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Protective Efficacy of Adenovirus/Protein Vaccines Against SIV Challenges in Rhesus Monkeys

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Abstract

Preclinical studies of viral vector-based HIV-1 vaccine candidates have previously shown partial protection against stringent virus challenges in rhesus monkeys. In this study, we evaluated the protective efficacy of adenovirus serotype 26 (Ad26) vector priming followed by boosting with a purified envelope (Env) glycoprotein. Rhesus monkeys primed with Ad26 vectors expressing SIVsmE543 Env/Gag/Pol antigens and boosted with AS01B-adjuvanted SIVmac32H Env gp140 demonstrated complete protection in 50% of vaccinated animals against a series of repetitive, heterologous, intrarectal SIVmac251 challenges that infected all controls. Protective efficacy correlated with the functionality of Env-specific antibody responses. Comparable protection was also observed with a similar Ad/Env vaccine against repetitive, heterologous, intrarectal SHIV-SF162P3 challenges. These data demonstrate robust protection by Ad/Env vaccines against acquisition of stringent virus challenges in rhesus monkeys.

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Supplementary Materials
Materials and Methods
Figure S1 to S15

Despite the urgent need for a safe and effective global HIV-1 vaccine, only four vaccine concepts have been evaluated for protective efficacy in humans in over 30 years (1, 2). In rhesus monkeys, vaccine protection has been reported against neutralization-sensitive viruses such as SIVsmE660 (3), but these data did not predict protective efficacy in humans (4), suggesting the importance of utilizing more stringent virus challenges for preclinical evaluation of HIV-1 and SIV vaccine candidates. We previously showed that priming with adenovirus vectors and boosting with poxvirus vectors expressing Env, Gag, and Pol resulted in a reduced per exposure acquisition risk following challenges with neutralization-resistant SIVmac251, but the majority of these animals were infected at the end of the challenge series (5, 6). To augment antibody responses, we evaluated the immunogenicity and protective efficacy of priming with adenovirus vectors and boosting with adjuvanted Env gp140 protein against SIVmac251 and SHIV-SF162P3 challenges in rhesus monkeys.

We immunized 32 adult rhesus monkeys (*M. mulatta*) that did not express the protective major histocompatibility complex (MHC) class I alleles *Mamu-A*01*, *Mamu-B*08*, or *Mamu-B*17* with adenovirus serotype 26 (Ad26) vectors (7) expressing SIVsmE543 Env/Gag/Pol antigens (5) followed by either SIVmac32H Env gp140 protein (8) (Ad/Env; N=12) or Ad35 vectors (9) expressing SIVsmE543 Env/Gag/Pol antigens (Ad Alone; N=12), and a control group received sham vaccines (Sham; N=8). Animals in the Ad/Env group were primed with 3×10^{10} viral particles (vp) Ad26-Env/Gag/Pol vectors (10^{10} vp per vector) by the intramuscular route at weeks 0 and 24 and were boosted with 0.25 mg Env gp140 with AS01B Adjuvant System at weeks 52, 56, 60. Animals in the Ad Alone group were primed with 3×10^{10} vp Ad26-Env/Gag/Pol vectors at weeks 0 and 24 and were boosted with 3×10^{10} vp Ad35-Env/Gag/Pol at week 52. One control animal died prior to challenge for reasons unrelated to the study protocol and was excluded from the analysis.

Binding antibody responses to heterologous SIVmac239 Env gp140 were detected by ELISA (10) in all vaccinated animals following Ad26 priming at weeks 4 and 28 (Fig. 1A). In the Ad/Env group, ELISA endpoint titers increased from 5.3 logs at week 28 to 6.4 logs following the SIV Env gp140 boosts at week 64 ($P < 0.0001$, Fig. 1A), confirming that the Env boost effectively augmented Ad26-primed antibody responses. Neutralizing antibody (NAb) responses assessed by TZM-bl assays (11) against tier 1 heterologous SIVmac251_TCLA.15 and homologous SIVsmE660 CP3C-P-A8 viruses also increased significantly following SIV Env gp140 boosting (Fig. S1). NAb responses against tier 2 viruses were borderline (Fig. S1).

In addition to neutralization, antibodies mediate a wide variety of additional antiviral functions through their ability to interact with Fc receptors, complement, and lectin-like proteins (12, 13). Previous studies showed that antibody-dependent cellular phagocytosis (ADCP) (14) and antibody-dependent complement deposition (ADCD) responses correlated with protective efficacy in rhesus monkeys (6). To perform a comprehensive analysis of vaccine-elicited antibody responses, we evaluated 150 independent antibody Fc parameters by high-throughput antibody profiling (G.A., M.E.A. et al., manuscript submitted), including multiple assessments of antibody Fc functionality (ADCP, ADCC, ADCD, and antibody-dependent NK cell expression of CD107a, interferon (IFN)- γ , and the chemokine CCL4), isotypes, glycosylation, complement binding, and Fc receptor binding (14–18). Integration

of all 3600 data points in a “systems serology” principal component analysis demonstrated that the Ad/Env vaccine and the Ad Alone vaccine elicited Env-specific antibodies that were phenotypically distinct ($P<0.0001$; Fig. 1B). A loadings plot (Fig. 1C) showed the distribution of all measured Fc features in the same multi-dimensional space, demonstrating the specific features that drove the separation of antibody profiles (red arrows). Partial least squares discriminant analysis (19) revealed that the six antibody Fc functions described above nearly completely separated these groups, with the majority of antibody Fc effector functions clustering with the Ad/Env vaccinated animals (Fig. 1D). Univariate analyses showed that these antibody Fc functions were all significantly increased in Ad/Env group as compared with the Ad Alone group (Fig. 1E), and a combined analysis demonstrated that the number of antibody Fc functions was significantly greater in Ad/Env vaccinated animals as compared with Ad Alone vaccinated animals (Fig. 1F, G). These data show that the protein boost resulted in a more polyfunctional antibody Fc effector profile.

Cellular immune responses measured by IFN- γ ELISPOT assays in response to heterologous SIVmac239 and homologous SIVsmE543 Env/Gag/Pol peptide pools were also detected in all animals following vaccination (Fig. S2). By multiparameter intracellular cytokine staining assays, SIV Env gp140 boosting primarily expanded Env-specific IFN- γ +CD4+ T lymphocyte responses in the Ad/Env group, whereas Ad35-Env/Gag/Pol boosting substantially expanded IFN- γ +CD8+ T lymphocyte responses in the Ad Alone group (Fig. S2). Both CD28+CD95+ central/transitional memory and CD28+CD95- effector memory CD4+ and CD8+ T lymphocyte responses (20, 21) were elicited by both vaccines (Fig. S3).

To evaluate the protective efficacy of these vaccine regimens, all animals were challenged with 6 repetitive, intrarectal inoculations with 500 TCID₅₀ of the heterologous, stringent, neutralization-resistant virus SIVmac251 (5, 22, 23) beginning at week 96 (Fig. 2A, B). All control animals were infected by this challenge protocol. The Ad Alone vaccine regimen resulted in a 75% reduction in the per exposure acquisition risk as compared with controls (1 – hazard ratio; $P=0.039$, Cox proportional hazard model), which is consistent with our prior studies (5). In contrast, the Ad/Env vaccine regimen afforded a 90% reduction in the per exposure acquisition risk as compared with controls ($P=0.001$). Moreover, 50% (6 of 12) of animals in this group also appeared uninfected at the end of this challenge protocol ($P=0.012$ compared with controls, chi-square test; $P=0.044$, Fisher’s exact test; Fig. 2B). Protection in the Ad/Env group was greater than that in the Ad Alone group ($P=0.042$, chi-square test; $P=0.097$, Fisher’s exact test). Binding antibody titers ($P<0.0001$; $R=0.75$) and antibody Fc polyfunctionality ($P=0.004$; $R=0.56$) best correlated with protection against acquisition of infection, as measured by the number of challenges required for infection (Fig. 2C). Individual antibody functions also correlated (ADCP, ADCC, CD107, CCL4) or trended (ADCD, IFN- γ) with protection (data not shown).

In the Ad/Env group, plasma viral loads were persistently negative in the 6 protected monkeys for 400 days following challenge (Fig. 2D). Of the 6 infected animals in this group, 4 animals developed measureable chronic setpoint viremia, whereas 2 monkeys exhibited transient acute viremia and subsequently became elite controllers with undetectable plasma viral loads (Fig. 2D; Fig. S4). In the Ad Alone group, plasma viral loads were persistently negative in 2 of 12 monkeys, and chronic viremia developed in 10 of

12 animals. In contrast, all sham controls developed high levels of chronic viremia with a median setpoint viral load from days 100–400 following infection of 6.03 log copies/ml, which was at least 1.65 logs higher than the median setpoint viral load in the animals in the Ad/Env group that became infected ($P=0.035$; Fig. S5).

We previously reported that progressive SIV infection correlated with a marked expansion of the enteric virome in rhesus monkeys, particularly for picornavirus reads (24–27). Metagenomics sequencing of stool samples in the present study demonstrated that the enteric virome expanded by week 28 but not by week 10 in the sham controls ($P=0.015$; Fig. S6). Both the Ad/Env and the Ad Alone vaccines reduced the expansion of enteric picornaviruses ($P=0.002$ and $P=0.042$, respectively; Fig. S6) and total enteric virus reads (data not shown). The Ad/Env vaccine also reduced AIDS-related mortality as compared with the sham controls ($P=0.020$; Fig. S7).

We next investigated whether the vaccinated animals that exhibited persistently negative plasma viral loads were completely protected by comprehensive tissue analyses, adoptive transfer studies, and immunologic assays. We necropsied the 6 protected animals in the Ad/Env group, the 2 protected animals in the Ad Alone group, and one of the elite controllers in the Ad/Env group at approximately 400 days following challenge (Fig. 2D). All of these animals exhibited negative plasma viral loads at the time of necropsy. We assessed 36 gastrointestinal, lymphoid, and reproductive tract tissues per animal (28 tissues in males) by ultrasensitive nested qPCR or qRT-PCR assays for SIV DNA and SIV RNA as previously described (28). Viral DNA and RNA were readily detectable in all tissues in the elite controller (red circles; Fig. 3A) but not in the 8 protected animals (black circles; Fig. 3A), except for one viral signal in a single animal, which is within the range of expected background false positive signals in similar analyses of naïve animals (28).

We next performed adoptive transfer studies and infused 60 million peripheral blood and lymph node mononuclear cells by the intravenous route from the 8 apparently protected animals and the 2 elite controllers into naïve rhesus monkey hosts. Cells from the elite controllers readily transferred infection and resulted in plasma viral loads of 6.87–7.12 log copies/ml in naïve recipients by day 14 following adoptive transfer (red lines; Fig. 3B). In contrast, cells from the protected animals failed to transfer infection (black lines; Fig. 3B).

Furthermore, the protected animals exhibited no increase in Env/Gag/Pol-specific cellular immune responses following challenge and also no responses to Vif, which was not included in the vaccine, whereas vaccinated animals that became infected developed massive anamnestic Env/Gag/Pol-specific cellular immune responses and primary Vif-specific responses (Fig. S8). The protected animals also exhibited no anamnestic Env-specific ELISA antibody responses following challenge. Taken together (Fig. 3, S8), these data strongly suggest that the Ad/Env vaccine afforded complete sterilizing protection in 50% of animals against the SIVmac251 challenge protocol and that the mechanism of protection involved primary blocking of acquisition of infection.

To confirm these findings with analogous vaccines expressing HIV-1 immunogens, we utilized a group of 20 rhesus monkeys that had been immunized previously at weeks 0 and

40 with Ad26 and Ad5HVR48 vectors expressing mosaic, consensus, or natural clade C HIV-1 Env/Gag/Pol immunogens (29). Two years following Ad priming, these animals were boosted six times with 0.25 mg HIV-1 clade C C97ZA012 Env gp140 (10, 30) with the AS01B Adjuvant System at weeks 156, 160, 164, 176, 180, and 184 (Ad/Env; N=20). A second group of animals received only 0.25 mg Env gp140 with AS01B at the same six timepoints (Env Alone; N=8), and a third control group received sham vaccines (Sham; N=12). The Ad/Env vaccine elicited greater antibody responses than did the Env Alone vaccine by ELISA (Fig. S9), functional non-neutralizing antibody assays (6, 31, 32) (Fig. S9), tier 1 NAb TZM-bl assays (Fig. S10), tier 2 NAb A3R5 assays (Fig. S11), and linear peptide microarray assays (33, 34), including V2-specific responses (35, 36) (Fig. S12). Protective efficacy was assessed by six intrarectal challenges with 500 TCID₅₀ of the heterologous, neutralization-resistant virus SHIV-SF162P3 (6) beginning at week 196 (Fig. 4A, B). While the Env Alone vaccine afforded only minimal protection, 40% (8 of 20) of Ad/Env vaccinated animals were completely protected against this challenge series (P=0.006 compared with controls, chi-square test; P=0.014, Fisher's exact test; Fig. 4B; Fig. S13–S15). Binding antibody titers (P=0.008) and ADCP responses (P=0.001) correlated with protection against acquisition of infection.

Our data demonstrate the protective efficacy of Ad/Env vaccine regimens against SIVmac251 and SHIV-SF162P3 challenges in rhesus monkeys and suggest that the Env protein boost improved protective efficacy by enhancing the functionality of vaccine-elicited, Env-specific antibody responses. In contrast, DNA/Ad5 vaccines afforded no protection against SIVmac251 challenges (3), reflecting the ability of DNA/Ad5 vaccines to block only neutralization-sensitive virus clones (37). Alphavirus vector prime, Env protein boost as well as Env alone vaccines afforded partial protection against the neutralization-sensitive virus SHIV-SF162P4 but were not evaluated against neutralization-resistant viruses (38). Rhesus cytomegalovirus (CMV) vectors failed to block acquisition of infection but afforded post-infection virologic control and eventual viral clearance in approximately half of animals following SIVmac239 challenges (28, 39).

The protective efficacy of Ad/Env vaccines against acquisition of stringent virus challenges in rhesus monkeys in the present study has important implications for HIV-1 vaccine development and suggests the potential of Env protein boosting following Ad vector priming. Nevertheless, important differences exist between SIV/SHIV infection in rhesus monkeys and HIV-1 infection in humans. Clinical efficacy studies are therefore required to determine the protective efficacy of these HIV-1 vaccine candidates in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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financial interests. D.H.B. is a named co-inventor on vector, antigen, and protein patents (PCT/EP2007/052463, PCT/US2009/060494, PCT/US2009/064999). Correspondence and requests for materials should be addressed to D.H.B. (dbarouch@bidmc.harvard.edu). Vectors, antigens, proteins, adjuvants, and viruses are subject to MTA. We acknowledge support from the National Institutes of Health (AI060354, AI078526, AI080289, AI084794, AI095985, AI096040, AI102660, AI102691, OD011170, HHSN261200800001E), the Bill and Melinda Gates Foundation (OPP1032817), and the Ragon Institute of MGH, MIT, and Harvard.

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Figure 1. Humoral immune responses elicited by the Ad26/Env SIV vaccine

(A) SIVmac239 Env-specific ELISA titers at weeks 0, 4, 28, 64, and 96. Mean log endpoint ELISA titers are shown. (B–G) Multi-dimensional systems serology analysis integrating 150 antibody Fc parameters per animal demonstrates differences in antibody Fc profiles. (B) Principal component analysis (PCA) of antibody Fc profiles from animals immunized with Ad/Env (red) or Ad Alone (blue) vaccines. (C) Loadings plot mirrors the multidimensional space in the PCA but shows the distribution of all measured Fc features, demonstrating the features that drove the separation of antibody profiles (red arrows). (D) Partial least squares discriminant analysis (PLSDA) of antibody profiles from Ad/Env and Ad Alone vaccinees.

(E) Univariate analyses of antibody functions identified in (D). A composite (F) dot plot and (G) pie chart show the overall functionality of antibody responses elicited by the Ad/Env and the Ad Alone vaccines. Error bars reflect s.e.m. P-values reflect Mann-Whitney tests.

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Figure 2. Protective efficacy of the Ad26/Env SIV vaccine against repetitive, intrarectal SIVmac251 challenges

(A) Number of challenges required for acquisition of infection in each vaccine group. (B) Statistical analyses include the hazard ratio with 95% confidence interval and the per exposure reduction of acquisition risk in each group, with P-values reflecting Cox proportional hazard models and log-rank tests. Additional statistical analyses include the percentage of completely protected animals at the end of the challenge series, with P-values reflecting chi-square tests and Fisher's exact tests. (C) Correlation of log ELISA titers and antibody Fc polyfunctionality at week 64 with the number of challenges required to establish infection. The plotted data reflect only vaccinated animals and do not include the sham controls. Values plotted as >6 challenges reflect animals that remained uninfected. P-values reflect Spearman rank-correlation tests. (D) Plasma SIV RNA copies/ml over time in infected and protected animals in each vaccine group. Red asterisks indicate elite controllers. + indicates mortality.

Figure 3. Tissue analyses and adoptive transfer studies in SIVmac251 challenged animals
(A) Ultrasensitive SIV DNA and SIV RNA assays in multiple tissues from one elite controller (red) and the 8 apparently completely protected monkeys (black) following necropsy. SIV copies/ 10^8 cell equivalents are shown. (B) Plasma SIV RNA copies/ml in previously naïve recipient monkeys following adoptive transfer of 60 million peripheral blood and lymph node mononuclear cells from the 2 elite controllers (red) or the 8 apparently completely protected monkeys (black). Only one week of follow-up was available for one of the protected animals.

Figure 4. Protective efficacy of the Ad/Env HIV-1 vaccine against repetitive, intrarectal SHIV-SF162P3 challenges

(A) Number of challenges required for acquisition of infection in each vaccine group. (B) Statistical analyses include the hazard ratio with 95% confidence interval and the per exposure reduction of acquisition risk in each group, with P-values reflecting Cox proportional hazard models and log-rank tests. Additional statistical analyses include the percentage of completely protected animals at the end of the challenge series, with P-values reflecting chi-square tests and Fisher's exact tests.