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Inactivated whole-virus vaccine derived from a proviral DNA clone of simian immunodeficiency virus induces high levels of neutralizing antibodies and confers protection against heterologous challenge

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ABSTRACT **We tested the ability of macaques vaccinated with inactivated whole simian immunodeficiency virus (SIV) to resist challenge with either homologous or heterologous cell-free uncloned SIV administered by the intravenous route. The vaccine virus was derived from a proviral DNA clone and thus was considered genetically homogeneous. Sixteen macaques received either hepatitis B surface antigen (n = 6) or the inactivated whole-SIV vaccine (n = 10) at weeks 0, 4, and 49 of the study. All SIV vaccine recipients developed high levels of homologous and heterologous neutralizing antibodies in response to vaccination. At the time of challenge (week 53), vaccinees were further stratified to receive either homologous (n = 10) or heterologous (n = 6) uncloned live SIV. The envelope glycoproteins of the homologous and heterologous challenge viruses were 94% and 81% identical to the vaccine virus, respectively. Regardless of challenge inoculum, all vaccinees in the control group (hepatitis B surface antigen) became infected, whereas all SIV vaccinees were protected against detectable infection. These data support the concept that an efficacious vaccine for HIV might be possible, and suggest that genetic variation of HIV might not be an insurmountable obstacle for vaccine development.**

Simian immunodeficiency virus (SIV) infection of macaques is a useful model system for studying the pathogenesis, treatment, and prevention of primate lentivirus infections. In the area of vaccine development, inactivated whole-virus vaccines can prevent infection from a low-dose [≈10 MID_{50} (macaque infectious dose, 50% endpoint)] intravenous challenge with cell-free virus that is closely related to the vaccine virus (1–5), but many important issues in SIV [and by analogy, human immunodeficiency virus (HIV)] vaccine development remain unanswered. For example, it is not known which component(s) of whole-virus vaccines is responsible for the induction of protective immunity. This knowledge is vital for the development of subunit vaccines (which might be viewed as safer than inactivated whole-virus vaccines). Also, it is not known if the immunity induced by whole-virus vaccines will protect from a challenge inoculum containing virus that is genetically distant from the vaccine virus (heterologous challenge).

In this study, we addressed two questions. (*i*) Can a whole-virus vaccine derived from a proviral DNA clone of SIV confer protection against challenge with cell-free virus that is closely related to the vaccine virus (homologous challenge)? (*ii*) If successful against homologous challenge, does protection extend to a heterologous challenge? Our data indicate that a whole-virus vaccine derived from a proviral DNA clone of SIV does protect against detectable infection by heterologous as well as homologous uncloned cell-free SIV.

MATERIALS AND METHODS

Preparation of the Vaccines. Infectious SIV was prepared by transfecting a proviral DNA clone of SIV from sooty mangabeys (SIVsm/H4) (6) into CEM×174 cells (7). Supernatants from expanded cultures were harvested, clarified, and concentrated 100-fold by ultrafiltration. The concentrated virus was further purified by ultracentrifugation through a 20% glycerol cushion. Pelleted, partially purified virus was resuspended in phosphate-buffered saline and stored at −70°C until inactivation. Inactivation was achieved by the addition of psoralen (trioxsalen, 4′-aminomethyl; Calbiochem; 10 μl at 5 mg/ml in 50% ethanol) to 5 ml of concentrated virus at room temperature in a 60-mm plastic Petri dish. This suspension was then exposed to UV light (365 nm at a distance of 5 cm) for 15 min. An additional three rounds of psoralen/UV light treatment were performed, for a total of 40 μl of psoralen and 1 hr of UV light. The inactivated virus was diluted in buffer, aliquoted, and stored in liquid nitrogen. Each dose of SIV vaccine contained 400 μg of total protein in 0.5 ml. Inactivation was confirmed by inoculating CEM×174 cells in culture with the vaccine preparation; after 1 month in culture, no evidence of infectious virus was detected. Further, there was no evidence of SIV infection in vaccinated macaques as judged by virus isolation or polymerase chain reaction (PCR) (data not shown).

Formalin-inactivated hepatitis B surface antigen (HBsAg) was prepared (8) and diluted in phosphate-buffered saline to give 20 μg per 0.5-ml dose. Both the SIV and HBsAg vaccines were mixed with an equal volume of adjuvant prior to deep intramuscular inoculation. The adjuvant (threonylmuramyl dipeptide) was dissolved in an emulsion vehicle (SAF, lot 10575-DL-142; Syntex, Palo Alto, CA) prior to mixing with the vaccine. Each dose of vaccine contained 500 μg of threonylmuramyl dipeptide.

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Abbreviations: SIV, simian immunodeficiency virus; HIV, human immunodeficiency virus; HBsAg, hepatitis B surface antigen; LTR, long terminal repeat; PBMC, peripheral blood mononuclear cell; C′-ADE, complement-mediated antibody-dependent enhancement; MID, macaque infectious dose(s); TCID, tissue culture infectious dose(s).

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Virus Stocks. Two stocks of live SIV were used as challenge viruses. SIVsm/E660 has been described (9). Briefly, SIVsm/E660 was recovered (in CEM×174 cells) from the spleen of a rhesus macaque that died of SIV-induced AIDS. Supernatants from an expanded culture were harvested at peak reverse transcriptase activity, filtered (0.22 μm), aliquoted, and stored in liquid nitrogen. Thawed aliquots were titered *in vitro* (CEM×174 cells) and *in vivo* (pig-tailed macaques). Both titrations gave an ID_{50} of $10^{4.5}$. The SIVmac/251(32H) stock has been described (4) and was the gift of Martin Cranage (Center for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, U.K.).

Macaques. Sixteen juvenile pig-tailed macaques (*Macaca nemestrina*) were used, and each received either the HBsAg or the SIV vaccine. After vaccination, complete physical examinations were performed at 4- to 8-week intervals (or as clinically indicated). At the time of examination, peripheral venous blood was taken for complete blood cell counts, isolation of peripheral blood mononuclear cells (PBMCs), and SIV-specific antibody determinations (see below). Also, circulating lymphocyte subsets were determined on heparinized whole blood by monoclonal antibody staining and fluorescence-activated cell sorting.

SIV-Specific Antibodies. Five assays were used to measure SIV-specific antibodies: (*i*) ELISA with whole virus as the antigen, (*ii*) neutralizing antibody assays, (*iii*) infection-enhancing assays, (*iv*) a radioimmunoprecipitation assay for anti-gp130 antibodies, and (*v*) Western blot. The whole-virus ELISA (Genetic Systems, Seattle) and Western blot assays (Cambridge Biotech, Gaithersburg, MD) were performed with commercially available HIV-2 kits according to manufacturers' protocols.

One assay for neutralizing antibodies (assay 1) has been described (10) and is outlined briefly here. Before dilution, each plasma sample (250 μl) was incubated overnight at 4°C with 2×10^6 CEM×174 cells to adsorb anti-cell antibodies; cells were removed by centrifugation prior to assay. Results obtained with or without adsorption were comparable, but endpoints were easier to interpret if the sample had been adsorbed (data not shown). Virus stocks were prepared from cell-free supernatants harvested from chronically infected H9 cells and were stored at −80°C until used. Twofold serial dilutions of plasma samples were made in triplicate in growth medium (100-μl final volume). Medium containing virus [50 μl, ≈3000 $TCID_{50}$ (tissue culture infectious dose, 50% endpoint)] was added to test wells, whereas growth medium was added to control wells. CEM×174 cells (5×10^4 in 100 μl of growth medium) were then added to each well to initiate the assay. Virus-induced cytopathic effect was quantitated by vital dye (neutral red) uptake at day 6 or 7, when >80% cytopathic effect was observed in the virus control wells. Percent protection was defined as the difference in light absorption (A_{540}) between test wells (cells plus test plasma plus virus) and virus control wells (cells plus virus) divided by the difference in light absorption between cell control wells (cells only) and virus control wells. The neutralizing antibody titer was defined as the reciprocal of the last dilution resulting in ≥50% protection.

A second assay for neutralizing antibodies (assay 2) was performed on selected samples and is described in detail elsewhere (11). Briefly, a standardized virus dose (≈100 syncytium-forming units) was incubated with serial dilutions of a test plasma prior to inoculation of CEM×174 cells in a 96-well microtiter plate. After 5 days, cells were treated with detergent and the lysate was quantitatively assayed for gp130 by a capture ELISA. Selected samples were assayed for SIV p27 Gag protein by a capture ELISA (Coulter); results obtained with the two capture ELISAs were consistent. The neutralization titer was determined by calculating the level of gp130 in each test well with plasma compared to the level of gp130 in wells without plasma and was expressed for a level of 90% inhibition.

Complement-mediated antibody-dependent enhancement (C'-ADE) of *in vitro* SIV infection was measured using MT-2 cells in a microdilution plate infection assay (12, 13). The *in vitro* challenge virus for all measurements was SIVmac/251, chosen because of shared C'-ADE epitopes with SIVsm (13).

Antibodies specific for the SIVsm/H4 and SIVmac/239 gp130 proteins were measured in a semiquantitative radioimmunoprecipitation assay. The recombinant gp130 glycoproteins were produced in CHO cells (11). Plasma (1 or 5 μl) from immunized macaques was mixed with ^{35}S-labeled recombinant gp130 in constant-volume reaction mixtures and precipitated with protein A-Sepharose (11). Relative titers were assigned by comparing band intensities on autoradiograms from assays of test sera with standard precipitations of various amounts of these same labeled antigens by sera from SIVmac/251- or SIVsm/H4-infected macaques.

Virus Isolation. Macaque PBMCs isolated by standard density gradient centrifugation were washed and resuspended in RPMI 1640 with 10% (vol/vol) heat-inactivated fetal bovine serum, penicillin, streptomycin, glutamine, phytohemagglutinin (10 μg/ml), and 10% (vol/vol) human interleukin 2 (Advanced Biotechnologies, Columbia, MD). After 2–4 days in culture, stimulated cells were cocultivated with an equal number of CEM×174 cells. Cultures were monitored for typical cytopathic effects (syncytium formation) and reverse transcriptase activity in supernatants (14). Cultures were routinely maintained for 6 weeks (or until positive for SIV). Viremia was defined as the recovery of SIV from cocultures of PBMCs.

PCR. SIV-specific nucleotide sequences in genomic DNA from macaque PBMCs were amplified by PCR using nested sets of oligonucleotide primers derived from two separate regions of the SIV genome. All PCR-related procedures (including the purification of genomic DNA from stimulated PBMCs) were carried out in a remote laboratory that was free of plasmid DNA containing SIV nucleotide sequences. All reagents and the DNA thermal cycler were from Perkin–Elmer/Cetus. The first-round reaction mixture contained genomic DNA (0.5 μg), an outer pair of primers (10 pmol each), dNTPs (200 μM each), KCl (50 mM), Tris·HCl (pH 8.3, 10 mM), $MgCl_2$ (2 mM), and *Taq* polymerase (2.5 units). Amplification cycles ($n = 30$) were programmed as follows: 94°C for 60 sec; 55°C for 90 sec; and 72°C for 30 sec. A second round of amplification was performed as follows: 10 μl from the first amplification mixture was placed in a new tube that contained the standard reaction components and a second set of inner primers (nested within the outer set). The amplification program for the second round was identical to the first round. Products of the amplification were electrophoresed in 1% agarose gels and then passively transferred to nitrocellulose membranes for hybridization with radiolabeled probes (see below).

Oligonucleotide primers were synthesized on an Applied Biosystems model 380B DNA synthesizer and used without further purification. Primers were chosen from highly conserved regions identified by a multiple alignment of SIVmac, SIVsm, and HIV-2 nucleotide sequences. Two separate regions were analyzed: the long terminal repeat (LTR) and *gag*. Nucleotide sequences of primers are given below; for each, the position of the 5' nucleotide in the complete SIVsm/H4 sequence (6) is given in parentheses: LTR outer forward, (521) 5'-CTCTGCGGAGAGGCTGGC-3'; LTR outer reverse, (776) 5'-GGGTCCTAACAGACCAGG-3'; LTR inner forward, (540) 5'-GATTGAGCCCTGGGAGGT-3'; LTR inner reverse, (730) 5'-ACCAGGCGGCGACTAGGA-3'; *gag* outer forward, (878) 5'-CAGTAAGGGCGGCAGGAA-3'; *gag* outer reverse, (1330) 5'-CACTTTCTCTTCTGCGTG-3'; *gag* inner forward, (1042) 5'-GTGGG-

AGATGGGCGCGAG-3'; *gag* inner reverse, (1203) 5'-CTCCAACAGGCTTTCTGC-3'.

Double-stranded DNA probes were synthesized by PCR amplification from a plasmid containing the SIVsm/H4 proviral DNA clone. Reaction products were electrophoresed in 1% agarose gels, and the band of expected size was excised and purified by phenol extraction and ethanol precipitation. Radiolabeling was performed by the random primer method (GIBCO/BRL). Oligonucleotide primers used to generate LTR and *gag* probes were positioned inside the "inner" primers described above and were as follows: LTR probe forward, (559) 5'-CTCTCCAGCACTAGCAGG-3'; LTR probe reverse, (712) 5'-GAGATGGGAACACACACT-3'; *gag* probe forward, (1069) 5'-CTTGTCAGGGAAGGAAGC-3'; *gag* probe reverse, (1163) 5'-TTGCTGCCCATACTACAT-3'.

RESULTS

Vaccination Protocol. Each of the 16 macaques was immunized three times, at weeks 0, 4, and 49. Six macaques received formalin-inactivated HBsAg (controls) and 10 received the SIV vaccine. All immunizations were clinically well tolerated. Complete blood counts, serum chemistries, and lymphocyte subsets did not vary significantly from baseline values during the 53 weeks prior to challenge (data not shown).

Development of SIV-Specific Antibodies in Response to Vaccination. (*i*) *Whole-virus ELISA antibodies.* During the 53 weeks prior to challenge, only macaques that received the SIV vaccine developed an SIV-specific ELISA antibody response (Table 1). At 8 weeks, all 10 SIV vaccine recipients had moderate levels of ELISA antibodies. These titers dropped significantly over the ensuing 7 months; in fact, 2 macaques (nos. 91 and 96) returned to baseline levels. At 53 weeks (4 weeks after the third immunization), each of the 10 macaques showed an amnestic response with antibody titers approximating those in experimentally infected macaques.

(*ii*) *Western blot antibodies.* As noted above, each SIV vaccine recipient developed SIV-specific antibodies that were present on the day of challenge. On Western blots (not shown), these antibodies appeared to react with all of the major SIV structural proteins, including Gag, Pol, and Env.

(*iii*) *Anti-gp130 antibodies.* To further characterize the antibody response to the gp130 subunit of Env, a semiquantitative radioimmunoprecipitation assay employing gp130 (produced in CHO cells) from SIVsm/H4 and SIVmac/239 was performed. On the day of challenge, 7 of the 10 SIV vaccinees had titers higher than those observed in experimentally infected macaques (Table 1). Moreover, SIV vaccinees tested against both SIVsm/H4 and SIVmac/239 had similar titers of antibodies against the heterologous antigen and the vaccine-strain antigen.

(*iv*) *Neutralizing antibodies.* Titers of *in vitro* neutralizing antibodies were determined against four SIV strains: SIVsm/H4, the vaccine virus; SIVsm/E660, the homologous challenge virus; SIVmac/251(32H), the heterologous challenge virus; and SIVmac/251(1A11), a virus derived from a molecular clone of SIVmac/251 (15). The first three viruses were used in assay 1, while the fourth virus was used in assay 2 (see *Materials and Methods*). All SIV vaccinees had neutralizing antibodies against the vaccine virus (SIVsm/H4) or the homologous challenge virus (SIVsm/E660) prior to challenge, and the titers of these antibodies equaled or exceeded titers observed in experimentally infected macaques (Table 1). The heterologous challenge virus [SIVmac/251(32H)] was not neutralized by plasma from SIV-vaccinated or experimentally infected macaques either before or after challenge. This result is in agreement with the observations of others who have found this stock of SIVmac to be resistant to neutralization *in vitro* (4). To further assess the neutralizing capacity of plasma samples taken from macaques challenged with SIVmac, another SIVmac/251 stock (1A11) was used in assay 2. In this assay, samples taken on the day of challenge from all 10 SIV vaccinees had neutralizing antibodies against the closely related SIVmac/251(1A11). After challenge, titers of neutralizing antibodies against SIVmac/251(1A11) fell in the SIV vaccinees; in HBsAg vaccinees, neutralization titers in this assay were typical of titers observed in SIVmac/251-infected macaques (11).

Challenge Protocol. Vaccine efficacy was tested by intravenous challenge with live, cell-free SIV. Two challenge viruses were used: 4 HBsAg and 6 SIV vaccinees were

Table 1. Titers of ELISA, neutralizing, and anti-gp130 antibodies in vaccinated macaques

			Whole-virus ELISA				Neutralizing								Anti-gp130			
							SIVsm/H4		SIVsm/E660		SIVmac/251(32H)		SIVmac/251(1A11)		SIVsm/H4		SIVmac/239	
Vaccine	Challenge SIV	Macaque	8	49	53	61	53	61	53	61	53	61	53	61	53	61	53	61
HBsAg	sm/E660	73			<80	2,560	<24	1536	<24	384			<4	5984	0	1		
		80			<80	>10,240	<24	3072	<24	768			<4	3055	0	5		
		81			<80	>10,240	<24	3072	<24	192			<4	774	0	6		
		82			<80	>10,240	<24	1536	<24	96			<4	887	0	5		
	mac/251(32H)	83			<80	>10,240	<24	1536			<24	<24	<4	1187	0	2	0	7
		84			<80	160	<24	192			<24	<24	50	3707	0	0	0	0
SIVsm/H4	sm/E660	85	5120	160	>10,240	2,560	1536	384	96	<24			225	<4	7	4		
		86	2560	320	>10,240	5,120	>3072	1536	768	96			245	21	7	5		
		87	5120	640	>10,240	2,560	>3072	1536	192	96			78	21	7	5		
		89	640	320	>10,240	5,120	>3072	1536	1536	96			1500	406	7	5		
		91	1280	80	5,120	2,560	1536	384	384	96			350	15	4	1		
		93	2560	320	10,240	2,560	>3072	1536	768	96			600	156	7	4		
	mac/251(32H)	94	1280	160	5,120	1,280	1536	1536			<24	<24	525	66	7	3	7	4
		95	1280	320	10,240	2,560	3072	768			<24	<24	750	118	7	3	3	1
		96	640	<80	5,120	1,280	>3072	768			<24	<24	100	150	4	1	3	2
		97	2560	640	10,240	2,560	1536	192			<24	<24	1500	22	3	0	5	0

Time of challenge was 53 weeks. Blanks indicate assays not performed. ELISA and neutralizing antibody data are expressed as reciprocal titer; anti-gp130 data are expressed as relative titer (see *Materials and Methods*). Preimmunization titers determined by ELISA were in all cases <80.

challenged with SIVsm/E660, and 2 HBsAg and 4 SIV vaccinees were challenged with SIVmac/251(32H). SIVsm/E660 was considered to be a homologous challenge virus stock because previous nucleotide sequence analyses (9) had shown that *env* genes from the E660 isolate encoded proteins that were, on average, 94% and 99% identical to the SIVsm/H4 gp130 and gp40, respectively. SIVmac/251(32H) was considered to be a heterologous challenge stock because the *env* genes of the SIVsm/H4 and SIVmac/251 molecular clones were only 81% identical at the amino acid level. Each virus stock was diluted to 50 MID_{50} before injection; the proper dilution factor was calculated for both stocks on the basis of *in vivo* ID_{50} determinations.

SIV-Specific Antibody Responses After Challenge. All macaques that received the HBsAg vaccine seroconverted to SIV by 8 weeks after challenge (week 61 of the protocol; Table 1). Macaque 84 developed very weak antibody responses in most tests despite being viremic; this profile has been associated with rapid progression to AIDS in experimentally infected macaques (29). Antibody titers of the 10 SIV vaccinees at 8 weeks after challenge (week 61 of the protocol) were in decline with respect to the levels on the day of challenge.

Evaluation of SIV Replication After Challenge. *Virus isolation.* Virus isolation was attempted from all 16 challenged macaques at 2, 4, 8, and 16 weeks after challenge (weeks 55, 57, 61, and 69 of the protocol). SIV was recovered from all 6 HBsAg vaccinees at each attempt. In contrast, SIV was not recovered from any of the 10 macaques that received the SIV vaccine.

PCR detection of SIV DNA. As another measure of infection, PCR amplification of SIV sequences was attempted with DNA from PBMCs of all challenged macaques at 2 and 8 weeks after challenge (weeks 55 and 61 of the protocol). PCR detection of SIV sequences was entirely concordant with the virus isolation data. Thus, all HBsAg vaccinees were positive, whereas all SIV vaccinees were negative. Fig. 1 shows the results of the 2-week postchallenge PCR assay. Note that the primers for amplification worked equally well on samples from macaques challenged with either SIVsm or SIVmac.

Transfusion into seronegative macaques. At 12 weeks after challenge, heparinized whole blood (8 ml) was drawn from each of the 10 SIV vaccinees. Each of 5 seronegative pig-tailed macaques received the blood from 2 of the challenged vaccinees (in separate infusions). None of the 5 transfusion recipients had shown any evidence of infection by 5 months after transfusion (negative for seroconversion, virus isolation, and PCR detection of SIV DNA).

Infection-Enhancing Antibody Responses in Vaccinated Macaques. A potentially undesirable antibody response to HIV and SIV is the production of infection-enhancing antibodies. Antibodies that enhance infection *in vitro* and that might lead to augmented disease and vaccine failure have been demonstrated in other virus systems, most notably dengue virus (16–19). Previous work has detected infection-enhancing antibodies in HIV-infected individuals (20–22) and SIV-infected macaques (12, 13), but the impact of such antibodies on natural infection or vaccine efficacy remains unclear. Therefore, we examined plasma samples from our vaccinated macaques for *in vitro* C′-ADE.

Prior to challenge, C′-ADE responses were elicited in all SIV vaccinees, but in none of the HBsAg vaccinees (data not shown). After challenge, titers of enhancing antibodies remained stable or declined in SIV vaccinees. In HBsAg vaccinees after challenge, enhancing antibodies were detected at levels less than or equal to titers in SIV vaccinees prior to challenge. Thus, the presence of *in vitro* C′-ADE response did not predict vaccine failure or enhanced infection, since all vaccinated macaques were protected.

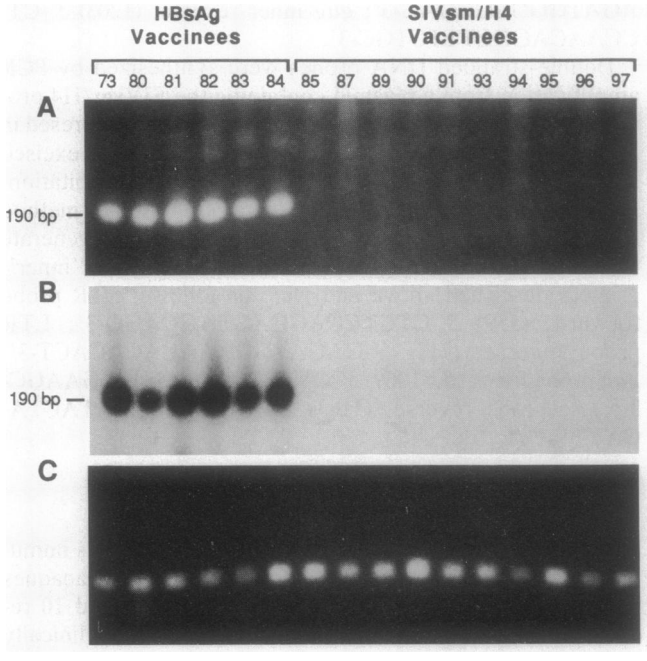

FIG. 1. PCR detection of SIV DNA in vaccinated macaques 2 weeks after challenge. Shown are results with nested primers in the LTR. (*A*) Ethidium bromide-stained agarose gel analysis of PCR products derived by amplification of DNA from circulating PBMCs. bp, Base pairs. (*B*) Southern blot analysis of the gel in *A*; the radiolabeled probe was specific for the SIV LTR. (*C*) Control showing that each DNA sample was a suitable template for amplification using primers derived from the β-globin gene (Perkin–Elmer/Cetus).

DISCUSSION

Inactivated Whole-Virus Vaccine Confers Broad Protection. This study demonstrates that an inactivated whole-virus vaccine derived from a proviral DNA clone can confer protection from infection against a homologous or heterologous SIV challenge. This conclusion is based on five observations. (*i*) SIV was not recovered in culture from any of the SIV-vaccinated macaques after challenge but was recovered from all controls (HBsAg vaccinees). (*ii*) SIV DNA was not detected by PCR in PBMCs taken from vaccinated macaques after challenge but was detected in all controls. (*iii*) Titers of SIV-specific antibodies in vaccinees were in decline after challenge, suggesting a lack of ongoing antigenic stimulation. (*iv*) A large volume of whole blood taken after challenge from the SIV vaccinees did not transmit SIV infection after transfusion into seronegative macaques. (*v*) The control macaques showed clinical evidence of SIV infection manifested by lymphadenopathy, rashes, and a significant decline in circulating $CD4^+$ cells; in addition, two of the six HBsAg vaccinees died of AIDS by 8 months after challenge. In contrast, the SIV vaccine recipients have remained clinically normal.

Studies of SIV vaccines in macaques have shown the efficacy of inactivated whole-virus vaccines against a challenge virus closely related to the vaccine virus (1–5). Our study confirms and extends these observations in several important aspects. The vaccine virus used in this study was derived from a proviral DNA clone and thus was of limited genetic diversity when compared with uncloned virus. Our data show that a vaccine derived from a molecular clone (SIVsm/H4) can confer protection against a highly related but uncloned challenge virus stock (SIVsm/E660). Furthermore, protection extended to challenge with a more distantly related uncloned virus stock [SIVmac/251(32H)] that was about 20% different from the vaccine virus (SIVsm/H4) in

Env amino acid sequence. This protection was reflected in the broad neutralization of these viruses *in vitro*. Thus, genetic variation of HIV might not be an insurmountable obstacle in the development of an efficacious HIV vaccine.

SIV Neutralizing and Infection-Enhancing Antibodies Are Induced by Immunization. Some SIV vaccine studies have failed to demonstrate the induction of SIV neutralizing antibodies in response to vaccination with inactivated whole-virus vaccines (2, 4). In contrast, plasma from macaques immunized in our study had high levels of SIV neutralizing activity. Because our vaccine was only partially purified and was produced in transformed human T cells, we were concerned that some of the neutralizing activity might arise from antibodies directed against human cellular proteins (23). However, plasma samples depleted of anti-cell antibodies retained high-titer *in vitro* neutralizing activity. Additional investigations revealed that the anti-cell antibodies in the plasma of SIV vaccinees did not (*i*) block binding of the OKT4a (anti-CD4) or OKB7 [anti-CR2 (complement receptor type 2)] monoclonal antibodies or (*ii*) block binding of live SIV to CD4-bearing human T cells (24). These data indicated that the anti-cell antibodies elicited in response to our whole-SIV vaccine did not play a major role in the *in vitro* neutralization of SIV. Additional studies will be required to rule out a role for anti-cell antibodies in the observed *in vivo* protection induced by our vaccine.

This study has examined infection-enhancing antibodies in SIV-vaccinated macaques that were challenged with heterologous virus. In previous SIV vaccine studies where homologous strains were used for challenge, the presence of C'-ADE did not predict vaccine efficacy (25). For dengue virus, a positive correlation between the presence of infection-enhancing antibodies and disease severity has been described, especially in cases of preexisting immunity followed by a heterotypic natural challenge (18, 19). Those data suggest that antibodies reactive with the homologous virus might be protective against homologous challenge, but the same antibodies might enhance infection with a heterologous virus. Our results suggest that *in vitro* C'-ADE does not portend failure of a particular vaccine and that the presence of heterologous antibodies does not enhance infection, since all macaques challenged with heterologous virus were protected. Thus, an *in vivo* role for antibodies that mediate C'-ADE remains to be elucidated.

Prospects for HIV Vaccine Development. Considered together, data from studies of inactivated whole-SIV vaccines suggest that the development of an efficacious HIV vaccine might be possible. Additional support for this position can be found in data from subunit-vaccine trials in chimpanzees and macaques (26–28). However, many significant hurdles lie ahead. (*i*) Inactivated whole-HIV vaccines may not be widely accepted because of valid concerns about the safety of such preparations. HIV subunit vaccines may be more acceptable and inexpensive to produce. However, the development of recombinant immunogens that mimic the oligomeric presentation of proteins found in virus particles may be difficult. (*ii*) More realistic (or natural) types of challenge inocula must be employed in efficacy trials in animals. For example, infected cells as a challenge might better represent an infectious inoculum likely to be encountered during natural transmission of HIV. (*iii*) Alternative routes of experimental inoculation, including vaginal and rectal challenges, should be tested using cell-free virus and infected cells. Finally, these potential concerns can be addressed in the SIV model and information derived from such studies may guide HIV vaccine development for humans.

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