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Inactivated Simian Immunodeficiency Virus Vaccine Failed To Protect Rhesus Macaques from Intravenous or Genital Mucosal Infection but Delayed Disease in Intravenously Exposed Animals

SUGANTO SUTJIPTO,[1]* NIELS C. PEDERSEN,[2] CHRISTOPHER J. MILLER,[1] MURRAY B. GARDNER,[3] CARL V. HANSON,[4] AGEGNEHU GETTIE,[1] MYRA JENNINGS,[3] JOANNE HIGGINS,[2] AND PRESTON A. MARX[1]

California Primate Research Center,[1] Department of Veterinary Medicine,[2] and Department of Medical Pathology,[3] University of California, Davis, California 95616, and Viral and Rickettsial Disease Laboratory, Department of Health Services,[4] Berkeley, California 94704

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Eight rhesus macaques were immunized four times over a period of 8 months with a psoralen–UV-light-inactivated whole simian immunodeficiency virus vaccine adjuvanted with threonyl muramyl dipeptide. Eight unvaccinated control animals received adjuvant alone. Only the vaccinated animals made antibodies before challenge exposure to the viral core and envelope as determined by Western blotting (immunoblotting) and virus-neutralizing antibodies. Ten days after the final immunization, one-half of the vaccinated and nonvaccinated monkeys were challenge exposed intravenously (i.v.) and one-half were challenge exposed via the genital mucosa with virulent simian immunodeficiency virus. All of the nonvaccinated control monkeys became persistently infected. In spite of preexisting neutralizing antibodies and an anamnestic antibody response, all of the immunized monkeys also became persistently infected. However, there was evidence that the clinical course in immunized i.v. infected animals was delayed. All four mock-vaccinated i.v. challenge-exposed animals died with disease from 3 to 9 months postchallenge. In contrast, only one of four vaccinated i.v. challenge-exposed monkeys had died by 11 months postchallenge.

Simian immunodeficiency virus (SIV) is a typical lentivirus with close genetic relationship to the human immunodeficiency virus (HIV) (6, 31). It causes a fatal disease in macaques that resembles the acquired immunodeficiency syndrome (AIDS) of humans (10). The simian disease, referred to as simian AIDS (SAIDS), occurs after a latency period that ranges from weeks to several years (7, 8, 11). Although the natural route of infection is unknown, monkeys can be experimentally infected by intravenous (i.v.), intramuscular, or mucosal exposure (7, 9, 20). The close genetic and pathogenic relationship of SIV to HIV makes it a particularly attractive model to test various HIV vaccine strategies.

Experience with retrovirus vaccines is somewhat limited. Conventional Formalin-inactivated whole-virus vaccines against type D retrovirus infection of rhesus macaques and type C retrovirus infection of domestic cats have induced protective immunity (17, 27). Based on these experiences, we decided upon a similar approach for an SIV vaccine. Instead of using Formalin, psoralen and UV light were utilized for inactivation. Psoralen–UV-light inactivation is gentler on the virus and may help to preserve more of the envelope structure (13; A. J. Watson, J. Klaniecki, and C. V. Hanson, AIDS Res. Hum. Retroviruses, in press). Once inactivated, the virus was combined with a threonyl muramyl dipeptide-containing adjuvant (2). This type of adjuvant was particularly effective with type D retrovirus vaccines in rhesus macaques (17).

We report here that rhesus macaques immunized with such a vaccine were not protected from infection with virulent SIV given by either the i.v. or genital mucosal route. However, the vaccine has thus far prevented the onset of clinical disease in three of four monkeys challenged by the i.v. route. These three animals were free of signs of disease at 11 months after challenge, whereas four of four mock-vaccinated controls had died with disease.

* Corresponding author.

MATERIALS AND METHODS

Virus. A fully virulent SIV_{MAC} (7) was adapted to grow in HUT-78 T-lymphoblastoid cells at the New England Regional Primate Research Center (NERPRC) and kindly provided for these studies. After several passages in HUT-78 cells at the California Primate Research Center, the virus was propagated in phytohemagglutinin- and interleukin-2-stimulated human peripheral blood mononuclear cells (PBMC) to prepare a live-virus challenge stock. Supernatant fluids were harvested by low-speed centrifugation as soon as the reverse transcriptase (RT) enzyme activity exceeded 65,000 cpm/ml (16). Two-milliliter samples of the human PBMC-propagated virus were quickly frozen without cryopreservatives and stored at −70°C. This human PBMC-propagated virus stock was used for all live-virus challenges. The virus for both the vaccine preparation and neutralization assays was grown in the HUT-78 T-cell line.

Cell lines. HUT-78 human T-lymphoblastoid cells were obtained originally from the laboratory of Robert Gallo, National Cancer Institute, Bethesda, Md. The CEM X-174 cell line was provided by James A. Hoxie, University of Pennsylvania, Philadelphia. These cells are a somatic hybrid of CEM human T-lymphoblastoid cells and an Epstein-Barr virus-transformed human B-cell line and express CD_4, CD_8, and DR antigens plus a variety of B-cell markers (30). A pronounced cytopathic effect consisting of giant-cell formation, cytoplasmic ballooning, and lysis occurred in SIV-infected cultures. All cell lines were propagated in RPMI

TABLE 1. In vivo titration of challenge stock of SIV_{MAC} in rhesus macaques

Virus dilution[a]	Route of infection	No. viremic animals/total no. animals[b]
Undiluted	i.v.[c]	2/2
10^{-1}	i.v.	2/2
10^{-3}	i.v.	4/4
10^{-4}	i.v.	2/2
10^{-5}	i.v.	0/2
10^{-7}	i.v.	0/2
Undiluted	Urethral mucosa[d]	6/6
Undiluted	Vaginal mucosa[d]	6/6
10^{-1}	Vaginal mucosa[e]	0/4

[a] Administered in 1-ml volumes.
[b] Animals were viremic at both 2 and 4 weeks postinoculation as detected by cocultivation of rhesus PBMC with CEM X-174 cells. The presence of virus was monitored by RT assay.
[c] All i.v. inoculations were done once.
[d] Four inoculations over a 2-week period.
[e] One inoculation.

medium containing 10% fetal bovine serum and 1× penicillin and streptomycin.

In vivo virus titration. A freshly thawed sample of the human PBMC virus stock was titrated for infectivity in juvenile rhesus macaques of both sexes. Following i.v. challenge exposure, 10 of 10 monkeys became persistently infected after receiving 1 ml of a stock virus preparation diluted 10^0 to 10^{-4} (Table 1). Four of four monkeys that were given 1 ml of a stock virus preparation diluted 10^{-5} or 10^{-7} remained aviremic and seronegative. Therefore, the stock virus inoculation contained approximately 10^4 100% animal infective doses (AID_{100}s) of SIV per ml. One milliliter of the freshly thawed stock virus inoculum contained 25 50% tissue culture infective doses of infectious virus when titrated in vitro in CEM X-174 cells by endpoint dilution. A similar finding concerning the difference in titer of the stock virus between the in vivo and the in vitro assays has also been reported previously (9, 22).

The same human PBMC virus stock was titrated for infectivity by the genital mucosal route (20). One milliliter of undiluted or diluted virus preparation was placed onto the urethral or vaginal mucosa of anesthetized animals with a soft plastic nasogastric feeding tube (8 French) (Table 1). This procedure was done four times in a 14-day period and infected six of six female and six of six male monkeys as measured by seroconversion and virus isolation from PBMC. None of the four female monkeys inoculated with 1 ml of a 10^{-1} dilution became either viremic or seropositive. The vaccinated and control animals were challenged with four inoculations of undiluted stock virus to ensure that all the control animals became infected.

Vaccine preparation. Cell-free supernatant fluids from the original HUT-78 T-cell cultures infected with virulent SIV_{MAC} were centrifuged at 10,000 × g for 10 min at 4°C to remove cellular and subcellular debris. The clarified infected supernatant fluid was then centrifuged at 100,000 × g for 1 h at 4°C to pellet the virus. The pelleted virus was suspended with phosphate buffered saline (PBS) at a 1,000× concentration and a total protein content of 2.6 mg/ml and stored at −70°C. No further purification of the virus preparation was carried out in an effort to preserve the major virus glycoprotein (gp120). The integrity of the virus core and envelope proteins in the concentrated preparation was confirmed by Western blotting (immunoblotting) as described previously (32).

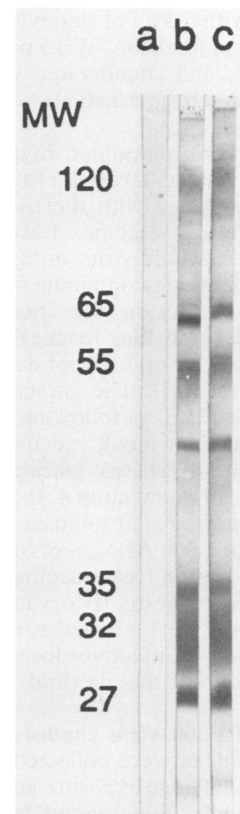

FIG. 1. Integrity of SIV_{MAC} proteins before and after psoralen–UV-light inactivation as determined by immunoblot. Lane a shows a negative reference serum; lanes b and c show SIV-positive sera reacted with SIV proteins before and after inactivation, respectively. MW, Molecular weight.

Psoralen–UV-light inactivation of the virus. The concentrated virus preparation was diluted fivefold with PBS and inactivated with 4′-aminomethyl-4,5′,8-trimethylpsoralen (AMT psoralen) (HRI Associates, Inc., Berkeley, Calif.) and UV light (13). 4′-Aminomethyl-4,5′,8-trimethylpsoralen (10 μg/ml) was added to the dilute virus preparation and then followed by a 5-min exposure to 20 mW of UV light per cm^2. Samples were irradiated from above and below in a layer no more than 5 mm deep in a horizontal polystyrene cell culture flask (Corning Glass Works, Corning, N.Y.) by using a UV exposure device (Dermacontrol, Dolton, Ill.) filled with UV lamps (type F587T12 BL HO). The cycle was repeated a second time. Western blots performed on preinactivated and inactivated virus preparations were identical, indicating that virion proteins were still intact following treatment (Fig. 1). The inactivated vaccine preparation was stored in 2-ml samples at −70°C.

To determine whether the inactivated vaccine preparation was free of residual infectious virus, 100-μl samples of 10-fold serial dilutions (10^{-1} to 10^{-6}) of the vaccine preparation were inoculated into 24-well tissue culture plates containing 5 × 10^5 HUT-78 or CEM X-174 cells per well. In order to enhance infectivity, the cells were pretreated for 30 min with 2 μg of polybrene per ml and then washed. The cultures were maintained for 8 weeks, and RT assays for the detection of virus in the supernatant fluids (14) were done weekly. After immunization 3, PBMC from immunized animals were also tested for virus infection. The PBMC were separated by gradient centrifugation on Ficoll-Hypaque (18),

stimulated for 72 h with 0.5 μg of staphylococcal enterotoxin A (Toxin Technology, Madison, Wis.) per ml, washed once with RPMI medium, and cocultivated with 2×10^6 CEM X-174 cells. Clarified cell supernatants were assayed weekly for RT for 2 months.

Immunization of rhesus macaques. Eight rhesus macaques (four males and four females) from 1.5 to 2 years of age, were immunized intramuscularly with the psoralen–UV-light-inactivated whole-SIV_{MAC} vaccine. Each dose of vaccine contained 0.5 mg of whole-virus antigen and 240 μg of threonyl muramyl dipeptide-containing adjuvant in 1 ml of a double oil-in-water emulsion (2). Eight control rhesus macaques (four males and four females) received an equal amount of threonyl muramyl dipeptide with PBS but no killed virus (mock vaccine). The animals received booster shots at 80, 140, and 235 days following primary immunization with either vaccine or mock vaccine.

Challenge exposure of rhesus macaques with infectious virus. Ten days after immunization 4, four of the vaccinated and four of the nonvaccinated animals were challenge exposed i.v. with 100 to 1,000 AID_{100}s of SIV_{MAC} (Table 1). In addition, four male rhesus (two vaccinated, 2 mock vaccinated) and four female rhesus (two vaccinated, two mock vaccinated) macaques were challenge exposed four times with 0.75 100% mucosal infective doses of SIV_{MAC} twice weekly for 2 weeks via the urethral (males) or vaginal mucosa (20).

Virus isolation after live-virus challenge exposure. PBMC from immunized animals were collected at 14, 30, 42, and 186 days following challenge exposure and cocultivated with fresh CEM X-174 cells. Supernatant fluids were then assayed weekly for RT, and the cells were observed twice weekly for cytopathic effect.

Determination of SIV-neutralizing antibody titers by MTT assay. A modification of an enzymatic cell viability assay was used to measure SIV-neutralizing antibodies in serum. Mitochondrial enzymes, present only in living cells, cleave 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) substrate to a dark blue formazan product that can be measured spectrophotometrically (21). SIV_{MAC} caused a rapid lytic infection of CEM X-174 cells; the ability of immune sera to protect the cells from the cytopathic effect produced by a known amount of SIV was a direct measure of neutralizing antibody activity. Serial twofold dilutions (60 μl) of test sera in RPMI medium were heated at 56°C for 30 min and then mixed with an equal volume of virus suspension (100 50% tissue culture infective doses of SIV_{MAC} grown in HUT-78 T cells in RPMI medium). Virus-serum mixtures were incubated at 37°C for 1 h, and 100 μl was added to each microtiter plate well containing 10^4 CEM X-174 cells in 100 μl of RPMI medium. Assays were run in triplicate. Control wells contained cells and serum alone or cells with virus and no serum. The cells were incubated at 37°C for 6 days, and 100 μl of the overlying culture supernatant fluid was gently removed from each well. Thirty microliters of the MTT substrate (5 mg/ml in PBS) (Sigma Chemical Co., St. Louis, Mo.) was added to each well, and the plates were incubated for 4 h at 37°C. The resultant formazan precipitate was dissolved with 100 μl of 0.01 N HCl-isopropanol solution, and the homogeneous blue solution was quantitated spectrophotometrically in an enzyme-linked immunosorbent assay plate reader using 570- and 630-nm wavelengths for test and reference samples, respectively. The virus-neutralizing antibody titer of serum was defined as the reciprocal of the serum dilution that reduced the cytopathic effect of the virus by 50%. Endpoint virus-neutralizing antibody titers determined by the MTT procedure were comparable to those measured by RT elaboration (data not shown).

Immunoblot. Sera or plasma taken following each immunization and after live-virus challenge exposure were screened for their antibody reactivity to SIV_{MAC} as determined by Western blotting (32).

Determination of T_4/T_8 and antigenemia assays. The T_4/T_8-cell ratio and absolute T_4-cell numbers (18) were done on PBMC collected prior to challenge-exposure and 31, 138, and 186 days thereafter. Briefly, PBMC from each monkey were separated from heparinized whole blood by Ficoll-Hypaque density gradient centrifugation for 30 min at 1,100 $\times$ *g*. Cells at the interface were collected, washed, and resuspended in RPMI medium supplemented with 100 U of penicillin per ml and 100 μg of streptomycin per ml at 4×10^6 cells per ml. Two hundred microliters of the cell suspension was incubated either with monoclonal antibody that was anti-T_4 and anti-T_8 (Becton Dickinson and Co., Mountain View, Calif.) or medium alone for 30 min at 4°C. The cells were washed with RPMI medium and incubated with fluorescein isothiocyanate-labeled goat $F(ab')_2$ anti-mouse immunoglobulin G (Tago, Burlingame, Calif.) for 30 min at 4°C. Cells were washed in PBS, centrifuged, and suspended in 1 ml of 2% paraformaldehyde in PBS, pH 7.2, for cytofluorographic analyses (50H cytofluorograph; Ortho Diagnostics, Inc., Raritan, N.J.). A complete blood count was done on each animal to determine total peripheral blood lymphocyte counts by using a System 9000 cell counter (Baker Instruments, Allentown, Pa.). Peripheral blood smears were stained with Wright-Leishman stain (Harleco, Gibbstown, N.J.), and differential counts were done on 100 cells. To determine the absolute number of T_4 or T_8 lymphocytes, the number of lymphocytes determined by complete blood count was multiplied by the percent T_4 or T_8. Plasma SIV-p24 antigenemia was measured with an HIV type 1 antigen enzyme immunoassay kit (Abbott Laboratories, North Chicago, Ill.) at 14, 92, and 138 days following challenge exposure.

Pathology. Animals were euthanatized because of severe clinical disease and were subjected to complete gross and microscopic postmortem examination. Euthanasia was accomplished with intramuscular ketamine hydrochloride sedation, followed by an overdose of i.v. administered barbiturate.

RESULTS

Immunoblot determination of humoral immune response of vaccinated animals before and after challenge exposure with live virus. No antibodies were detected in the sera of immunized monkeys 30 days following vaccination 1 (data not shown). However, after immunization 2 (154 days post-primary immunization), all vaccinated animals showed strong antibody responses to the major gag polypeptide, p27, its precursor p55, and the p65 RT protein. A weak antibody reactivity was detected against the major envelope glycoproteins, gp120 and gp32 (data not shown). The antibody reactivity toward p27, p55, and p65 before challenge exposure remained strong at 235 days post-primary immunization, and antibodies to p18, p16, and gp32 were now detectable (Fig. 2, lanes c to j). The weak immunoblot reactivity to gp120 remained unchanged. Unknown antigens with molecular masses of 10, 14, 24, 41, and 45 kilodaltons were also recognized by the immune sera, but they may have been of cellular origin. No immunoblot antibody reactivity was

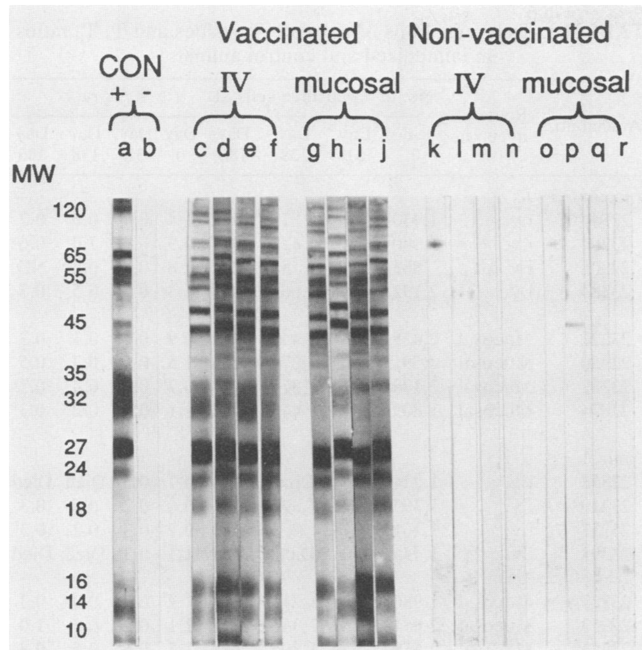

FIG. 2. Humoral immune responses as determined by immunoblot. Lanes a and b show positive and negative SIV reference sera; lanes c to j show sera from vaccinated animals (22844, 23204, 22905, 23384, 23221, 22950, 23382, and 22874); and lanes k to r are sera from mock-vaccinated (adjuvant alone) animals (22852, 23219, 23217, 23393, 23227, 22953, 23383, and 23092) at 235 days after primary immunizations. CON, Control; IV, i.v.; MW, molecular weight.

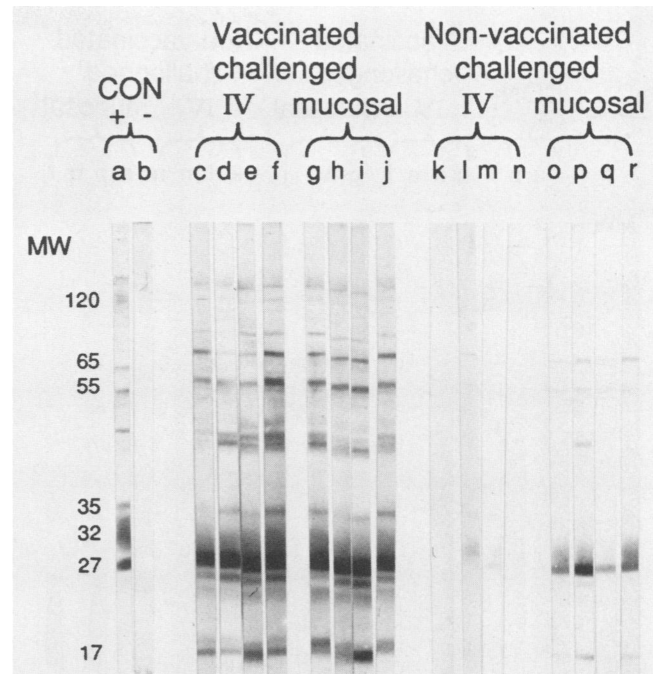

FIG. 3. Humoral immune responses as determined by immunoblot. Lanes a and b show positive and negative SIV reference sera; lanes c to f show sera from vaccinated monkeys i.v. (IV) challenge exposed (22844, 23204, 22905, and 23384); lanes g to j show sera from vaccinated monkeys challenge exposed mucosally (23221, 22950, 23382, and 22874); lanes k to n show sera from control monkeys challenged i.v. (22852, 23219, 23217, and 23393) and via the genital mucosa (23227, 22953, 23383, and 23902). All sera were collected at 40 days after challenge exposure. CON, Control; MW, molecular weight.

detected in sera of animals that received the mock vaccine before challenge exposure (Fig. 2, lanes k to r).

The eight vaccinated animals showed no change in Western blot antibody reactivity toward the core proteins by 40 days following challenge exposure, and reactivity to gp120 was still weak (Fig. 3, lanes c to j). However, all eight sera now reacted strongly to gp32 (Fig. 3, lanes c to j). The mucosally infected mock-vaccinated control animals (Fig. 3, lanes o to r) had seroconverted strongly to p27 by this time but only weakly to p55, p65, and gp32 (Fig. 3, lanes o, p, and r). Two animals failed to seroconvert in the control group that was i.v. challenge exposed, while two others had weak antibody responses by postinfection day 40 (Fig. 3, lanes k to n). No antibody reactivity to gp120 was detected after challenge exposure in any of the mock-vaccinated control animals.

The Western blot serum profiles of vaccinated animals were unchanged at 140 (data not shown) and 186 days following infection (Fig. 4). However, one vaccinated, mucosally challenge-exposed animal (Fig. 3 and 4, lanes g) died at 269 days postchallenge. Even though the antibody response seen in the animal did not correlate with the clinical outcome, it lived longer than the animals that failed to seroconvert. Three of the mock-vaccinated control animals which had been mucosally infected and seroconverted strongly at 40 days postinfection now had immunoblot patterns comparable to those of the vaccinated group. Two i.v. challenge-exposed mock-vaccinated control animals which weakly seroconverted at 40 days following infection (Fig. 3, lanes l and m) were antibody negative at days 140 and 186. These animals died with SAIDS at days 248 and 260. Two other i.v. exposed control animals that failed to seroconvert at 40 days following infection (Fig. 3, lanes k to n) continued to be seronegative and died of SAIDS after 91 and 127 days.

Virus isolation after live-virus challenge. Infectious virus was recovered from cocultivated PBMC of all vaccinated and control monkeys at 14, 30, 42, and 186 days following challenge exposure (data not shown).

Determination of neutralization antibody titers. All vaccinated animals had 50% endpoint neutralizing antibody titers of 1:8 to 1:128 just prior to challenge exposure, while mock-vaccinated control monkeys were antibody negative (Table 2). At 30 days following infection, six of eight of the vaccinated animals had neutralizing antibody titers that were higher than the preexposure level, and only one animal (22844) showed a drop in antibody titer (Table 2). However, this animal still had strong immunoblot reactivity to SIV antigens (Fig. 3, lane c). Seven of eight vaccinated animals had a significant drop of neutralizing antibody titers by 138 and 186 days postinfection. The only mock-vaccinated control monkeys that produced detectable neutralizing antibody were the four mucosally exposed animals.

Determinations of T_4/T_8-cell ratio, absolute T_4-cell counts, and antigenemia after live-virus challenge. The T_4/T_8 ratio and absolute T_4-cell counts were depressed at 31 days postinfection in six of seven vaccinated and eight of eight mock-vaccinated animals (Table 3). Although transient increases occurred in several of the vaccinees and controls over the next 5 months, the T_4/T_8 ratio and absolute T_4 levels never returned to preinfection values in any of the monkeys.

Antigenemia was present in eight of eight vaccinated animals and four of eight mock-vaccinated animals at 14

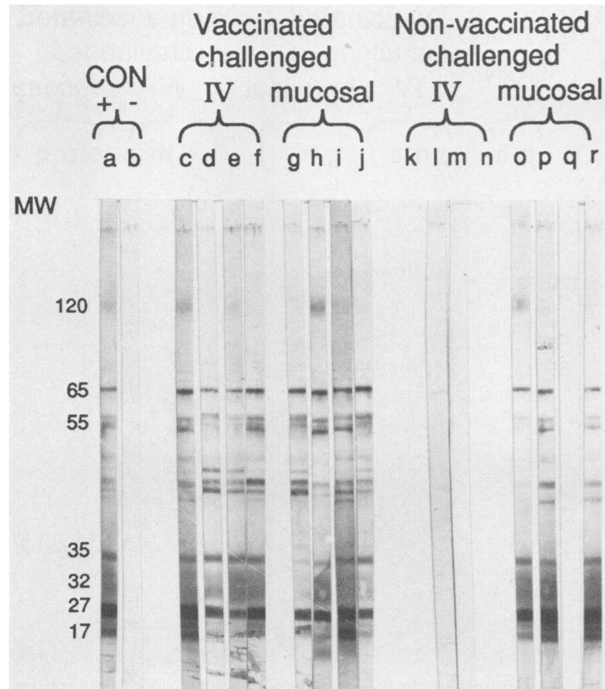

FIG. 4. Humoral immune responses as determined by immunoblot. All lanes and abbreviations are the same as in Fig. 3 but show the antibody responses at 186 days after live-virus challenge. The animals for lanes k and n died 127 and 91 days postinfection, respectively, and are therefore not represented in the figure. Note the absence of seroconversion for animals represented in lanes l and m.

TABLE 3. Determinations of absolute T_4 values and T_4/T_8 ratios in immunized and control animals

Animal no.	Route of infection	No. of T_4-positive cells/μl				T_4/T_8 ratio			
		Day 0	Day 31	Day 138	Day 186	Day 0	Day 31	Day 138	Day 186
Vaccinated									
22844	i.v.	1,430	854	575	147	1.4	0.4	0.5	0.2
23204	i.v.	1,940	1,342	2,425	327	0.9	0.4	1.0	0.6
22905	i.v.	863	669	188	ND[a]	1.8	0.4	0.8	ND
23384	i.v.	2,192	565	665	448	1.3	0.5	0.5	0.7
23221	Mucosal	2,425	1,084	440	422	0.9	0.4	0.1	0.3
22950	Mucosal	2,591	ND	1,274	275	1.5	0.5	0.7	0.5
23382	Mucosal	1,186	874	373	203	0.7	0.5	0.4	0.2
22874	Mucosal	1,805	2,373	443	273	1.1	0.4	0.2	0.3
Control									
22852	i.v.	1,224	867	Died	Died	0.7	0.5	Died	Died
23219[b]	i.v.	1,395	476	1,397	115	0.6	0.2	0.5	0.3
23217[b]	i.v.	1,161	278	273	185	0.7	0.3	0.2	0.3
23393	i.v.	1,425	506	Died	Died	1.1	0.3	Died	Died
23227	Mucosal	1,986	670	1,213	348	1.2	0.3	0.9	0.3
22953	Mucosal	2,051	737	1,343	852	1.1	0.2	0.5	1.0
23383[b]	Mucosal	2,548	879	1,304	618	1.5	0.6	0.5	0.8
23092	Mucosal	1,826	909	857	1,279	1.0	0.4	0.6	1.0

[a] ND, Not done.
[b] Died between 214 and 260 days postchallenge.

days following challenge exposure (Table 4). However, by 138 days postinfection, no vaccinated and only two of eight mock-vaccinated monkeys were still antigenemic. Antigenemia persisted in only one mock-vaccinated monkey (22852), which failed to seroconvert postinfection and died soon afterward with SAIDS.

Clinical outcome. Two of eight vaccinated and five of eight

TABLE 2. MTT neutralization assay determination of 50% neutralizing antibody titers[a] of immunized animals before and after live-virus challenge

Animal no.	Route of infection	Virus-neutralizing antibody titer[b]				Day of death
		Day 0	Day 30	Day 138	Day 186	
Vaccinated						
22844	i.v.	1:8	<1:8	1:8	<1:8	
23204	i.v.	1:64	1:8,192	<1:8	<1:8	194
22905	i.v.	1:8	1:512	<1:8	<1:8	
23384	i.v.	1:32	1:32	<1:8	1:8	
23221	Mucosal	1:8	1:4,096	<1:8	<1:8	269
22950	Mucosal	1:8	1:2,048	1:128	1:16	
23382	Mucosal	1:16	1:4,096	1:8	1:32	
22874	Mucosal	1:128	1:8,192	1:8	1:8	
Control						
22852	i.v.	<1:8	<1:8			127
23219	i.v.	<1:8	<1:8	<1:8	<1:8	248
23217	i.v.	<1:8	<1:8	<1:8	<1:8	260
23393	i.v.	<1:8	<1:8			91
23227	Mucosal	<1:8	<1:8	1:512	1:512	
22953	Mucosal	<1:8	<1:8	1:8	<1:8	
23383	Mucosal	<1:8	1:2,096	<1:8	<1:8	214
23092	Mucosal	<1:8	1:8	<1:8	<1:8	

[a] Defined as the reciprocal of serum dilution which neutralized the virus activity by 50%.
[b] Average values of three culture wells.

TABLE 4. Determination of antigenemia of immunized and control rhesus monkeys

Animal no.	Route of infection	Antigenemia[a] (pg/ml)		
		Day 14	Day 92[b]	Day 138[c]
Vaccinated				
22844	i.v.	118	<31	<26
23204	i.v.	35	<31	<26
22905	i.v.	122	<31	<26
23384	i.v.	48	<31	<26
23221	Mucosal	77	<31	Died
22950	Mucosal	78	<31	<26
23382	Mucosal	105	49	<26
22874	Mucosal	93	<31	<26
Control				
22852	i.v.	176	492	Died
23219[d]	i.v.	58	36	36
23217[d]	i.v.	<31	<31	32
23393	i.v.	39	<31	Died
23227	Mucosal	<31	<31	<26
22953	Mucosal	<31	<31	<26
23383[d]	Mucosal	<31	49	<26
23092	Mucosal	278	<31	<26

[a] Detected by an HIV type 1 enzyme immunoassay kit.
[b] Negative cutoff value was 31 pg/ml.
[c] Negative cutoff value was 26 pg/ml.
[d] Died between 214 and 260 days postchallenge.

TABLE 5. Significant pathologic findings of animals with SAIDS[a]

Animal no.	Weight loss (>10%)	Enterocolitis	Interstitial pneumonia with giant cells	Cholecystitis	Opportunistic infection with		
					Cytomegalovirus	Crytosporidia	Campylobacters
Vaccinated							
23204	+	−	+	+	+	+	+
23221	+	−	+	+	−	−	+
Control							
22852	−	−	+	−	−	−	−
23219	+	+	+	+	−	−	+
23217	+	+	−	−	−	−	+
23393	−	−	+	+	−	+	+
23383	+	+	+	−	+	−	+

[a] +, Positive for the finding; −, negative for the finding. Each animal exhibited lymphoid atrophy.

mock-vaccinated monkeys died within 9 months of challenge exposure (Table 2). The gross and microscopic pathologies in these seven animals were identical to those previously described for macaques with SIV-induced SAIDS (3, 20). The six vaccinated and three mock-vaccinated monkeys that survived all maintained strong immunoblot reactivity against SIV_{MAC} core antigen. The control animals that died had little or no detectable SIV antibody response following challenge exposure with live SIV_{MAC} or lost reactivity before death. The vaccinated monkeys that died had a drop in neutralizing antibody levels before death but were still nonantigenemic. The levels of neutralizing antibody prior to challenge exposure, antigenemia, and T_4/T_8 and absolute T_4 counts after infection did not correlate with the clinical outcome in either vaccinated or mock-vaccinated monkeys. However, protection from clinical disease was seen in the i.v. challenged groups. All four mock-vaccinated i.v. challenge-exposed animals died with disease by 9 months following infection (Table 2). In contrast, only one of four vaccinated i.v. challenge-exposed monkeys had died during 11 months postchallenge. The pathologic findings of animals with SAIDS is shown in Table 5.

DISCUSSION

The failure of a psoralen-inactivated whole-SIV vaccine to protect rhesus macaques against challenge exposure with a virulent homologous virus was not surprising. Lentivirus infections cause lifelong infections in exposed individuals (10, 23, 26), and these infections persist in the presence of host immunity (12, 26). One fundamental concept of vaccinology is that successful vaccines are against diseases to which the hosts have mounted a successful immune response in a significant proportion of cases (26). This concept has been borne out by experience with other retrovirus infections (11). Both inactivated whole virus and genetically engineered vaccinia virus expressing the envelope glycoprotein will provide protection against virulent simian type D retrovirus infection in macaques (14a, 17). Vaccines have also been used to protect cats against feline leukemia virus, a type C retrovirus (27). The pathogeneses of type D retrovirus infections of rhesus macaques and type C retrovirus infection of cats are similar; a significant proportion of naturally and experimentally infected animals mounted an immune response and recovered from infection (15, 17, 27, 28). In contrast, vaccine-induced protection against lentivirus infections has been difficult to demonstrate (23, 25, 26). Similarly, subunit and vaccinia recombinant HIV type 1 vaccines failed to protect chimpanzees against virulent HIV type 1 challenge (5). Inactivated whole-virus vaccines have also failed to protect sheep against ovine lentivirus infection (25).

Recently, researchers at the NERPRC and the Delta Regional Primate Research Center (DRPRC) were able to protect rhesus macaques against virulent-SIV challenge by using inactivated whole-SIV vaccines similar to the one described herein (9, 22). The NERPRC vaccine protected two of six animals, while the DRPRC vaccine protected eight of nine rhesus macaques. The differences in efficacy between these two successful vaccines and the present study could not be explained merely on the basis of postinfection vaccination virus-neutralizing antibody. In fact, the neutralizing antibody titers induced by the unsuccessful NERPRC vaccine were 1:16 to 1:32, while the endpoint titers in the partially successful trials were 1:160 to 1:320. The latter titers may have been somewhat higher than the 50% endpoint titers of 1:8 to 1:128 observed in the present study. The antibody titers for the successful DRPRC were 1:20 to 1:80, which is considerably lower than the partially successful study at the NERPRC. However, neutralization antibody titers between different laboratories may not be directly comparable because of differences in methodology. Formalin was used to inactivate the virus in the two successful studies, while the present vaccine was inactivated with psoralen and UV light. The types of adjuvants used in the three studies were similar, but the doses of antigen were slightly higher in the successful vaccines. The DRPRC vaccine was given over a prolonged period, with the final immunization given 13 months later. This may have allowed for a better memory cellular response. The most significant difference among the three vaccines was in the challenge exposure dose of virulent SIV given to the animals. Animals given the highly successful DRPRC vaccine were given a very low dose of virus (1 to 10 ID_{50}s), while animals given the partially successful NERPRC vaccine were challenged with 200 AID_{100}s. The virus dose used in the present study was somewhere between 100 and 1,000 AID_{100}s. Significantly, an earlier vaccine study at the NERPRC was unsuccessful when a 1,000-times-greater challenge exposure dose of virus was used (9). Therefore, it is possible that the low challenge exposure doses may have been largely neutralized at the site of inoculation before virus could attach to susceptible target cells, a possibility mentioned by investigators from the NERPRC (9). If the challenge exposure dose of virus can influence the outcome of SIV vaccine studies, it will be important to take this into consideration in future vaccine experiments by challenging animals at two dose

levels. A low dose given to the animals will give the best indication of immunization potential, while a higher dose will provide additional information on the strength of immune responses of the animals. The question of how well a vaccine can protect against a given challenge dose of virus under laboratory conditions and its efficacy against the same disease in nature is difficult to answer. The average infectious dose of HIV in nature is unknown, but it is believed to be small. As such, vaccines that protect against minimal amounts of infectious virus in the laboratory may be sufficient. However, successful vaccines of the past have been able to withstand large challenge exposure doses of the virus (10^5 to 10^9 50% lethal doses) (1, 4, 17, 19, 24, 29).

The present experiments failed to demonstrate protection against SIV infection; however, vaccinated animals that were challenge exposed by the i.v. route did have a significant measure of protection against clinical disease. The abrupt rise in neutralizing antibody titers after challenge exposure indicates that prior vaccination had immunologically primed the animals and allowed them to avoid the acute fatal disease seen in some of the nonvaccinated control rhesus macaques. It appears that a strong, immediate immune response to SIV can delay the onset of clinical SAIDS. The importance of the route of infection in determining the disease course was also evident in the present study. Rhesus monkeys that were infected by the i.v. route were much more likely to develop the acute form of SIV-induced disease. Four of four nonvaccinated rhesus macaques that were challenge exposed by the i.v. route died within 91 to 260 days, whereas only one of four monkeys infected by the genital mucosal route died within this same period. It appears, therefore, that the disease course is altered in some way by mucosal presentation of the virus. It is possible that mucosal inoculation causes an initial localized infection of sufficient duration to provide systemic immunity. Once the virus does reach the blood, ongoing immunity may slow the disease course. If the virus is given initially by the i.v. route, this initial localized immune response is bypassed and the infection is essentially systemic from the onset.

There was no indication that vaccinated rhesus macaques fared any better following a minimal mucosal challenge exposure than they did against a much greater i.v. infection. All of the vaccinated and unvaccinated animals became infected following mucosal challenge, and one monkey in each group died from SAIDS within the observation period. We did not monitor local immune responses to vaccination, and it is possible that this particular vaccine did not induce sufficient levels of local immunity.

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