and 20) and clade E AE244 and clade F 14/00/4 (week 18) V1V2-specific antibody titers. The strongest correlations were with clade B JRFL V1V2-specific binding antibodies (Table 2) measured both at week 18 ($P = 0.0080$) and when the challenge phase started at week 20 ($P = 0.0074$).

In addition, 1086.C Env-specific IgG responses in buccal secretions at week 18 correlated with systemic humoral responses ($P < 0.04$ versus all week 18 and week 20 V1V2 binding antibody titers; $P < 0.04$ versus 1086.C week 18 and week 20 binding antibody titers; $P < 0.05$ versus week 18 N-Luc.1157ipEL neutralization; $P = 0.03$ versus week 18 ADCVI) in the SAd7 vaccine group only (data not shown). There was also a positive trend between SAd7 buccal IgG responses and the time to infection ($P = 0.068$ in the SAd7 group versus $P = 0.923$ in the Ad4 group) (data not shown).

TABLE 2 Study correlations[a]

Vaccine	Time to infection		pVL AUC		Peak pVL		Peak CAVL	
	Spearman's *r*	*P*	Spearman's *r*	*P*	Spearman's *r*	*P*	Spearman's *r*	*P*
SAd7								
Wk18 1086.C V1V2	0.764	**0.0175**	−0.638	**0.0497**	−0.602	0.0676	−0.769	**0.0051**
Wk20 1086.C V1V2	0.583	0.0794	−0.444	0.1932	−0.407	0.2349	−0.640	**0.0298**
Wk18 JRFL V1V2	0.797	**0.0111**	−0.766	**0.0116**	−0.790	**0.0080**	−0.705	**0.0148**
Wk20 JRFL V1V2	0.871	**0.0032**	−0.772	**0.0108**	−0.796	**0.0074**	−0.756	**0.0067**
Wk18 AE 244 V1V2	0.724	**0.0254**	−0.650	**0.0446**	−0.675	**0.0352**	−0.679	**0.0196**
Wk20 AE 244 V1V2	0.690	**0.0349**	−0.608	0.0639	−0.584	0.0774	−0.718	**0.0120**
Wk18 14/00/4 F V1V2	0.724	**0.0254**	−0.638	**0.0497**	−0.687	**0.0312**	−0.653	**0.0265**
Wk20 14/00/4 V1V2	0.690	**0.0349**	−0.535	0.1099	−0.535	0.1099	−0.666	**0.0231**
Ad4								
Wk18 1086.C V1V2	−0.566	0.0627	0.419	0.2267	0.602	0.0707	0.197	0.5826
Wk20 1086.C V1V2	−0.509	0.0976	0.097	0.7911	0.328	0.3520	−0.025	0.9097
Wk18 JRFL V1V2	−0.133	0.6076	0.061	0.8713	0.170	0.6371	−0.062	0.8275
Wk20 JRFL V1V2	−0.165	0.5456	0.006	0.9924	0.201	0.5755	−0.105	0.7352
Wk18 AE 244 V1V2	−0.521	0.0898	0.310	0.3796	0.553	0.1013	0.240	0.4990
Wk20 AE 244 V1V2	−0.426	0.1671	0.128	0.7247	0.359	0.3061	0.098	0.7879
Wk18 14/00/4 F V1V2	−0.483	0.1167	0.426	0.2203	0.547	0.1064	0.437	0.2052
Wk20 14/00/4 V1V2	−0.388	0.2056	0.353	0.3156	0.498	0.1456	0.412	0.2343

[a]Time to infection was determined as the number of weeks required for productive infection. The pVL AUC is the plasma viral load measured as the area under the curve. The peak pVL is the peak plasma viral load. The peak CAVL is the peak cell-associated viral load. Statistically significant values ($P < 0.05$) are indicated in boldface.

DISCUSSION

This complex antigen vaccine challenge study investigated the use of a combination of three immunogens HIV-1 Env, SIV Gag, and GBV-C E2 as Ad-vectored constructs and two recombinant proteins (HIV-1 Env and GBV-C E2) to elicit protective immunity against SHIV mucosal exposure. This combination delivered in either SAd7 or Ad4 vectors elicited humoral and cellular immune responses that led to protection or tight virological control in 7/20 (35%) animals after repeated low-dose SHIV i.r. challenge. These seven animals showed no integrated virus in PBMCs and no anamnestic response to Env after challenge. None of the sham-vaccinated controls exhibited viral control after infection with SHIV for the duration of the study, and all seroconverted and had cell-associated virus loads in the range of 1.9×10^3 to 5.1×10^4 viral copies/10^6 cells. Notably, for the SAd7 group, the time to infection was significantly longer than for the sham group, and the viral outcome was associated with Env V1V2-specific binding antibodies measured at the time of challenge. A nonsignificant trend toward a decreased infection rate was observed in the Ad4 group. Furthermore, the plasma viral burden in the infected vaccinated macaques was significantly lower in the SAd7 group than in the Ad4 group. Taken together, these results suggest that the simian SAd7-immunized macaques developed more potent and effective immunity than those immunized with Ad4 and that this immunity contributed to improved viral control in this group. We speculate that the modest advantage of SAd7 versus Ad4 vectors was due, in part, to localized replication of the simian vector in the simian host. However, the virus, as measured by quantitative PCR, did not persist at the nasal inoculation site (data not shown). Although the contribution of GBV-C E2 antibodies cannot be determined, the greater levels of E2 antibodies in the SAd7 group raises the possibility that these antibodies may have contributed to the lower HIV load observed in these animals.

The vaccine candidates were immunogenic in macaques, eliciting both modest humoral and strong cellular immune responses. Env-specific binding antibodies were rapidly elicited in both groups after just one Ad inoculation and were boosted by subsequent immunizations. Similar kinetics, albeit with lower titers, were observed for the Env V1V2-specific binding antibody responses. Both vaccines also stimulated Env-specific IgG responses in buccal secretions. However, no significant IgA responses were measured. No autologous neutralization of tier 2 1086.C pseudovirus was mea-

sured in the TZM-bl assay format. These results are consistent with the previous immunogenicity study where rabbits vaccinated with 1086.C gp160 Ad4 weakly neutralized the 1086.C pseudovirus in the A3R5 assay format (21), which is less stringent than the TZM-bl assay format. Tier 1 heterologous NAbs were measured after the first Ad inoculation, and these were boosted after additional vaccinations. Neutralization of clade C MW965 and clade B SF162 after the last protein boost was comparable to the titers elicited after two Ad primes and three protein boosts (7). ADCVI activity was detected prior to challenge and may be contributing to viral control. Other vaccine regimens have also elicited ADCVI responses after Ad26/MVA or MVA/Ad26 prime/boost immunizations (27) and Ad5/Env prime boosts (28). Despite the relatively modest humoral responses generated, we observed apparent sterilizing protection in three macaques. It is generally accepted that challenge with tier 1 SHIVs is more easily defended than tier 2 SHIVs, a concept that will require testing in subsequent experiments. We do not know the contribution of the GBV-C E2 to the protection against SHIV acquisition in this study, in part since all vaccinated NHPs received this immunogen via Ad and recombinant protein during the vaccination period and the sham-vaccinated group did not. Further studies would be required to address the importance of GBV-C E2 as an HIV-1 vaccine component. Nonetheless, the tight viral control that was seen after this tier 1 challenge suggests that the vaccines were effective in limiting viral infectivity and/or seeding at the time of exposure, a highly desirable outcome if sterilizing immunity is not achieved.

Strong Env-specific IFN-γ cellular responses were elicited after the first Ad inoculation in both vaccine groups and were boosted to exceptionally high levels by the second Ad inoculation/protein immunization. These Env-specific IFN-γ responses are higher than in previously published studies (7, 27). These responses were maintained after the third immunization. In contrast, the Gag-specific IFN-γ cellular responses were weaker than the Env-specific ones and were not further boosted by Ad exposure. The weaker Gag-specific responses may be due to immune interference from the Env immunogen, since both immunogens were delivered simultaneously at the same location in the macaques. This phenomenon has been previously observed in macaques and mice immunized with plasmid DNA (29–31) and in macaques immunized with ALVAC- or Ad5-vectored constructs (32).

Our results show partial protective efficacy of SAd7/Env and Ad4/Env vaccine strategies against a tier 1 repeated low-dose SHIV i.r. challenge. The level of protection is similar to that observed in the RV144 clinical trial and in macaque preclinical studies designed to mimic the RV144 trial (33, 34). This level is also comparable to published studies (7), where 50% of macaques immunized with Ad26/Env or Ad26/Ad5HVR48/Env were protected from SIVsmE660 or SHIV-SF162P3 challenge, respectively. Thus, Ad vectors, similar to poxvirus vectors, can stimulate immunity effective in blocking viral acquisition, in contrast to cytomegalovirus (CMV) vectors that do not block infection but provide tight control of infection in 50% of the vaccinated NHPs (8, 35). The time to infection was significantly longer in the SAd7 vaccine group than in the sham group, whereas this difference nearly reached significance between the Ad4 vaccine and sham groups. While vaccination in RV144 did not affect viral loads in infected subjects (33), the plasma viremia in our study was significantly lower in infected vaccinated macaques than in controls, similar to results obtained with a different SHIV and other adenovirus vectors (7). We speculate that the significant control of plasma viremia in animals that became infected is initiated by the presence of antibodies that limit the virus inoculum or eliminate early infectious centers, as observed with potent human neutralizing monoclonal antibodies (MAbs) administered therapeutically (36). Control of the infection by the attenuated inoculum is then assisted by Env-specific T cells and potentially abetted by *de novo* antibodies. However, there was no correlation in either vaccine group between ELISPOT responses and viral set point, suggesting that they may have been active postinfection but not causative of reduced viremia set points. In contrast, there was a positive trend between the time to infection and the Env-specific IgG responses in buccal secretions in the SAd7 vaccine group only. Because the Env

V1V2-specific binding antibody titers correlated with viral control only in the SAd7 vaccine group, these data provide a potential mechanism for antibodies in contributing to the control of viral spread early in infection. A correlation with V1V2 binding antibody titers was also measured in both preclinical studies and the RV144 clinical trial (17, 27), but we do not know whether this response or other functional antibodies contributed to blocking infection.

In summary, our data demonstrate that recombinant Ad plus protein vaccines generated significant humoral and cellular immunity that is effective in partial protection from SHIV challenge by either preventing mucosal virus acquisition or by reducing viremia in animals that became infected, thus supporting the additional development of replicating Ad vectors as HIV vaccines.

MATERIALS AND METHODS

Ethics statement. This study was carried out in strict accordance with the recommendations described in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health, the Office of Animal Welfare, and the U.S. Department of Agriculture. All animal work was approved by the Oregon Health and Science University (OHSU) Institutional Animal Care and Use Committee. The facilities at the Oregon National Primate Research Center (ONPRC) are accredited by the American Association for Accreditation of Laboratory Animal Care. All efforts were made to minimize animal suffering, and all procedures involving potential pain were performed with the appropriate anesthetic or analgesic. The number of animals used in this study was scientifically justified based on statistical analyses of virological and immunological outcomes.

Animals. Thirty male *M. mulatta* (rhesus macaques) between 2.5 and 4.5 years of age were housed at the ONPRC in Beaverton, OR. All animals were free of cercopithicine herpesvirus 1, D-type simian retrovirus, simian T-lymphotrophic virus type 1, and SIV infection at the start of the study. All macaques were genotyped for MHC class I alleles Mamu-A*01, -B*08, and -B*17. They were also serotyped for SAd7 and Ad4 preexisting immunity (NAbs). All procedures were performed according to rules and protocols approved by the Institutional Animal Care and Use Committee at the OHSU.

Vector and protein immunogens. The adenovirus vectors used in this study express either clade C HIV-1 envelope 1086.C glycoprotein (Env gp150, transmembrane Env glycoprotein with truncated cytoplasmic domain), SIVmac239 Gag p55 (SIVgag), or GBV-C E2 truncated protein (soluble fragment). Recombinant Ad4 viral vectors were generated by homologous recombination of an adenoviral plasmid and a shuttle plasmid containing either the Env, SIVgag, or GBV-C E2 transgene with flanking Ad4 sequences in *Escherichia coli* as described previously (21, 37). Recombinant SAd7 viral vectors were generated by direct cloning of the CMV and transgene cassettes into the adenoviral plasmid using homing endonucleases I-CeuI and PI-SceI. The SAd7 vector encoding the SIVgag transgene was provided by J. M. Wilson, University of Pennsylvania. Replication-competent viruses were generated by transfecting PacI-linearized recombinant Ad4 plasmid DNA into A549 cells (ATCC, CCL-185) or SwaI-linearized recombinant SAd7 plasmid DNA into HEK293 cells (ATCC, CRL-1573) on 70 to 80% confluent 100-mm cell culture dishes using FuGene HD transfection reagent (Roche Applied Sciences). *In vitro* characterization of the Ad vector capacity to express transgene included: (i) Western blot analysis for Env, SIVgag, and GBV-C E2 proteins and (ii) broadly neutralizing antibody (PGT123, 3BNC117, 10E8, and PG9) binding to Env glycoproteins expressed on the surfaces of A549 or 293 cells after recombinant AdEnv vector infection. Briefly, for protein expression, HEK293 cells were infected with 5×10^8 vp/ml of SAd7 Env gp150 or Ad4 Env gp150 recombinant vectors. Cells were harvested 48 h later and resuspended in radioimmunoprecipitation assay buffer (Thermo Scientific) supplemented with protease inhibitors. Cell lysates samples were run on 4 to 10% SDS-PAGE gels and transferred to nitrocellulose (iBlot; Life Technologies) prior to probing with MAb VRC-C 3B3 (primary Ab; B. Haynes) and horseradish peroxidase-conjugated anti-mouse IgG antibody (secondary Ab; Southern Biotech). A SuperSignal West Femto blotting detection system (Thermo Scientific) was used for development. The adenoviruses were expanded and purified to obtain sufficient amounts of viral particles (vp) for vaccination, i.e., 1×10^{13} to 3×10^{13} vp for each vector.

Recombinant 1086.C Env140 transgene was modified to express protein with a lysine-to-asparagine modification at amino acid position 160 in the V2 loop to acquire PG9 and PGT145 recognition. Recombinant GBV-C E2 protein was generated by transfecting 293F cells with a mammalian expression plasmid. The GBV-C E2 protein was extracted from the insoluble fraction and buffer exchanged in preparation for immunization.

Immunization and challenge. Macaques were assigned to three experimental groups as shown in Table 1. Groups were balanced for MHC class I alleles, and NHPs seropositive for SAd7 or Ad4 were only included in the sham groups. NHPs were inoculated i.n. (0.5×10^{11} vp) with a mucosal atomization device MAD300 (Teleflex Medical) and i.m. (1×10^{11} vp) with SAd7 or Ad4 vectors at weeks 0 and 4. They also received i.m. protein boosts (25 μg of 1086.C Env and 25 μg of GBV-C E2) in the presence of Adjuplex adjuvant (Sigma) at weeks 4 and 16. The protein-adjuvant mixture was delivered into a different muscle group than the Ad vector combinations. Regular blood draws were used to monitor immune responses in plasma and PBMCs during the immunogenicity phase. Inguinal lymph node biopsies were performed 3 weeks after the second and third immunizations. One month after the last immunization, weekly i.r. challenges with the tier 1 clade C SHIV-1157ipEL-p virus (8,000 $TCID_{50}$) were performed as previously

described (22, 23), until all controls were infected. Blood was obtained to monitor immune responses in plasma and PBMCs during the challenge phase.

Antibody ELISAs. HIV-1 Env binding antibody titers were measured in plasma samples collected at regular intervals against cognate 1086.C gp140 and SF162 gp140 (data not shown) according to methods previously established (21, 38). Env V1V2 enzyme-linked immunosorbent assays (ELISAs) using Avi TAG polypeptide coating antigens were performed on plasma samples as previously described (21). GBV-C ELISAs were performed using similar methodology as described above, and crude GBV-C E2 was harvested from an Integra CELLine bioreactor as the coating antigen. The concentration of GBV-C E2 protein used for coating was 0.5 μg/100 μl, previously determined using a standard purified GBV-C E2 sample from Chiron. Buccal and rectal secretions collected 2 weeks after the second and third immunizations were assayed in an ELISA against cognate 1086.C gp140 Env protein as previously described (39).

Neutralization assay. The TZM-bl neutralization assay was performed as previously described (40). All values were calculated with respect to virus-only wells as "[(RLU value for virus only minus cells only) − (RLU value for serum minus cells only)]/(RLU value for virus minus cells only)," where RLU represents the relative light units.

ADCVI assay. ADCVI was performed as previously reported (41, 42). Briefly, SHIV-1157ipEL-p-infected CEM.NKr-CCR5 target cells were mixed with a 1:100 dilution of study animal plasma (prevaccination and 18 weeks postvaccination) and with human PBMC effector cells (effector/target = 10:1). After 3 days, wells were washed to remove unbound antibody, and 7 days later the supernatant fluid was collected to measure p27 by ELISA. Each sample was assayed in triplicate, and the percent ADCVI was calculated relative to wells containing infected target cells, effector cells, and medium (without plasma).

ELISPOT assay. IFN-γ ELISPOT assay was performed using fresh PBMCs as previously described (43). Briefly, 10^5 PBMCs were incubated in duplicate overnight with 10 μM concentrations of the indicated Env or Gag 15-mer overlapping peptides. Results are expressed as IFN-γ spot-forming cells (SFCs) per 10^6 PBMCs after subtraction of the duplicate wells with medium only (negative control) and are considered positive if greater than twice the background and greater than 50 SFCs/10^6 PBMCs.

Follicular helper CD4 T cell (T_{FH}) intracellular cytokine staining. Lymphocytes were isolated from inguinal lymph node biopsy specimens 3 weeks after the second and third immunizations. Purified lymphocytes (5×10^6) were incubated with soluble gp140 Env (5 μg) and stained as previously described (38). Cells were analyzed on a BD LSRII flow cytometer, and T_{FH} cells were defined as $CD3^+$ $CD4^+$ $CD95^+$ $ICOS^+$ PD-1^{hi}. Env-specific responses were measured using interleukin-21 (IL-21) and IFN-γ.

$CD4^+$ T cell counts. Whole blood was stained for Naive, central memory and effector memory $CD4^+$ T cells as previously described (44). Complete blood counts (CBCs) were determined and used to assess CD4 T cell subset frequencies.

Viral loads. Quantitative PCR assays were performed on coded plasma or PBMC DNA samples using methods described in detail previously (36).

Statistical analyses. For longitudinal binding and neutralizing antibody data, repeated-measures analysis of variance (ANOVA) with the vaccine group as a between-group factor and the number of vaccinations as a within-group factor was used to explore the effect of vaccine over time during the immunogenicity phase (weeks 0 to 20). Repeated-measures ANOVA was also used to test the vaccination groups and time effects for the viral load during the challenge phase. The Bayesian information criterion was used to determine the optimal spatial/temporal correlation structure within subjects. AR(1), autoregressive order 1, was chosen to be a within-subject covariance structure. A Tukey multiple-comparison correction was used. Prior to applying repeated-measures ANOVA, the data were transformed using the logarithmic function with base 10. Due to the estimability of the data, data up to 16 weeks were included in the statistical model. Kruskal-Wallis tests, followed by Dunn's post tests, were used to assess differences in plasma AUC, peak plasma, and cell-associated viral loads. The Mann-Whitney test was also used to compare the AUC plasma viral loads in infected vaccinated macaques. A log-rank test, followed by Bonferroni's post test, was used to analyze the Kaplan-Meier curve and assess differences in time to SHIV infection between groups. The Spearman's test was used to assess correlations of protection. The statistical analyses were performed with either the GraphPad Prism or the SAS software packages.

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REFERENCES

1. Robert-Guroff M. 2007. Replicating and nonreplicating viral vectors for vaccine development. Curr Opin Biotechnol 18:546–556. https://doi.org/10.1016/j.copbio.2007.10.010.
2. Yu B, Zhou Y, Wu H, Wang Z, Zhan Y, Feng X, Geng R, Wu Y, Kong W, Yu X. 2012. Seroprevalence of neutralizing antibodies to human adenovirus type 5 in healthy adults in China. J Med Virol 84:1408–1414. https://doi.org/10.1002/jmv.23325.
3. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, Gilbert PB, Lama JR, Marmor M, Del Rio C, McElrath MJ, Casimiro DR, Gottesdiener KM, Chodakewitz JA, Corey L, Robertson MN. 2008. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the STEP study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 372:1881–1893. https://doi.org/10.1016/S0140-6736(08)61591-3.
4. Duerr A, Huang Y, Buchbinder S, Coombs RW, Sanchez J, del Rio C, Casapia M, Santiago S, Gilbert P, Corey L, Robertson MN. 2012. Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (STEP study). J Infect Dis 206:258–266. https://doi.org/10.1093/infdis/jis342.
5. Gurwith M, Lock M, Taylor EM, Ishioka G, Alexander J, Mayall T, Ervin JE, Greenberg RN, Strout C, Treanor JJ, Webby R, Wright PF. 2013. Safety and immunogenicity of an oral, replicating adenovirus serotype 4 vector vaccine for H5N1 influenza: a randomised, double-blind, placebo-controlled, phase 1 study. Lancet Infect Dis 13:238–250. https://doi.org/10.1016/S1473-3099(12)70345-6.
6. Ludwig SL, Brundage JF, Kelley PW, Nang R, Towle C, Schnurr DP, Crawford-Miksza L, Gaydos JC. 1998. Prevalence of antibodies to adenovirus serotypes 4 and 7 among unimmunized US Army trainees: results of a retrospective nationwide seroprevalence survey. J Infect Dis 178:1776–1778. https://doi.org/10.1086/314498.
7. Barouch DH, Alter G, Broge T, Linde C, Ackerman ME, Brown EP, Borducchi EN, Smith KM, Nkolola JP, Liu J, Shields J, Parenteau L, Whitney JB, Abbink P, Ng'ang'a DM, Seaman MS, Lavine CL, Perry JR, Li W, Colantonio AD, Lewis MG, Chen B, Wenschuh H, Reimer U, Piatak M, Lifson JD, Handley SA, Virgin HW, Koutsoukos M, Lorin C, Voss G, Weijtens M, Pau MG, Schuitemaker H. 2015. Protective efficacy of adenovirus/protein vaccines against SIV challenges in rhesus monkeys. Science 349:320–324. https://doi.org/10.1126/science.aab3886.
8. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, Whizin N, Oswald K, Shoemaker R, Swanson T, Legasse AW, Chiuchiolo MJ, Parks CL, Axthelm MK, Nelson JA, Jarvis MA, Piatak M, Jr, Lifson JD, Picker LJ. 2011. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature 473:523–527. https://doi.org/10.1038/nature10003.
9. Rowe WP, Huebner RJ, Hartley JW, Ward TG, Parrott RH. 1955. Studies of the adenoidal-pharyngeal-conjunctival (APC) group of viruses. Am J Hyg (Lond) 61:197–218.
10. Vahidnia F, Petersen M, Stapleton JT, Rutherford GW, Busch M, Custer B. 2012. Acquisition of GB virus type C and lower mortality in patients with advanced HIV disease. Clin Infect Dis 55:1012–1019. https://doi.org/10.1093/cid/cis589.
11. Schwarze-Zander C, Blackard JT, Rockstroh JK. 2012. Role of GB virus C in modulating HIV disease. Expert Rev Anti Infect Ther 10:563–572. https://doi.org/10.1586/eri.12.37.
12. Jung S, Eichenmuller M, Donhauser N, Neipel F, Engel AM, Hess G, Fleckenstein B, Reil H. 2007. HIV entry inhibition by the envelope 2 glycoprotein of GB virus C. AIDS 21:645–647. https://doi.org/10.1097/QAD.0b013e32803277c7.
13. Koedel Y, Eissmann K, Wend H, Fleckenstein B, Reil H. 2011. Peptides derived from a distinct region of GB virus C glycoprotein E2 mediate strain-specific HIV-1 entry inhibition. J Virol 85:7037–7047. https://doi.org/10.1128/JVI.02366-10.
14. Mohr EL, Xiang J, McLinden JH, Kaufman TM, Chang Q, Montefiori DC, Klinzman D, Stapleton JT. 2010. GB virus type C envelope protein E2 elicits antibodies that react with a cellular antigen on HIV-1 particles and neutralize diverse HIV-1 isolates. J Immunol 185:4496–4505. https://doi.org/10.4049/jimmunol.1001980.
15. Tillmann HL, Heiken H, Knapik-Botor A, Heringlake S, Ockenga J, Wilber JC, Goergen B, Detmer J, McMorrow M, Stoll M, Schmidt RE, Manns MP. 2001. Infection with GB virus C and reduced mortality among HIV-infected patients. N Engl J Med 345:715–724. https://doi.org/10.1056/NEJMoa010398.
16. Williams CF, Klinzman D, Yamashita TE, Xiang J, Polgreen PM, Rinaldo C, Liu C, Phair J, Margolick JB, Zdunek D, Hess G, Stapleton JT. 2004. Persistent GB virus C infection and survival in HIV-infected men. N Engl J Med 350:981–990. https://doi.org/10.1056/NEJMoa030107.
17. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH. 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 366:1275–1286. https://doi.org/10.1056/NEJMoa1113425.
18. Karasavvas N, Billings E, Rao M, Williams C, Zolla-Pazner S, Bailer RT, Koup RA, Madnote S, Arworn D, Shen X, Tomaras GD, Currier JR, Jiang M, Magaret C, Andrews C, Gottardo R, Gilbert P, Cardozo TJ, Rerks-Ngarm S, Nitayaphan S, Pitisuttithum P, Kaewkungwal J, Paris R, Greene K, Gao H, Gurunathan S, Tartaglia J, Sinangil F, Korber BT, Montefiori DC, Mascola JR, Robb ML, Haynes BF, Ngauy V, Michael NL, Kim JH, de Souza MS. 2012. The Thai Phase III HIV type 1 vaccine trial (RV144) regimen induces antibodies that target conserved regions within the V2 loop of gp120. AIDS Res Hum Retroviruses 28:1444–1457. https://doi.org/10.1089/aid.2012.0103.
19. Rolland M, Edlefsen PT, Larsen BB, Tovanabutra S, Sanders-Buell E, Hertz T, deCamp AC, Carrico C, Menis S, Magaret CA, Ahmed H, Juraska M, Chen L, Konopa P, Nariya S, Stoddard JN, Wong K, Zhao H, Deng W, Maust BS, Bose M, Howell S, Bates A, Lazzaro M, O'Sullivan A, Lei E, Bradfield A, Ibitamuno G, Assawadarachai V, O'Connell RJ, deSouza MS, Nitayaphan S, Rerks-Ngarm S, Robb ML, McLellan JS, Georgiev I, Kwong PD, Carlson JM, Michael NL, Schief WR, Gilbert PB, Mullins JI, Kim JH. 2012. Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. Nature 490:417–420. https://doi.org/10.1038/nature11519.
20. Roy S, Clawson DS, Adam VS, Medina A, Wilson JM. 2011. Construction of gene transfer vectors based on simian adenovirus 7. J Gen Virol 92:1749–1753. https://doi.org/10.1099/vir.0.032300-0.
21. Alexander J, Mendy J, Vang L, Avanzini JB, Garduno F, Manayani DJ, Ishioka G, Farness P, Ping LH, Swanstrom R, Parks R, Liao HX, Haynes BF, Montefiori DC, LaBranche C, Smith J, Gurwith M, Mayall T. 2013. Preclinical development of a recombinant, replication-competent adenovirus serotype 4 vector vaccine expressing HIV-1 envelope 1086 clade C. PLoS One 8:e82380. https://doi.org/10.1371/journal.pone.0082380.
22. Siddappa NB, Hemashettar G, Wong YL, Lakhashe S, Rasmussen RA, Watkins JD, Novembre FJ, Villinger F, Else JG, Montefiori DC, Ruprecht RM. 2011. Development of a tier 1 R5 clade C simian-human immunodeficiency virus as a tool to test neutralizing antibody-based immunoprophylaxis. J Med Primatol 40:120–128. https://doi.org/10.1111/j.1600-0684.2010.00454.x.
23. Siddappa NB, Watkins JD, Wassermann KJ, Song R, Wang W, Kramer VG, Lakhashe S, Santosuosso M, Poznansky MC, Novembre FJ, Villinger F, Else JG, Montefiori DC, Rasmussen RA, Ruprecht RM. 2010. R5 clade C SHIV strains with tier 1 or 2 neutralization sensitivity: tools to dissect env evolution and to develop AIDS vaccines in primate models. PLoS One 5:e11689. https://doi.org/10.1371/journal.pone.0011689.
24. Forthal D, Hope TJ, Alter G. 2013. New paradigms for functional HIV-specific nonneutralizing antibodies. Curr Opin HIV AIDS 8:393–401. https://doi.org/10.1097/COH.0b013e328363d486.
25. Forthal DN, Moog C. 2009. Fc receptor-mediated antiviral antibodies. Curr Opin HIV AIDS 4:388–393. https://doi.org/10.1097/COH.0b013e32832f0a89.
26. Yant LJ, Friedrich TC, Johnson RC, May GE, Maness NJ, Enz AM, Lifson JD, O'Connor DH, Carrington M, Watkins DI. 2006. The high-frequency major histocompatibility complex class I allele Mamu-B*17 is associated with

control of simian immunodeficiency virus SIVmac239 replication. J Virol 80:5074–5077. https://doi.org/10.1128/JVI.80.10.5074-5077.2006.

27. Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, Lynch DM, Iampietro MJ, SanMiguel A, Seaman MS, Ferrari G, Forthal DN, Ourmanov I, Hirsch VM, Carville A, Mansfield KG, Stablein D, Pau MG, Schuitemaker H, Sadoff JC, Billings EA, Rao M, Robb ML, Kim JH, Marovich MA, Goudsmit J, Michael NL. 2012. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. Nature 482:89–93. https://doi.org/10.1038/nature10766.
28. Xiao P, Patterson LJ, Kuate S, Brocca-Cofano E, Thomas MA, Venzon D, Zhao J, DiPasquale J, Fenizia C, Lee EM, Kalisz I, Kalyanaraman VS, Pal R, Montefiori D, Keele BF, Robert-Guroff M. 2012. Replicating adenovirus-simian immunodeficiency virus (SIV) recombinant priming and envelope protein boosting elicits localized, mucosal IgA immunity in rhesus macaques correlated with delayed acquisition following a repeated low-dose rectal SIV(mac251) challenge. J Virol 86:4644–4657. https://doi.org/10.1128/JVI.06812-11.
29. Valentin A, Li J, Rosati M, Kulkarni V, Patel V, Jalah R, Alicea C, Reed S, Sardesai N, Berkower I, Pavlakis GN, Felber BK. 2015. Dose-dependent inhibition of Gag cellular immunity by Env in SIV/HIV DNA vaccinated macaques. Hum Vaccin Immunother 11:2005–2011. https://doi.org/10.1080/21645515.2015.1016671.
30. Bockl K, Wild J, Bredl S, Kindsmuller K, Kostler J, Wagner R. 2012. Altering an artificial Gagpolnef polyprotein and mode of ENV co-administration affects the immunogenicity of a clade C HIV DNA vaccine. PLoS One 7:e34723. https://doi.org/10.1371/journal.pone.0034723.
31. Toapanta FR, Craigo JK, Montelaro RC, Ross TM. 2007. Reduction of anti-HIV-1 Gag immune responses during coimmunization: immune interference by the HIV-1 envelope. Curr HIV Res 5:199–209. https://doi.org/10.2174/157016207780077057.
32. Valentin A, McKinnon K, Li J, Rosati M, Kulkarni V, Pilkington GR, Bear J, Alicea C, Vargas-Inchaustegui DA, Jean Patterson L, Pegu P, Liyanage NP, Gordon SN, Vaccari M, Wang Y, Hogg AE, Frey B, Sui Y, Reed SG, Sardesai NY, Berzofsky JA, Franchini G, Robert-Guroff M, Felber BK, Pavlakis GN. 2014. Comparative analysis of SIV-specific cellular immune responses induced by different vaccine platforms in rhesus macaques. Clin Immunol 155:91–107. https://doi.org/10.1016/j.clim.2014.09.005.
33. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH. 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 361:2209–2220. https://doi.org/10.1056/NEJMoa0908492.
34. Pegu P, Vaccari M, Gordon S, Keele BF, Doster M, Guan Y, Ferrari G, Pal R, Ferrari MG, Whitney S, Hudacik L, Billings E, Rao M, Montefiori D, Tomaras G, Alam SM, Fenizia C, Lifson JD, Stablein D, Tartaglia J, Michael N, Kim J, Venzon D, Franchini G. 2013. Antibodies with high avidity to the gp120 envelope protein in protection from simian immunodeficiency virus SIV(mac251) acquisition in an immunization regimen that mimics the RV-144 Thai trial. J Virol 87:1708–1719. https://doi.org/10.1128/JVI.02544-12.
35. Hansen SG, Piatak M, Jr, Ventura AB, Hughes CM, Gilbride RM, Ford JC, Oswald K, Shoemaker R, Li Y, Lewis MS, Gilliam AN, Xu G, Whizin N, Burwitz BJ, Planer SL, Turner JM, Legasse AW, Axthelm MK, Nelson JA, Fruh K, Sacha JB, Estes JD, Keele BF, Edlefsen PT, Lifson JD, Picker LJ. 2013. Immune clearance of highly pathogenic SIV infection. Nature 502:100–104. https://doi.org/10.1038/nature12519.
36. Hessell AJ, Jaworski JP, Epson E, Matsuda K, Pandey S, Kahl C, Reed J, Sutton WF, Hammond KB, Cheever TA, Barnette PT, Legasse AW, Planer S, Stanton JJ, Pegu A, Chen X, Wang K, Siess D, Burke D, Park BS, Axthelm MK, Lewis A, Hirsch VM, Graham BS, Mascola JR, Sacha JB, Haigwood NL. 2016. Early short-term treatment with neutralizing human monoclonal antibodies halts SHIV infection in infant macaques. Nat Med 22:362–368. https://doi.org/10.1038/nm.4063.
37. Alexander J, Ward S, Mendy J, Manayani DJ, Farness P, Avanzini JB, Guenther B, Garduno F, Jow L, Snarsky V, Ishioka G, Dong X, Vang L, Newman MJ, Mayall T. 2012. Preclinical evaluation of a replication-competent recombinant adenovirus serotype 4 vaccine expressing influenza H5 hemagglutinin. PLoS One 7:e31177. https://doi.org/10.1371/journal.pone.0031177.
38. Hessell AJ, Malherbe DC, Pissani F, McBurney S, Krebs SJ, Gomes M, Pandey S, Sutton WF, Burwitz BJ, Gray M, Robins H, Park BS, Sacha JB, LaBranche CC, Fuller DH, Montefiori DC, Stamatatos L, Sather DN, Haigwood NL. 2016. Achieving potent autologous neutralizing antibody responses against tier 2 HIV-1 viruses by strategic selection of envelope immunogens. J Immunol 196:3064–3078. https://doi.org/10.4049/jimmunol.1500527.
39. Hessell AJ, McBurney S, Pandey S, Sutton W, Liu L, Li L, Totrov M, Zolla-Pazner S, Haigwood NL, Gorny MK. 2016. Induction of neutralizing antibodies in rhesus macaques using V3 mimotope peptides. Vaccine 34:2713–2721. https://doi.org/10.1016/j.vaccine.2016.04.027.
40. Malherbe DC, Doria-Rose NA, Misher L, Beckett T, Puryear WB, Schuman JT, Kraft Z, O'Malley J, Mori M, Srivastava I, Barnett S, Stamatatos L, Haigwood NL. 2011. Sequential immunization with a subtype B HIV-1 envelope quasispecies partially mimics the in vivo development of neutralizing antibodies. J Virol 85:5262–5274. https://doi.org/10.1128/JVI.02419-10.
41. Forthal DN, Landucci G. 1998. In vitro reduction of virus infectivity by antibody-dependent cell-mediated immunity. J Immunol Methods 220: 129–138. https://doi.org/10.1016/S0022-1759(98)00152-5.
42. Bialuk I, Whitney S, Andresen V, Florese RH, Nacsa J, Cecchinato V, Valeri VW, Heraud JM, Gordon S, Parks RW, Montefiori DC, Venzon D, Demberg T, Guroff MR, Landucci G, Forthal DN, Franchini G. 2011. Vaccine induced antibodies to the first variable loop of human immunodeficiency virus type 1 gp120, mediate antibody-dependent virus inhibition in macaques. Vaccine 30:78–94. https://doi.org/10.1016/j.vaccine.2011.10.040.
43. Jaworski JP, Kobie J, Brower Z, Malherbe DC, Landucci G, Sutton WF, Guo B, Reed JS, Leon EJ, Engelmann F, Zheng B, Legasse A, Park B, Dickerson M, Lewis AD, Colgin LM, Axthelm M, Messaoudi I, Sacha JB, Burton DR, Forthal DN, Hessell AJ, Haigwood NL. 2013. Neutralizing polyclonal IgG present during acute infection prevents rapid disease onset in simian-human immunodeficiency virus SHIVSF162P3-infected infant rhesus macaques. J Virol 87:10447–10459. https://doi.org/10.1128/JVI.00049-13.
44. Wilson NA, Keele BF, Reed JS, Piaskowski SM, MacNair CE, Bett AJ, Liang X, Wang F, Thoryk E, Heidecker GJ, Citron MP, Huang L, Lin J, Vitelli S, Ahn CD, Kaizu M, Maness NJ, Reynolds MR, Friedrich TC, Loffredo JT, Rakasz EG, Erickson S, Allison DB, Piatak M, Jr, Lifson JD, Shiver JW, Casimiro DR, Shaw GM, Hahn BH, Watkins DI. 2009. Vaccine-induced cellular responses control simian immunodeficiency virus replication after heterologous challenge. J Virol 83:6508–6521. https://doi.org/10.1128/JVI.00272-09.
45. Song RJ, Chenine AL, Rasmussen RA, Ruprecht CR, Mirshahidi S, Grisson RD, Xu W, Whitney JB, Goins LM, Ong H, Li PL, Shai-Kobiler E, Wang T, McCann CM, Zhang H, Wood C, Kankasa C, Secor WE, McClure HM, Strobert E, Else JG, Ruprecht RM. 2006. Molecularly cloned SHIV-1157ipd3N4: a highly replication-competent, mucosally transmissible R5 simian-human immunodeficiency virus encoding HIV clade C Env. J Virol 80:8729–8738. https://doi.org/10.1128/JVI.00558-06.