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Immune Response of Rhesus Macaques to Recombinant Simian Immunodeficiency Virus gp130 Does Not Protect from Challenge Infection

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Simian immunodeficiency virus (SIV) infection of rhesus macaques is a model for human immunodeficiency virus (HIV) infection in humans. Inactivated and modified live whole-virus vaccines have provided limited protective immunity against SIV in rhesus macaques. Because of safety concerns in the use of inactivated and live whole-virus vaccines, we evaluated the protective immunity of vaccinia virus recombinants expressing the surface glycoprotein (gp130) of SIVmac and subunit preparations of gp130 expressed in mammalian cells (CHO). Three groups of animals were immunized with recombinant SIV gp130. The first group received SIV gp130 purified from genetically engineered CHO cells (cSIVgp130), the second group was vaccinated with recombinant vaccinia virus expressing SIVmac gp130 (vSIVgp130), and the third group was first primed with vSIVgp130 and then given a booster immunization with cSIVgp130. Although anti-gp130 binding antibodies were elicited in all three groups, neutralizing antibodies were transient or undetectable. None of the immunized animals resisted intravenous challenge with a low dose of cell-free virus. However, the group primed with vSIVgp130 and then boosted with cSIVgp130 had the lowest antigen load (p27) compared with the other groups. The results of these studies suggest that immunization of humans with HIV type 1 surface glycoprotein may not provide protective immunity against virus infection.

The development of an effective vaccine against the human immunodeficiency virus (HIV) is a major public health priority. Many vaccine strategies have been targeted to the HIV envelope glycoprotein (gp160). The gp120 (extracellular) surface domain of the envelope glycoprotein binds to the CD4 receptor as the first step in infection. Thus, gp120 plays a role in viral tropism for T cells and macrophages (41). In healthy HIV-infected individuals, neutralizing determinants and T-cell epitopes are located in hypervariable as well as conserved domains of the gp120 molecule (14, 33). In some but not all studies, the presence of antibodies to surface gp120 epitopes correlates with a lack of viral transmission from an infected mother to infants (8). Furthermore, chimpanzees immunized with recombinant HIV gp120 were protected from intravenous challenge with HIV (1). However, this model is limited to prevention of infection since HIV does not cause clinical disease in chimpanzees.

Simian immunodeficiency virus (SIV) is a T-lymphotropic lentivirus genetically related to HIV, and SIV causes an AIDS-like disease in rhesus macaques (6, 10). Accordingly, SIV in macaques is a model for the study of HIV infection and disease in humans. This model also offers opportunities to test and develop vaccine strategies as well as antiviral drugs (11).

The SIV envelope precursor, gp160, is cleaved into the surface gp130 and the transmembrane gp41. Macaques have been protected against SIV and HIV type 2 (HIV-2) infection by immunization with a whole inactivated virus (2, 7, 19, 24, 30, 35, 38), although the observed protection may have been due to immune responses to cellular as well as viral antigens (20, 37). Such immunized animals had envelope-specific neutralizing (SN) antibodies that bound to SIVmac recombinant gp130 expressed in CHO cells (13). Protection has also been achieved with a modified live SIV vaccine (26), and partial protection has been achieved with SIV gp130 prepared from virions (31, 32). Thus, gp130 is a logical candidate for the development of a subunit vaccine against SIV.

For vaccine studies in the SIV-macaque system, we focused on the external domain of the SIVmac1A11 envelope gp130. The molecular clone SIVmac1A11 was obtained from the biological isolate SIVmac251. SIVmac1A11 is infectious and induces syncytia (23). The introduction of an *Nco*I site to provide an initiation codon (ATG) for gp130, the insertion of a stop codon (TAG) in the processing site between gp130 and gp41, and excision of the gp130-coding sequence from plasmid pGEM SIVmacgp130-II by digestion with *Nco*I and *Sal*I restriction endonucleases have been described previously (34). To prepare a vaccinia virus (VV) recombinant that expresses SIV gp130 (vSIVgp130), we cloned the DNA fragment of the gp130-coding sequence into the *Sma*I site of pVY6 transfer vector to generate pVYSIV macgp130. The pVY6 vector directs the insertion of genes into the hemagglutinin region of the VV genome (9). vSIVgp130 was generated by homologous recombination between VV (WR) and pVYSIVmacgp130 in CV-1 cells (3). Expression and purification of gp130 in mammalian (CHO) cells (cSIVgp130) has been described previously (34).

For the construction of baculovirus expression vector, the

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TABLE 1. Schedule for immunization of rhesus macaques with SIVmac1A11 gp130

Group[a]	Week				
	0	4	8	22	24
A	vSIVgp130[b]		vSIVgp130	cSIVgp130	SIVmac[d]
B	vSIVgp130			vSIVgp130	SIVmac
C	cSIVgp130[c]	cSIVgp130	cSIVgp130	cSIVgp130	SIVmac
D					SIVmac

[a] Each group had four juvenile rhesus macaques (*M. mulatta*) that were seronegative for SIV, simian type D retrovirus, and simian T-lymphotropic virus.

[b] Animals receiving vaccinia virus that expresses SIVmac1A11 gp130 (vSIVgp130) were inoculated with 10^8 PFU in 100 μl by intradermal inoculation and scarification.

[c] Animals receiving SIVmac1A11 gp130 expressed in CHO cells (cSIVgp130) were immunized with 50 μg of purified protein in SAF-M adjuvant.

[d] The challenge stock was cell-free, pathogenic SIVmac titered in vivo; animals received 1 to 10 ID_{100} doses intravenously on week 24 after primary immunization (25).

plasmid pGEM SIVmacgp130-II was digested with *Nco*I, treated with Klenow DNA polymerase, and digested again with *Eco*RI to remove the gp130-coding sequence. It was then ligated to a *Sma*I-*Eco*RI-treated baculovirus transfer vector, pVL1393, to produce pVLgp130. Homologous recombination between pVLgp130 and *Autographa californica* nuclear polyhedrosis virus DNA was performed by standard procedures (39). Inclusion body-negative plaques were selected, and the expression of SIVgp130 (bSIVgp130) was confirmed by Western immunoblot analysis with serum from an SIV-infected monkey.

In Western blots, SIV gp130 expressed in all three systems strongly reacted with serum from an SIV-infected rhesus macaque. SIV gp130 expressed by both recombinant viruses had a molecular mass of 110 kDa. Both CHO- and VV-derived gp130 bound to recombinant, soluble human CD4 (data not shown). Binding studies of bSIVgp130 to human CD4 were not performed.

Immunization of rhesus macaques. Colony-bred juvenile rhesus macaques (*Macaca mulatta*) seronegative for SIV, type D retrovirus, and simian T-lymphotropic virus were used. The immunization schedule for 12 rhesus macaques is outlined in Table 1. Eight animals were vaccinated by intradermal inoculation and scarification with 10^8 PFU of vSIVgp130 in 0.1 ml of phosphate-buffered saline. Pock lesions developed in all animals by day 5 and completely healed 2 weeks postvaccination. However, some animals had enlarged pock lesions that resolved after 1 month. After 8 weeks, four of these animals (group A) received a booster inoculation with the same dose of vSIVgp130. On week 22, group A animals were given a second booster injection with 50 μg of cSIVgp130, and the remaining macaques (group B) were reinoculated with vSIVgp130. Booster vaccinations with vSIVgp130 occasionally induced small pock lesions (data not shown). Four animals in group C were vaccinated intramuscularly with 50 μg of cSIVgp130 in Syntex adjuvant formulation (SAF-M; Syntex, Palo Alto, Calif.). Booster administrations of cSIVgp130 with the same dose, adjuvant, and route were given on weeks 4, 8, and 22 after the primary vaccination.

Anti-SIV gp130 antibodies were determined by enzyme-linked immunosorbent assay (ELISA) with bSIVgp130 as a coating antigen (Fig. 1). The choice of this antigen over cSIVgp130 (antigen used for immunization) minimized anti-

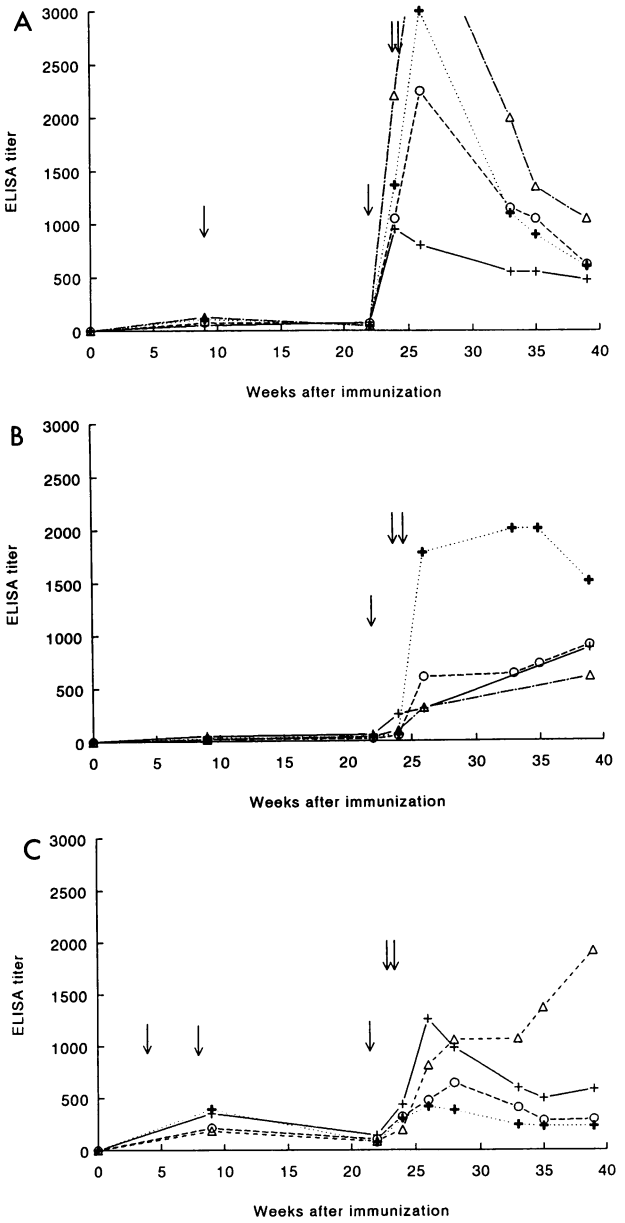

FIG. 1. Antienvelope ELISA titers in immunized macaques. Antibody titers against SIV gp130 were measured by ELISA with bSIVgp130 in microtiter plates. The anti-SIV gp130 titers were read as the reciprocal of the serum dilution giving greater than two times the optical density reading of the negative control serum. (A) Rhesus macaques immunized with vSIVgp130 and cSIVgp130. Symbols: +, 17081; △, 18505; ○, 21773; +, 21974. (B) Macaques immunized with vSIVgp130 alone. Symbols: +, 18291; △, 19837; ○, 20143; +, 21678. (C) Macaques immunized with cSIVgp130 alone. Symbols: +, 19014; △, 21169; ○, 21465; +, 22378. Primary immunizations were given on day 0; single arrows indicate day of booster immunization, and double arrows indicate challenge date.

bodies directed against contaminating mammalian cellular proteins. Briefly, Sf9 cells were infected with a recombinant *A. californica* nuclear polyhedrosis virus expressing SIV gp130 at a multiplicity of infection of 10 and were maintained in fetal calf serum-free Ex-Cell 400 (JRH Biosciences, Lenexa, Kans.) medium for 72 h. The supernatant was har-

TABLE 2. Virus neutralization titers in immunized and control macaques

Group[a]	Neutralization of SIVmac1A11[b] at wk:			Neutralization of VV[c] at wk:		
	0	24	26	0	10	24
A-17081	<8	<8	<8	<10	530	160
A-18505	<8	64	<8	<10	830	100
A-21773	<8	<8	8	<10	690	115
A-21974	<8	<8	<8	<10	590	150
B-18291	<8	<8	<8	<10	104	1,220
B-19837	<8	<8	<8	<10	105	820
B-20143	<8	<8	<8	<10	88	420
B-21678	<8	<8	<8	<10	110	570
C-19014	<8	8	<8	ND[d]	ND	ND
C-21169	<8	<8	8	ND	ND	ND
C-21465	<8	<8	<8	ND	ND	ND
C-22378	<8	<8	<8	ND	ND	ND
D-8106	<8	<8	<8	ND	ND	ND
D-8246	<8	<8	<8	ND	ND	ND

[a] Group A was vaccinated with vSIVgp130 on weeks 0 and 8, and group B on weeks 0 and 22.

[b] 90% reduction in envelope production compared with virus-alone controls (31).

[c] Titer was the reciprocal of the dilution of serum which gave 50% plaque reduction of VV on BSC-1 cells (42). Neutralization titers of serum dilutions of <10 were not determined.

[d] ND, neutralization titers of serum dilutions of <8 were not determined.

vested, and the gp130 concentration (adjusted to 2 μg/ml in carbonate-bicarbonate buffer [pH 9.5]) was used to coat microtiter plates (Immulon II; Dynatech Laboratories, Alexandria, Va.). Test sera were serially diluted (twofold), and horseradish peroxidase-labelled antimonkey immunoglobulin was used as the secondary antibody (Cappel, Inc., West Chester, Pa.). The anti-SIV gp130 titers were read as the reciprocal of the serum dilution giving greater than two times the optical density reading of the negative control serum. Animals in group A (vSIVgp130 plus cSIVgp130) developed anti-gp130 titers of <100 after both vSIVgp130 immunizations. However, there was a dramatic increase in anti-gp130 titer (>1,500) after booster injection with cSIVgp130. In contrast, group B animals (vSIVgp130 alone) had low anti-gp130 titers of 100 throughout the immunization phase of the study. Animals in group C (cSIVgp130) had an anti-gp130 titer of <500 after three consecutive immunizations (Fig. 1). The titer dropped to <100 2 weeks before challenge, and the last immunization of the group resulted in an anamnestic response with titers of 600.

Anti-SIVmac1A11 SN antibodies were determined by measuring inhibition of SIV gp130 production as previously described (34). No neutralizing antibodies to SIVmac1A11 were detected in any of the monkeys before the challenge date. Anti-VV SN antibodies were measured by a plaque reduction assay. Serial twofold dilutions of each serum sample were incubated with 80 PFU of VV at 37°C and then plated on monolayers of BSC-1 cells. After 24 to 48 h, the plates were stained with crystal violet and the plaques were counted. The titer of each sample was measured as the reciprocal of the highest dilution that reduced plaque counts by 50% (42). Although there was no detectable SN antibody response to SIV, anti-VV SN antibodies were readily detected in both groups A and B. Measurements of the immune responses of both groups to recombinant VV showed a low titer of SN antibody induced after the first inoculation, and an anamnestic response followed the booster immunization (Table 2).

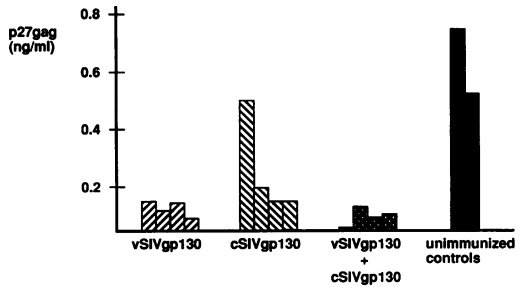

FIG. 2. SIV antigenemia (p27 in plasma) in vaccinated and challenged macaques 2 weeks after challenge with SIVmac. Antigenemia levels were measured by a commercial SIV core (p27) antigen capture ELISA (Coulter SIV Core Antigen Assay). The sensitivity of this assay allows the detection of 0.050 ng of SIV p27 per ml.

Challenge of rhesus macaques with pathogenic SIV. The preparation and in vivo titration of cell-free SIVmac challenge stock have been described (28). All immunized animals and two naive macaques were challenged by intravenous inoculation with 1 to 10 100% animal infectious doses (ID_{100}) 2 weeks after the last booster injection (i.e., 24 weeks after primary immunization).

Blood samples were collected in heparinized tubes at various time intervals after challenge, and peripheral blood mononuclear cells (PBMC) from these samples were isolated on a Ficoll gradient and cocultured with CEM-X-174 cells. Cultures were monitored for SIV replication by an antigen capture ELISA with the monoclonal antibody 55F12, which is specific for SIVmac p27 (21). One week after challenge, SIV was isolated from PBMC of the two naive controls and 11 of 12 immunized macaques. Two weeks postchallenge, PBMC from all were positive for virus and remained positive for the duration of the study (4 months). Antigenemia levels were measured by a commercial SIV core (p27) antigen capture ELISA (Coulter SIV Core Antigen Assay; Retrovirology Coulter Corp., Hialeah, Fla.). The sensitivity of this assay allows the detection of 0.050 ng of SIV p27 per ml. SIV p27-Gag antigenemia was detected 2 weeks after challenge in the two controls and 11 of the 12 immunized animals; macaque 17081 (vSIVgp130 plus cSIVgp130) was negative until week 15 after challenge. Although the antigenemia pattern was similar for immunized and control macaques, the antigen load appeared to be higher for nonimmunized animals (Fig. 2). After challenge, unimmunized animals had values ranging from 0.464 to 3.26 ng/ml compared with 0.059 to 0.141 ng/ml for the immunized groups. Macaque 21773 (vaccinated with vSIVgp130 plus cSIVgp130) showed an increased level of antigenemia 9 weeks after challenge.

After challenge, the anti-gp130 antibody titers steadily increased in all groups of animals, presumably due to an anamnestic response associated with the replication of challenge virus (Fig. 1). In group A animals (vSIVgp130 plus cSIVgp130), the titers peaked at >2,500 by 2 weeks after challenge as a result of anamnestic responses to the last booster vaccination and replication of challenge virus. Later, the anti-gp130 titer steadily declined to a level of 500 by 40 weeks after primary immunization. In group B (vSIVgp130 alone), the anamnestic response to both booster vaccination and replication of challenge virus steadily increased to a titer of 1,000 in three of four animals by 40

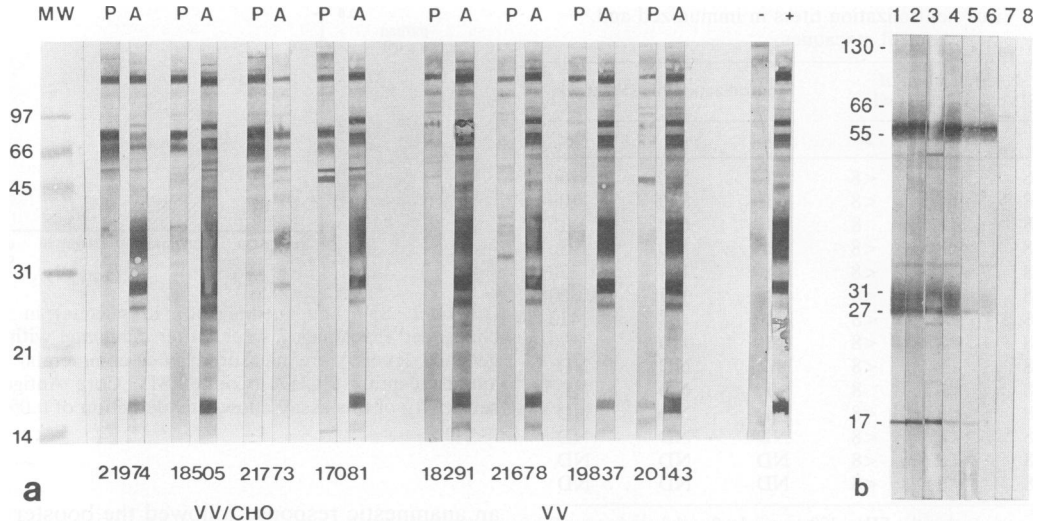

FIG. 3. (a) Immunoblots of sera from macaques immunized with recombinant SIV gp130. Sera from macaques were analyzed by Western blot against purified SIVmac proteins on the day of challenge (P) and 16 weeks after challenge (A). VV/CHO designates animals vaccinated with a combination of vSIVgp130 and cSIVgp130, while those vaccinated with vSIVgp130 alone are indicated by VV. Serum used as a positive control from an SIV-infected macaque is indicated by +, while serum used as a negative control from a known SIV-uninfected animal is indicated by −. Numbers on left show molecular weight ($\times 10^3$). (b) Immunoblot of macaque sera after challenge infection. Sera from macaques 12 weeks postchallenge (lanes 1 to 6) and on the day of challenge (lane 7) and from a negative control animal (lane 8) were assayed on Western blots against purified SIVmac. Lanes: 1 to 4, animals 19014, 21169, 21465, and 22378, respectively, immunized with cSIVgp130; 5 and 6, unimmunized animals 7168 and 8106, respectively; 7, macaque 22378 on the day of challenge; 8, negative control. SIV proteins are indicated on the left.

weeks after primary immunization. One animal (21678) had a peak titer of 2,000 by 2 weeks after challenge that declined to 1,500 by 40 weeks after primary immunization. Three of the four animals in group C (cSIVgp130 alone) had immune responses similar to those in group A except that the peak ELISA titers were lower (1,000). The fourth animal had a steadily increasing titer, approaching 2,000 by 40 weeks after primary immunization.

Anti-SIV SN antibodies were transient or not detected in group A and C macaques and completely absent in group B (Table 2). The immune response of macaques was studied further by Western blot analysis. All immunized animals showed antibodies directed exclusively against gp130 before challenge. Samples taken 4 months after challenge showed the presence of antibodies directed against other SIV proteins as well (Fig. 3). Specific, reactive bands were more intense, however, in vaccinated animals and weak in controls. This observation has also been noted for other viruses. For instance, priming of mice with a single synthetic T-cell epitope, located in the hepatitis B virus nucleocapsid protein, helped B cells to produce antibodies against envelope antigens as well as nucleocapsid protein, even though these antigens are found on separate molecules (27). The presence of T-helper-cell epitopes in SIV gp130 was not shown consistently in T-cell proliferation assays. However, this could explain why macaques immunized with gp130 developed higher antibody titers against other SIV antigens when compared with unimmunized controls (Fig. 3b). We observed an inverse relationship between the levels of antigenemia and antibody. This is best illustrated by macaque 21773 (vSIVgp130 plus cSIVgp130), the animal exhibiting the weakest Western blot response along with increasing antigenemia (0.162 to 0.251 ng/ml) 9 weeks after challenge (Fig. 3a).

PBMC were obtained from heparinized blood by centrifugation in Ficoll-Hypaque gradients. T-cell proliferation assays were performed on macaque PBMC with concanavalin A and cSIVgp130 as the stimulants (34). All three groups developed variable and inconsistent proliferative immune responses against cSIVgp130 during the immunization period. After challenge, no proliferative response to cSIV gp130 was detected (data not shown).

Evaluation of gp130 as immunogen. The potential of SIV gp130 as an effective vaccine against live virus challenge was tested in rhesus macaques immunized with various preparations of the antigen. SIV gp130 expressed in CHO cells and in VV was used as the immunogen, and these experiments included immunization of macaques with cSIVgp130, vSIV gp130, and a combination of both preparations.

We chose the WR strain of VV and the hemagglutinin gene for the insertion of the coding sequence of gp130 in order to obtain higher levels of expression of the protein. The WR strain replicates and reaches titers at least 1 log higher than the Wyeth strain, and VV is less attenuated by insertional inactivation of the hemagglutinin gene than by the thymidine kinase gene (12). Presumably due to the reduced level of attenuation, about one-half of the animals developed severe skin lesions after primary immunization. These lesions healed in 1 month and did not recur after a second immunization with vSIVgp130.

Serum ELISA antibody titers to gp130 were significantly higher in animals vaccinated with vSIVgp130 and then boosted with cSIVgp130 than in animals vaccinated with either immunogen alone. Immunologic priming with a VV recombinant followed by booster immunization with a particulate antigen to obtain a strong immune response has been described previously (11a, 17). Protective immunization against SIV in rhesus macaques has been achieved with inactivated or modified live whole-virus vaccines (2, 7, 19, 30) or inactivated-SIV-infected cell vaccine (38). Partial

protection has been accomplished with viral subunit preparations such as native envelope-enriched vaccine (31, 32) and a combination of β-galactosidase–SIV Env epitope fusion proteins (36). More recently, Hu et al. (17) reported that macaques immunized with VV recombinants expressing SIVmne gp160 and then boosted with baculovirus-expressed SIVmne gp160 were protected against intravenous challenge with the homologous, biologically cloned SIVmne. There are a number of differences in our study that could account for the lack of protection we found. In the study of Hu et al. (17), macaques (*Macaca fasicularis*) were vaccinated with the immunogen obtained from SIVmne and then challenged with the homologous, biological clone. We used a different species, *M. mulatta*, and the source of both immunogen and challenge virus was SIVmac. Differences in the virulence of SIV isolates and/or differences in the immune responses of macaque species may affect the elicitation of protective immunity against SIV infection. The same dose of the identical challenge stock of SIVmac used in our experiment has been shown to produce persistent, fatal infection in unvaccinated rhesus macaques inoculated intravenously; all animals died with clinical signs of immunodeficiency within 22 to 41 weeks postchallenge (25). In contrast, the biological clone of SIVmne used for challenge in the study of Hu et al. (17) has low genetic diversity and induces an intermittent viremia in about one-half of the unvaccinated cynomolgus macaques (17). The period for development of fatal immunodeficiency with SIVmne can take much longer than for SIVmac (11).

In our study, the gp130-coding sequence was derived from SIVmac1A11, a nonpathogenic molecular clone of SIVmac, the biological isolate used as a challenge virus. Tissue culture isolates of SIVmac do not account for the complete spectrum of genotypes present in an infected macaque (15). Therefore, the SIVmac1A11 genome probably represents a low proportion of the genomes found in the biological, uncloned SIVmac. In consequence, the conditions of our challenge can be defined as heterogeneous. In other lentivirus systems, it has been demonstrated that a single amino acid substitution at a critical residue in the envelope glycoprotein resulted in the alteration of a major biological function of a molecule. For example, a point mutation in the V3 loop of HIV-1 gp120 alters the pattern of cell tropism and susceptibility to neutralization (4).

Another important difference that could account for protection in the study of Hu et al. (17) is the immunization of macaques with the external and transmembrane domains of the SIV envelope glycoprotein (gp160). Our monkeys were immunized with the external envelope glycoprotein (gp130) only. It is possible that immune responses directed toward the transmembrane domain are essential for protection against SIV. In all the successful experimental protocols, the envelope glycoprotein was present either as precursor gp160 or as mature proteins gp130 and gp41. Even when conserved epitopes were used for immunization, they represented portions of both gp130 and gp41 (36). In the present study, we report the lack of protection in groups of animals vaccinated with cSIVgp130, vSIVgp130, or a combination of both preparations. The absence of gp41 in our vaccine preparation might have contributed to the failure of protection. In this regard, experiments are in progress to evaluate gp160 as a protective immunogen.

Finally, no SN antibodies were detected in our studies, and they may be required for protective immunity. The role of SN antibodies in protection is still controversial. Correlation between protection and neutralization with inactivated whole-virus vaccine has been observed but not consistently (11). Furthermore, we did not measure or determine the role of enhancing antibodies which might have contributed to lack of protection. However, it is known that inactivated SIV vaccines can protect macaques from infection even in the presence of detectable enhancing antibodies (29).

Our data did not corroborate the successful protection studies against HIV-1 infection in chimpanzees vaccinated with HIV gp120 expressed in CHO cells (1). Several differences exist between the two studies that might affect the results. First, there are distinct immunochemical differences between SIV gp130 and HIV gp120 (5). For example, an equivalent immunodominant V3 loop of HIV gp120 has not been found in SIV gp130 (18). The primary amino acid sequences and the glycosylation pattern of the two molecules are also different (16, 22).

In our study, delay in the onset of simian AIDS has not been included as one of the parameters for evaluating the effectiveness of a vaccine. A number of whole-virus vaccine preparations have significantly delayed the onset of clinical disease in infected vaccinates (25, 32, 40).

In summary, recombinant SIV gp130 delivered either alone as a live recombinant vaccine in VV or boosted with a purified cSIVgp130 failed to provide protective immunity to rhesus macaques challenged with a low dose of pathogenic SIVmac 251. However, the group primed with vSIVgp130 and then boosted with cSIVgp130 had the lowest virus load (p27) compared with the other groups. Experiments are in progress to evaluate the immunogenicity and efficacy of other novel recombinant SIV vaccines.

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REFERENCES

1. **Berman, P. W., T. J. Gregory, L. Riddle, G. R. Nakamura, M. A. Champe, J. P. Porter, F. M. Wurm, R. D. Hershberg, E. K. Cobb, and J. W. Eichberg.** 1990. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Nature (London) **345:** 622–625.
2. **Carlson, J. R., T. P. McGraw, E. Keddie, J. L. Yee, A. Rosenthal, A. J. Langlois, R. Dickover, R. Donovan, P. A. Luciw, M. B. Jennings, and M. B. Gardner.** 1990. Vaccine protection of rhesus macaques against simian immunodeficiency virus infection. Aids Res. Hum. Retroviruses **6:**1239–1246.
3. **Chakrabarti, S., K. Brechling, and B. Moss.** 1985. Vaccinia virus expression vector: co-expression of beta-galactosidase provides visual screening of recombinant virus plaques. Mol. Cell. Biol. **5:**3403–3408.
4. **Cheng-Mayer, C., T. Shioda, and J. A. Levy.** 1991. Host range, replicative, and cytopathic properties of human immunodeficiency virus type 1 are determined by very few amino acid changes in tat and gp120. J. Virol. **65:**6931–6941.
5. **Clapham, P. R., D. Blanc, and R. A. Weiss.** 1991. Specific cell surface requirements for the infection of CD4-positive cells by human immunodeficiency virus types 1 and 2 and by simian immunodeficiency virus. Virology **181:**703–715.
6. **Desrosiers, R. C.** 1990. The animal immunodeficiency viruses. Annu. Rev. Immunol. **8:**557–578.
7. **Desrosiers, R. C., M. S. Wyand, T. Kodama, D. J. Ringler, L. O. Arthur, P. K. Sehgal, N. L. Letvin, N. W. King, and M. D. Daniel.** 1989. Vaccine protection against simian immunodefi-

ciency virus infection. Proc. Natl. Acad. Sci. USA **86**:6353–6357.
8. **Devash, Y., T. A. Calvelli, D. G. Wood, K. J. Reagan, and A. Rubinstein.** 1990. Vertical transmission of human immunodeficiency virus is correlated with the absence of high-affinity/avidity maternal antibodies to the gp120 principal neutralizing domain. Proc. Natl. Acad. Sci. USA **87**:3445–3449.
9. **Flexner, C., S. S. Broyles, P. Earl, S. Chakrabarti, and B. Moss.** 1988. Characterization of the human immunodeficiency virus gag/pol gene products expressed by recombinant vaccinia viruses. Virology **166**:339–349.
10. **Gardner, M. B.** 1990. SIV infection of macaques: a model for AIDS vaccine development. Dev. Biol. Stand. **72**:259–266.
11. **Gardner, M. B., and S. L. Hu.** 1991. SIV vaccines, 1991—a year in review. AIDS **5**(Suppl. 2):S115–S127.
11a. **Giavedoni, L. D.** Unpublished data.
12. **Giavedoni, L. D., L. Jones, C. Mebus, and T. D. Yilma.** 1991. A vaccinia virus double recombinant expressing the F and H genes of rinderpest virus protects cattle against rinderpest and causes no pock lesions. Proc. Natl. Acad. Sci. USA **88**:8011–8015.
13. **Haigwood, N. L., S. M. Chin, M. Blair, V. Planelles, C. J. Scandella, K. S. Steimer, M. B. Gardner, T. Yilma, V. M. Hirsch, and P. R. Johnson.** 1992. Characterization of group specific antibodies in primates: studies with SIV envelope in macaques. J. Med. Primatol. **21**:82–90.
14. **Haigwood, N. L., J. R. Shuster, G. K. Moore, H. Lee, P. V. Skiles, K. W. Higgins, P. J. Barr, C. George-Nascimento, and K. S. Steimer.** 1990. Importance of hypervariable regions of HIV-1 gp120 in the generation of virus neutralizing antibodies. AIDS Res. Hum. Retroviruses **6**:855–869.
15. **Hirsch, V. M., P. M. Zack, and P. R. Johnson.** 1990. Molecular characterization of SIV in tissues from experimentally infected macaques. J. Med. Primatol. **19**:287–294.
16. **Holschbach, C., J. Schneider, and H. Geyer.** 1990. Glycosylation of the envelope glycoprotein gp130 of simian immunodeficiency virus from sooty mangabey (Cercocebus atys). Biochem. J. **267**:759–766.
17. **Hu, S. L., K. Abrams, G. N. Barber, P. Moran, J. M. Zarling, A. J. Langlois, L. Kuller, W. R. Morton, and R. E. Benveniste.** 1992. Protection of macaques against SIV infection by subunit vaccines of SIV envelope glycoprotein gp160. Science **255**:456–459.
18. **Javaherian, K., A. J. Langlois, S. Schmidt, M. Kaufmann, N. Cates, J. P. Langedijk, R. H. Meloen, R. C. Desrosiers, D. P. W. Burns, D. P. Bolognesi, G. J. LaRosa, and S. C. Putney.** 1992. The principal neutralization determinant of simian immunodeficiency virus differs from that of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **89**:1418–1422.
19. **Johnson, P. R., D. C. Montefiori, S. Goldstein, T. E. Hamm, J. Zhou, S. Kitiv, N. L. Haigwood, L. Misher, W. T. London, J. L. Gerin, A. Allison, R. H. Purcell, R. M. Chanock, and V. M. Hirsch.** 1992. Inactivated whole-virus vaccine derived from proviral DNA clone of simian immunodeficiency virus induces high levels of neutralizing antibodies and confers protection against heterologous challenge. Proc. Natl. Acad. Sci. USA **89**:2175–2179.
20. **Langlois, A. J., K. J. Weinhold, T. J. Matthews, M. L. Greenberg, and D. P. Bolognesi.** 1992. The ability of certain SIV vaccines to provoke reactions against normal cells. Science **255**:292–293.
21. **Lohman, B. L., J. Higgins, M. L. Marthas, P. A. Marx, and N. C. Pedersen.** 1991. Development of simian immunodeficiency virus isolation, titration, and neutralization assays which use whole blood from rhesus monkeys and antigen capture enzyme-linked immunoadsorbent assay. J. Clin. Microbiol. **29**:2187–2192.
22. **Luke, W., C. Fendrich, D. Schreiner, R. Sprenger, J. Rietschel, and G. Hunsmann.** 1991. Structural comparison of the external glycoproteins of human and simian immunodeficiency virus. Intervirology **32**:198–203.
23. **Marthas, M. L., B. Banapour, S. Sutjipto, M. E. Siegel, P. A. Marx, M. B. Gardner, N. C. Pedersen, and P. A. Luciw.** 1989. Rhesus macaques inoculated with molecularly cloned simian immunodeficiency virus. J. Med. Primatol. **18**:311–319.
24. **Marthas, M. L., C. J. Miller, S. Sutjipto, J. Higgins, J. Torten, B. Lohman, R. Unger, R. Ramos, H. Kiyono, J. McGee, P. Marx, and N. Pedersen.** Efficacy of live-attenuated and whole-inactivated simian immunodeficiency virus vaccines against vaginal challenge with virulent SIV. J. Med. Primatol., in press.
25. **Marthas, M. L., S. Sutjipto, J. Higgins, B. Lohman, J. Torten, P. A. Luciw, P. A. Marx, and N. C. Pedersen.** 1990. Immunization with a live, attenuated simian immunodeficiency virus (SIV) prevents early disease but not infection in rhesus macaques challenged with pathogenic SIV. J. Virol. **64**:3694–3700.
26. **Marthas, M. L., S. Sutjipto, C. J. Miller, J. Higgins, J. Torten, B. Lohman, R. Unger, P. Marx, N. Pedersen, H. Kiyono, and J. McGee.** 1992. Efficacy of live-attenuated and whole-inactivated SIV vaccines against intravenous and vaginal challenge, p. 117–122. *In* R. M. Chanock, H. Giensburg, R. Lerner, and F. Brown (ed.), Vaccines 90. Modern approaches to new vaccines including prevention of AIDS. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Milich, D. R., A. McLachlan, G. B. Thorton, and J. L. Hughes.** 1992. Antibody production to the nucleocapsid and envelope of the hepatitis B virus primed by a single synthetic T cell site. Nature (London) **329**:547–549.
28. **Miller, C. J., N. J. Alexander, S. Sutjipto, S. M. Joye, A. G. Hendrickx, M. Jennings, and P. A. Marx.** 1990. Effect of virus dose and nonoxynol-9 on the genital transmission of SIV in rhesus macaques. J. Med. Primatol. **19**:401–409.
29. **Montefiori, D. C., W. E. Robinson, Jr., V. M. Hirsch, A. Modliszewski, W. M. Mitchell, and P. R. Johnson.** 1990. Antibody-dependent enhancement of simian immunodeficiency virus (SIV) infection in vitro by plasma from SIV-infected rhesus macaques. J. Virol. **64**:113–119.
30. **Murphey-Corb, M., L. N. Martin, B. Davison-Fairburn, R. C. Montelaro, M. Miller, M. West, S. Ohkawa, G. B. Baskin, J. Y. Zhang, and S. D. Putney.** 1989. A formalin-inactivated whole SIV vaccine confers protection in macaques. Science **246**:1293–1297.
31. **Murphey-Corb, M., L. N. Martin, B. Davison-Fairburn, R. C. Montelaro, M. Miller, M. West, S. Ohkawa, G. B. Baskin, J. Y. Zhang, and S. D. Putney.** 1990. A formalin inactivated whole SIV vaccine and a glycoprotein-enriched subunit vaccine confers protection against experimental challenge with pathogenic live SIV in rhesus monkeys. Dev. Biol. Stand. **72**:273–285.
32. **Murphey-Corb, M., R. C. Montelaro, M. A. Miller, M. West, L. N. Martin, B. Davison-Fairburn, S. Ohkawa, G. B. Baskin, J. Y. Zhang, and G. B. Miller.** 1991. Efficacy of SIV/deltaB670 glycoprotein-enriched and glycoprotein-depleted subunit vaccines in protecting against infection and disease in rhesus monkeys. AIDS **5**:655–662.
33. **Norley, S. G., and R. Kurth.** 1991. Neutralizing antibodies and antigens in AIDS. Infection **19**(Suppl. 2):S83–S88.
34. **Planelles, V., N. L. Haigwood, M. L. Marthas, K. A. Mann, C. Scandella, W. D. Lidster, J. R. Shuster, R. Van Kuyk, P. A. Marx, M. B. Gardner, and P. A. Luciw.** 1991. Functional and immunological characterization of SIV envelope glycoprotein produced in genetically engineered mammalian cells. Aids Res. Hum. Retroviruses **7**:889–898.
35. **Putkonen, P., R. Thorstensson, L. Ghavamzadeh, J. Albert, K. Hild, G. Biberfeld, and E. Norrby.** 1991. Prevention of HIV-2 and SIV_{sm} infection by passive immunization in cynomolgus monkeys. Nature (London) **352**:436–438.
36. **Shafferman, A., P. B. Jahrling, R. E. Benveniste, M. G. Lewis, T. J. Phipps, F. Eden-McCutchan, J. Sadoff, G. A. Eddy, and D. S. Burke.** 1991. Protection of macaques with a simian immunodeficiency virus envelope peptide vaccine based on conserved human immunodeficiency virus type 1 sequences. Proc. Natl. Acad. Sci. USA **88**:7126–7130.
37. **Stott, E. J.** 1991. Anticell antibody in macaques. Nature (London) **253**:393.
38. **Stott, E. J., W. L. Chan, K. H. G. Mills, M. Page, F. Taffs, M. Cranage, P. Greenaway, and P. Kitchin.** 1990. Preliminary report: protection of cynomolgus macaques against simian im-

munodeficiency virus by fixed infected-cell vaccine. Lancet **336:**1538–1541.

39. **Summers, M. D., and G. E. Smith.** 1987. A manual of methods for baculovirus vectors and insect cells. Culture procedures. Texas Agricultural Experimental Station Bulletin no. 1555. Texas A&M University, College Station.
40. **Sutjipto, S., N. C. Pedersen, C. J. Miller, M. B. Gardner, C. V. Hanson, A. Gettie, M. Jennings, J. Higgins, and P. A. Marx.** 1990. Inactivated simian immunodeficiency virus vaccine failed to protect rhesus macaques from intravenous or genital mucosal infection but delayed disease in intravenously exposed animals. J. Virol. **64:**2290–2297.
41. **Weiss, R. A., P. R. Clapham, M. O. McClure, J. A. McKeating, A. McKnight, A. G. Dalgleish, Q. J. Sattentau, and J. N. Weber.** 1988. Human immunodeficiency viruses: neutralization and receptors. J. Acquired Immune Defic. Syndr. **1:**536–541.
42. **Yilma, T., M. Mackett, J. K. Rose, and B. Moss.** 1985. Vaccinia virus recombinants expressing vesicular stomatitis genes immunize mice and cattle, p. 187–200. *In* G. V. Quinnan (ed.), Vaccinia viruses as vectors for vaccine antigens. Elsevier-North Holland, New York.