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Protective Efficacy of a Single Immunization of a Chimeric Adenovirus Vector-Based Vaccine against Simian Immunodeficiency Virus Challenge in Rhesus Monkeys▽

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Rare serotype and chimeric recombinant adenovirus (rAd) vectors that evade anti-Ad5 immunity are currently being evaluated as potential vaccine vectors for human immunodeficiency virus type 1 and other pathogens. We have recently reported that a heterologous rAd prime-boost regimen expressing simian immunodeficiency virus (SIV) Gag afforded durable partial immune control of an SIV challenge in rhesus monkeys. However, single-shot immunization may ultimately be preferable for global vaccine delivery. We therefore evaluated the immunogenicity and protective efficacy of a single immunization of chimeric rAd5 hexon hypervariable region 48 (rAd5HVR48) vectors expressing SIV Gag, Pol, Nef, and Env against a homologous SIV challenge in rhesus monkeys. Inclusion of Env resulted in improved control of peak and set point SIV RNA levels following challenge. In contrast, DNA vaccine priming did not further improve the protective efficacy of rAd5HVR48 vectors in this system.

Heterologous prime-boost vaccine regimens have proven substantially more immunogenic than single vector immunizations in a variety of experimental models, but a single-shot vaccine would presumably be ideal for eventual global delivery. The potential utility of single-shot vaccines against pathogenic simian immunodeficiency virus (SIV) challenges in rhesus monkeys has not been well characterized. We therefore evaluated the protective efficacy of a single immunization of recombinant chimeric adenovirus type 5 (rAd5) hexon hypervariable region 48 (rAd5HVR48) vectors (15) expressing SIV Gag, Pol, Nef, and Env against a pathogenic SIV challenge in rhesus monkeys. These vectors contain the HVRs of the rare Ad48 serotype and have been shown to evade dominant Ad5 hexon-specific neutralizing antibodies (NAbs) (15). We also assessed the potential utility of inclusion of Env as an immunogen (6, 7, 17) and the degree to which DNA vaccine priming would enhance the protective efficacy afforded by a single rAd5HVR48 immunization (2, 7, 18, 21).

Thirty adult rhesus monkeys (n = 6/group) lacking the *Mamu-A*01*, *Mamu-B*17*, and *Mamu-B*08* class I alleles were primed with plasmid DNA vaccines and boosted with rAd5HVR48 vectors as follows: (1) adjuvanted DNA prime, rAd5HVR48 boost; (2) DNA prime, rAd5HVR48 boost; (3) rAd5HVR48 alone; (4) rAd5HVR48 alone (excluding Env); and (5) sham controls. Monkeys in groups 1 to 3 received vectors expressing SIVmac239 Gag, Pol, Nef, and Env, whereas monkeys in group 4 received vectors expressing only Gag, Pol, and Nef. The DNA vaccine adjuvants in group 1 were plasmids expressing the rhesus chemokine MIP-1α and Flt3L, which have been shown to increase recruitment of dendritic cells and to improve DNA vaccine immunogenicity (20). Monkeys were primed intramuscularly with a total dose of 4 mg of DNA vaccines at weeks 0, 4, and 8. All animals then received a single intramuscular immunization of 4×10^{10} viral particles (vp) of rAd5HVR48 at week 24. At week 52, animals were challenged intravenously (i.v.) with 100 monkey infectious doses of SIVmac251 (7, 10).

Vaccine-elicited immune responses. We monitored vaccine-elicited, SIV-specific cellular immune responses prior to challenge by gamma interferon (IFN-γ) enzyme-linked immunospot (ELISPOT) assays following stimulation with SIV Gag, Pol, Nef, and Env peptide pools (8). As shown in Fig. 1A, monkeys that received a single immunization of the rAd vaccines alone at week 24 developed IFN-γ ELISPOT responses to the encoded SIV antigens by week 26. As expected, animals that were primed with the DNA vaccines developed higher responses than those elicited by the rAd alone vaccines (16, 18). The adjuvanted DNA vaccines elicited a trend toward higher peak responses compared with the unadjuvanted DNA vaccines, but these differences were not sustained at week 52.

As shown in Fig. 1B, DNA priming augmented both $CD8^+$ and $CD4^+$ T-lymphocyte responses, as determined by cell-

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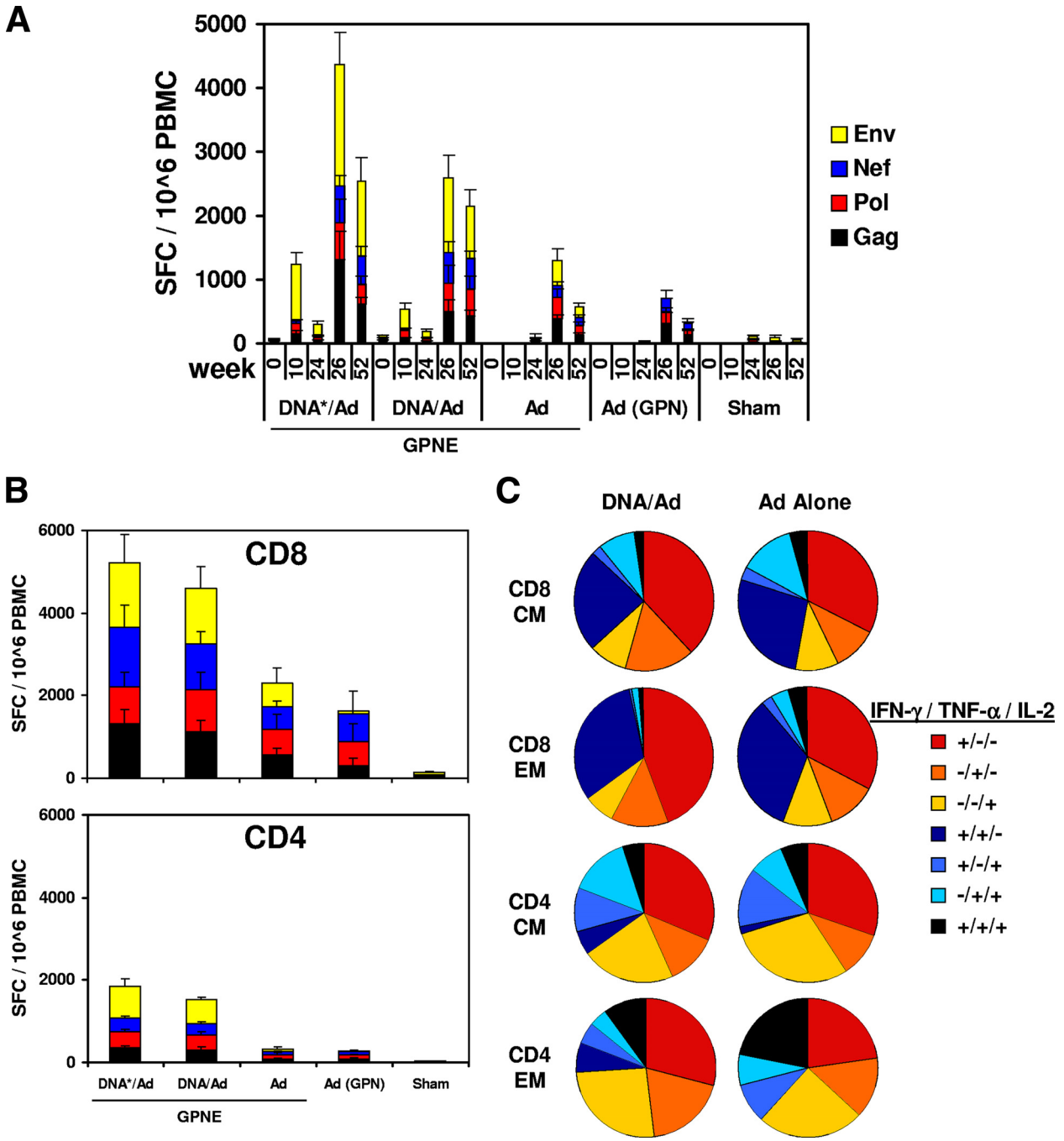

FIG. 1. Immunogenicity of vaccine regimens. Rhesus monkeys were primed at weeks 0, 4, and 8 with adjuvanted DNA vaccines (DNA*) or unadjuvanted DNA vaccines and were boosted at week 24 with a single immunization of rAd5HVR48 expressing SIV Gag, Pol, Nef, and Env (GPNE). One group of monkeys received rAd5HVR48 vectors expressing only SIV Gag, Pol, and Nef (GPN). (A) SIV-specific IFN-γ ELISPOT assays were performed at weeks 0, 10, 24, 26, and 52 following immune priming. (B) SIV-specific $CD8^+$ (top panel) and $CD4^+$ (bottom panel) T-lymphocyte responses were evaluated at week 28 by CD4-depleted and CD8-depleted ELISPOT assays, respectively. Data represent mean responses with standard errors. (C) The functionality of SIV-specific $CD8^+$ and $CD4^+$ central memory CM ($CD28^+$ $CD95^+$) and effector memory EM ($CD28^-$ $CD95^+$) T-lymphocyte responses to all antigens elicited by DNA/rAd5HVR48 regimens and rAd5HVR48 alone regimens was assessed by eight-color ICS assays. Proportions of IFN-γ, TNF-α, and IL-2 responses are depicted individually and in all possible combinations for each cellular subpopulation.

depleted IFN-γ ELISPOT assays ($P = 0.01$ and $P = 0.001$, respectively [Wilcoxon rank-sum tests]). Multiparameter intracellular cytokine staining (ICS) assays (8, 13, 14) confirmed these findings but did not reveal other consistent phenotypic differences in terms of IFN-γ, tumor necrosis factor alpha (TNF-α), and interleukin 2 (IL-2) secretion in $CD8^+$ and $CD4^+$ central memory (CM [$CD28^+$ $CD95^+$]) and effector memory (EM [$CD28^-$ $CD95^+$]) T-lymphocyte subpopula-

tions (Fig. 1C). The ICS assays utilized SIV Gag, Pol, Nef, and Env peptide pools and the following monoclonal antibodies: anti-CD3-Alexa700 (SP34), anti-CD4-AmCyan (L200), anti-CD8-allophycocyanin-Cy7 (anti-CD8-APC-Cy7) (SK1), anti-CD28-PerCP-Cy5.5 (L293), anti-CD95-phycoerythrin (anti-CD95-PE) (DX2), anti-IFN-γ–PE-Cy7 (B27), anti-IL-2–APC (MQ1-17H12), and anti-TNF-α–fluorescein isothiocyanate (anti-TNF-α–FITC) (Mab11).

Immune responses following SIV challenge. Six months after the rAd boost immunization, monkeys were challenged i.v. with the essentially homologous virus SIVmac251 (7, 10). As shown in Fig. 2A, all vaccinated animals developed rapid and potent anamnestic SIV-specific cellular immune responses by week 2 following challenge, as determined by IFN-γ ELISPOT assays. SIV-specific humoral immune responses were evaluated by luciferase-based pseudovirus neutralization assays (12) and antibody-dependent cell-mediated virus inhibition (ADCVI) assays (5). All animals developed virus-specific NAbs against laboratory-adapted SIVmac251 but not against primary isolate SIVmac251 following challenge (Fig. 2B). Animals that received vaccines containing SIV Env (groups 1 to 3) also developed more rapid kinetics of ADCVI than animals that received sham controls or vaccines that did not include Env (Fig. 2C).

We next monitored the $CD4^+$ T-lymphocyte dynamics in these animals following challenge (10) utilizing the following monoclonal antibodies: anti-CD3-Alexa700 (SP34), anti-CD4-AmCyan (L200), anti-CD8-APC-Cy7 (SK1), anti-CD28-PerCP-Cy5.5 (L293), anti-CD95-APC (DX2), anti-CCR5-PE (3A9), anti-HLA-DR–PE-Cy7 (L243), and anti-Ki67-FITC (B56). As shown in Fig. 3, control animals exhibited slightly decreased levels of $CD4^+$ CM T lymphocytes, substantially decreased levels of $CCR5^+$ $CD4^+$ CM T lymphocytes, and markedly increased levels of $Ki67^+$ proliferation of $CCR5^+$ $CD4^+$ CM T lymphocytes following SIV challenge, consistent with our prior results (10). In contrast, animals vaccinated with rAd alone had fewer dramatic perturbations of these $CD4^+$ T-lymphocyte subsets.

Protective efficacy. To assess the protective efficacy of these vaccine regimens, we monitored SIV RNA levels and survival in these animals following challenge, as depicted in Fig. 4. SIV RNA levels for each individual animal (Fig. 4A) as well as peak (day 14) (Fig. 4B) and set point (median days 112 to 392) (Fig. 4C) SIV RNA levels are shown. The rAd alone vaccine expressing only Gag, Pol, and Nef afforded only minimal control of SIV replication, but the rAd alone vaccine expressing Gag, Pol, Nef, and Env resulted in a 1.34 log reduction of peak SIV RNA levels (Fig. 4B) ($P = 0.001$ [Wilcoxon rank-sum test]) and a 1.08 log reduction of set point SIV RNA levels compared with sham controls (Fig. 4C) ($P = 0.05$). These data suggest that the addition of Env improved control of SIV replication following challenge in this study. The rAd alone vaccines also afforded trends toward improved survival compared with sham controls for 500 days following the challenge (Fig. 4D) ($P = 0.04$ and $P = 0.07$ [unadjusted two-sided log-rank tests comparing rAd-Gag/Pol/Nef/Env and rAd-Gag/Pol/Nef regimens, respectively, versus sham controls]). Moreover, in a prespecified exploratory analysis, the combined rAd alone vaccinated animals ($n = 12$) exhibited significantly improved survival compared with the sham controls ($P = 0.003$ [two-sided log-rank test]).

The monkeys that were primed with DNA vaccines or adjuvanted DNA vaccines prior to the rAd immunization, however, did not exhibit improved protection compared with the animals that received rAd alone vaccines. The DNA/rAd regimens afforded 0.94 to 1.11 log reductions of peak SIV RNA levels (Fig. 4B) ($P = 0.004$ [Wilcoxon rank-sum test]) but no significant reductions of set point SIV RNA levels (Fig. 4C) ($P =$ nonsignificant) and only marginal improvements of survival (Fig. 4D) ($P =$ nonsignificant) compared with the sham controls. These data show that DNA vaccine priming did not improve the protective efficacy afforded by rAd5HVR48 vectors in this study. These results are consistent with previous SIV challenge studies that have similarly shown no significant and durable benefit of DNA vaccine priming prior to rAd5 boosting in terms of set point SIV RNA levels or survival following challenge (2, 7). Casimiro et al. (2) reported that neither DNA/rAd5 regimens nor rAd5 alone regimens expressing SIV Gag resulted in significant reductions of set point SIV RNA levels following SIV challenge of *Mamu-A*01*-positive rhesus monkeys, although a transient 0.8 log reduction of peak SIV RNA levels was noted in the DNA/rAd5 group. Similarly, Letvin et al. (7) reported that neither DNA/rAd5 regimens nor rAd5 alone regimens expressing SIV Gag, Pol, and Env resulted in significant reductions of set point SIV RNA levels following SIV challenge of *Mamu-A*01*-negative monkeys, although a transient 1.1 log reduction of peak SIV RNA levels and a survival advantage were observed in a post hoc analysis of all vaccinated groups combined.

One possible explanation for these observations is that the DNA vaccines utilized in these studies were simply not sufficiently potent to enhance protective efficacy in this stringent challenge model. Improved DNA vaccine delivery technologies, such as in vivo electroporation (9, 11) and the use of genetic adjuvants (1, 3), may therefore prove useful in this regard. An alternative possibility is that DNA priming skewed the phenotypes of the vaccine-elicited cellular immune responses. It has been reported that DNA priming prior to rAd5 boosting increased virus-specific $CD4^+$ T-lymphocyte responses in both rhesus monkeys (16) and humans (4), and our data are consistent with these observations (Fig. 1B). It is therefore possible that SIV-specific $CD4^+$ T-lymphocyte responses that were augmented by DNA priming may have resulted in increased numbers of viral targets for infection (19). Our current data, however, are insufficient to address this possibility conclusively; thus, further studies will be required to address these hypotheses.

The present findings show that a single-shot immunization with rAd5HVR48 vectors afforded a detectable but relatively modest level of protective efficacy against a homologous SIV challenge in rhesus monkeys. Overall, the protective efficacy observed in this study was less impressive than that seen in a previous study in which we utilized a heterologous rAd26 prime, rAd5 boost regimen expressing SIV Gag (10). A direct comparison of the results of these two studies, however, is not possible as a result of the different vectors and inserts utilized. Nevertheless, it appears likely that a single-shot vaccine regimen may not afford as robust protection as an optimal heterologous rAd prime-boost regimen. In our previous study of the rAd26/rAd5 regimen, we observed a significant inverse corre-

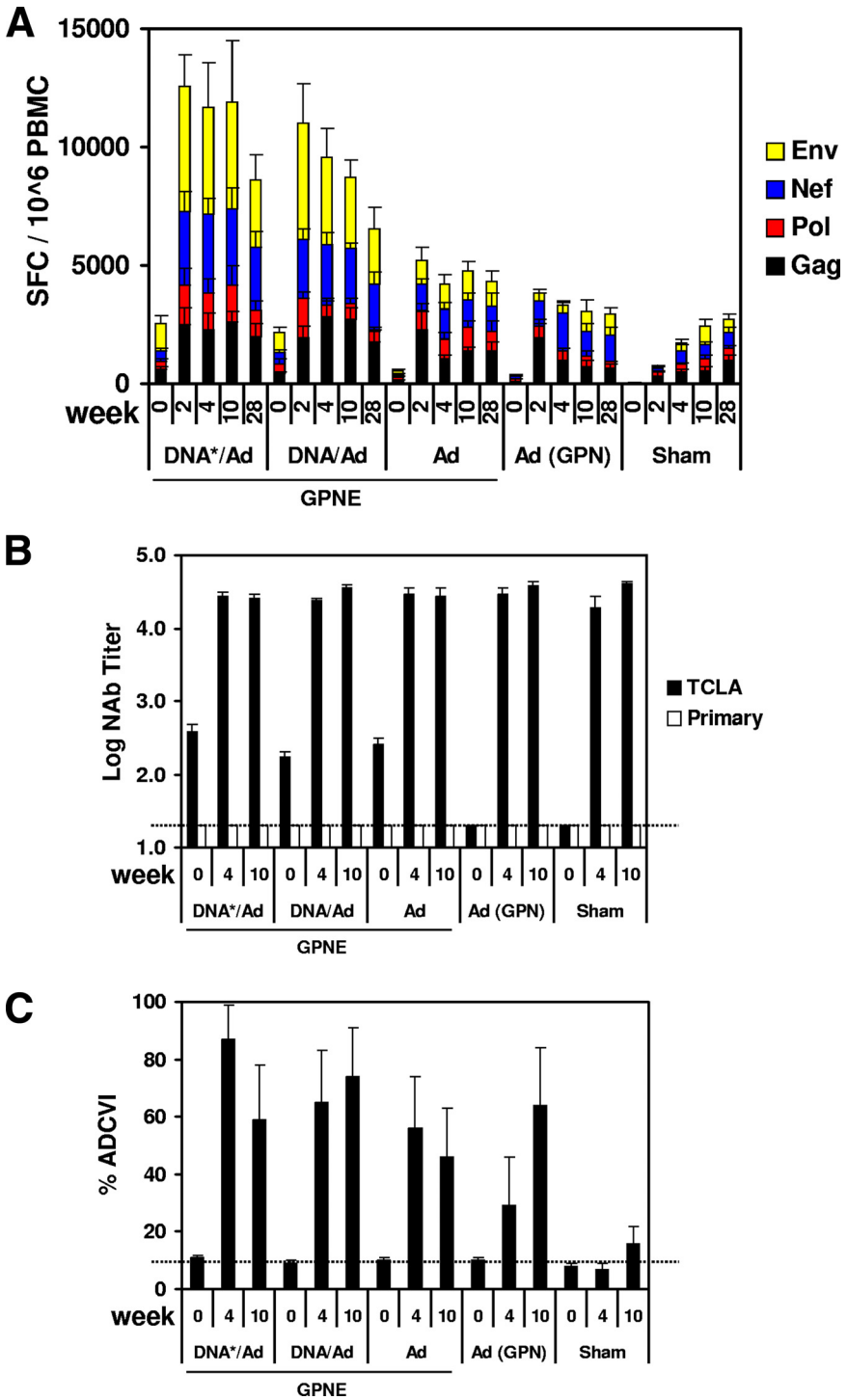

FIG. 2. Cellular and humoral immune responses following challenge. (A) IFN-γ ELISPOT assays, (B) SIV-specific neutralizing antibody assays against T-cell laboratory-adapted (TCLA) and primary isolate SIVmac251, and (C) ADCVI assays at a 1:100 dilution were performed at multiple time points following challenge. GPNE, SIV Gag, Pol, Nef, and Env; GPN, SIV Gag, Pol, and Nef; SFC, spot-forming cells; PBMC, peripheral blood mononuclear cells.

lation between the magnitude and breadth of Gag-specific cellular immune responses and set point viral loads (10). In contrast, we did not detect statistically significant correlations between vaccine-elicited immune responses and set point viral loads in the present study. This may reflect the fact that DNA priming could have opposing effects in terms of augmenting potentially useful cellular immune responses but also potentially increasing viral targets for infection, thus complicating analyses of immune correlates of protection.

In the present study, peak and set point SIV RNA levels

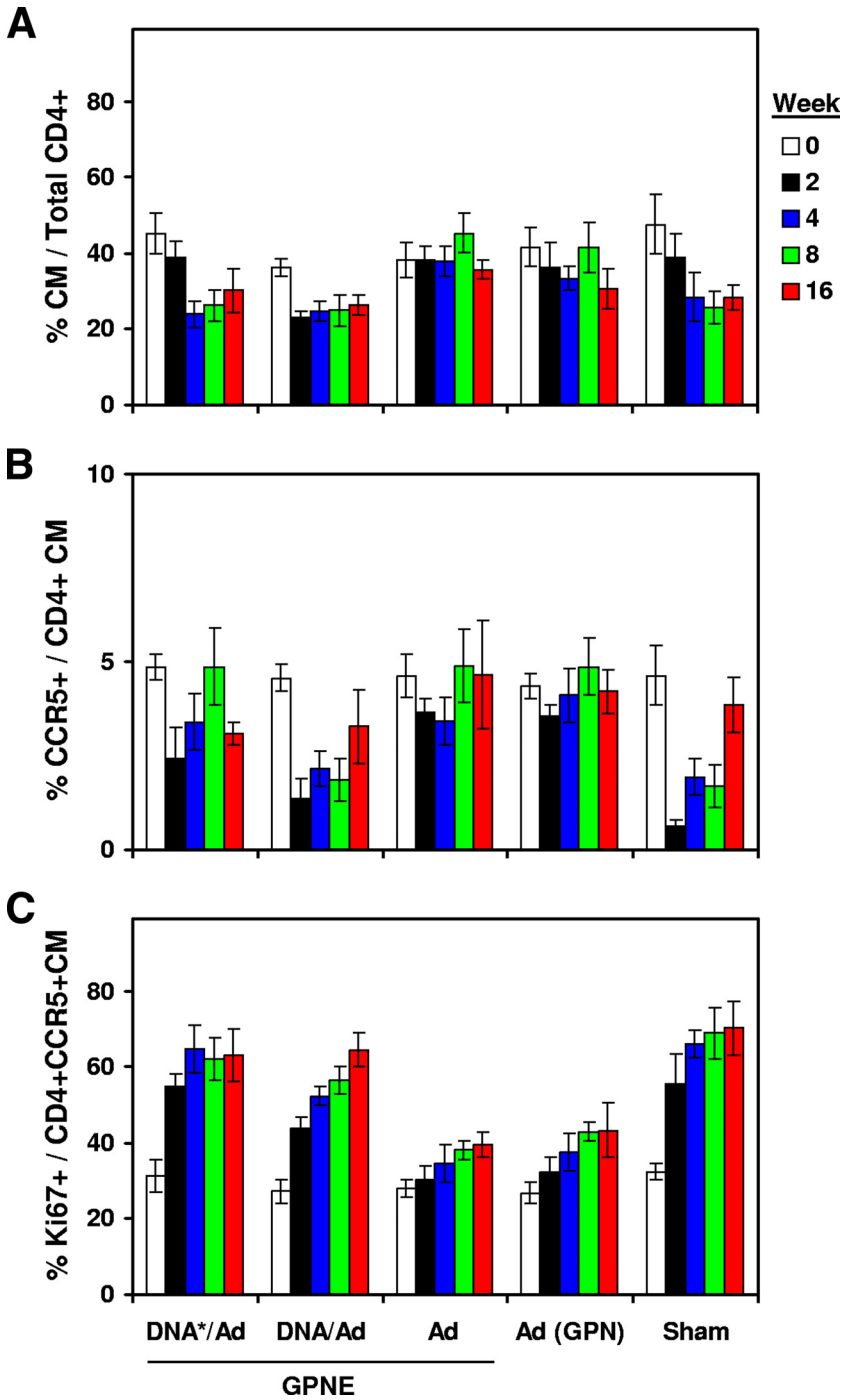

FIG. 3. $CD4^+$ T-lymphocyte dynamics following challenge. (A) CM ($CD28^+$ $CD95^+$) $CD4^+$ T lymphocytes and (B) $CCR5^+$ $CD4^+$ CM T lymphocytes were assessed at multiple time points following challenge. (C) Ki67 staining of $CD4^+$ $CCR5^+$ CM T lymphocytes was also determined. Data represent mean responses with standard errors. GPNE, SIV Gag, Pol, Nef, and Env; GPN, SIV Gag, Pol, and Nef.

were lower in animals that received rAd5HVR48 vectors expressing Gag, Pol, Nef, and Env than in those that received rAd5HVR48 vectors expressing only Gag, Pol, and Nef. These data indicate that inclusion of Env resulted in improved control of SIV replication in this study. We suspect that Env-specific cellular immune responses likely contributed to the improved protective efficacy, since Env-specific NAbs against primary isolate SIVmac251 were not observed either prior to challenge or following challenge. More rapid kinetics of Env-specific ADCVI activity, however, was observed in the groups administered vaccine containing Env; thus, it is possible that nonneutralizing Env-specific antibodies also contributed to protection. A limitation is that the challenge virus was essentially homologous to the antigens contained in the vac-

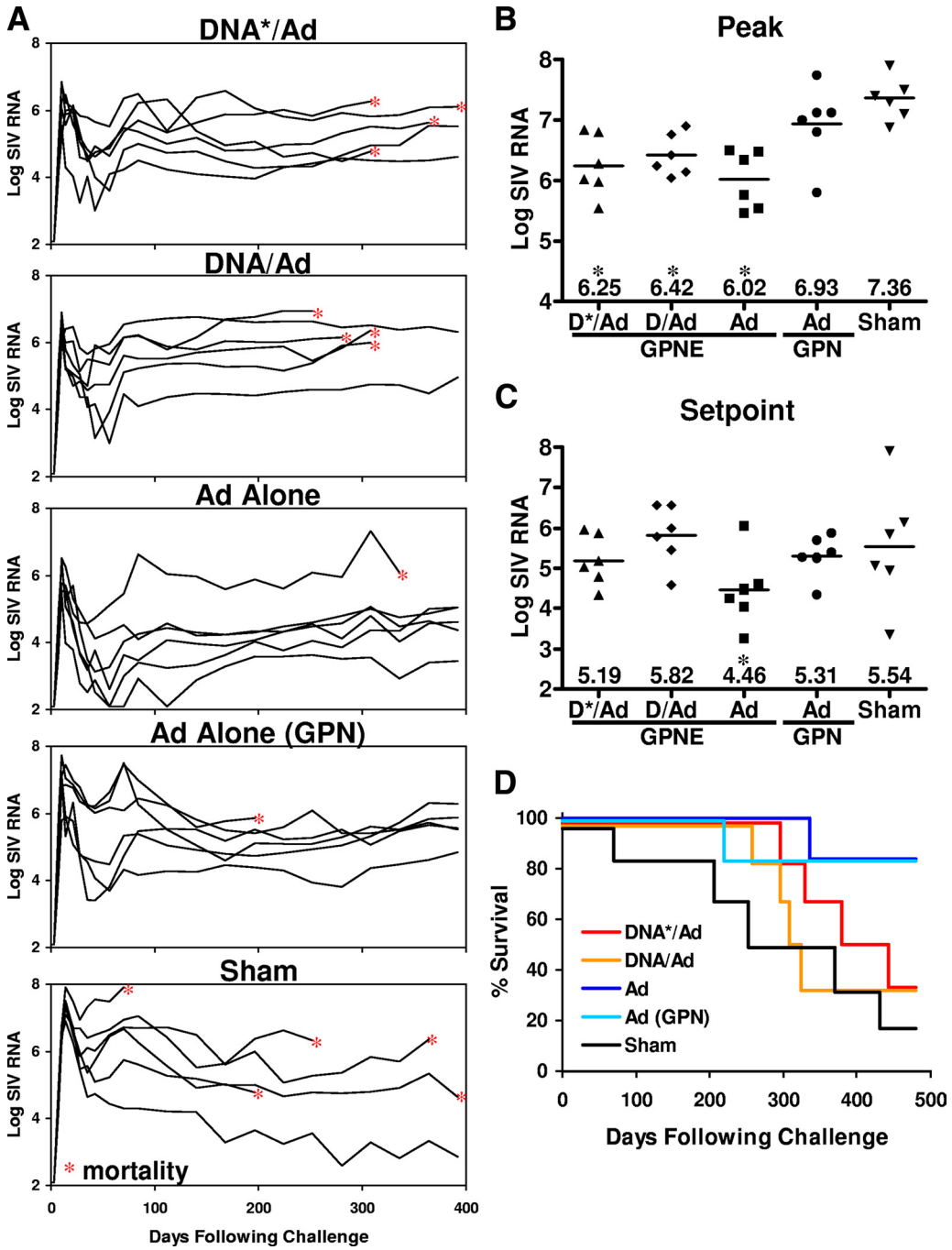

FIG. 4. Protective efficacy of vaccine regimens. Monkeys were challenged i.v. with SIVmac251, and protective efficacy was monitored by SIV RNA levels (A to C) and clinical disease progression and mortality following challenge (D). (A to C) SIV RNA levels are depicted longitudinally for each group (A), and peak (day 14) (B) and set point (median days 112 to 392) (C) SIV RNA levels are summarized for each group. Red asterisks indicate mortality. The numbers at the bottom of the graphs indicate mean SIV RNA levels in each group. Black asterisks indicate significant differences compared with sham controls as assessed by two-tailed Wilcoxon rank-sum tests. (D) Survival curve for 500 days following challenge. GPNE, SIV Gag, Pol, Nef, and Env; GPN, SIV Gag, Pol, and Nef.

cine. Thus, future studies utilizing larger numbers of animals will be required to evaluate the potential utility of Env against heterologous SIV challenges. The protective efficacy of single immunizations of optimal vaccine vectors should also continue to be explored.

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We declare no competing financial interests.

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