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Immunization with a Live, Attenuated Simian Immunodeficiency Virus (SIV) Prevents Early Disease but Not Infection in Rhesus Macaques Challenged with Pathogenic SIV

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An infectious, virulence-attenuated molecular clone of simian immunodeficiency virus (SIV), $SIV_{MAC-1A11}$, was derived from an SIV isolate that causes fatal immunodeficiency in rhesus macaques. When inoculated intravenously in rhesus macaques, $SIV_{MAC-1A11}$ induced transient viremia (1 to 6 weeks) without clinical disease and a persistent humoral antibody response. The antibodies were directed mainly against the viral envelope glycoproteins, as determined by immunoblots and virus neutralization. The potential of this virulence-attenuated virus to protect against intravenous challenge with a pathogenic SIV_{MAC} strain was assessed. Five rhesus macaques were each given two intravenous inoculations with $SIV_{MAC-1A11}$ 7 months apart. Three of the five immunized monkeys and four naive control animals were then challenged with 100 to 1,000 100% animal infectious doses of pathogenic SIV_{MAC}. All seven animals became persistently viremic following the challenge. Four of four unimmunized animals developed severe clinical signs of simian acquired immunodeficiency syndrome by 38 to 227 days after challenge and were euthanatized 91 to 260 days postchallenge. However, no signs of illness were seen in immunized monkeys until 267 to 304 days postchallenge, when two of three immunized animals developed mild thrombocytopenia and lymphopenia; one of these animals died with clinical signs of simian immunodeficiency disease at 445 days after challenge. The two $SIV_{MAC-1A11}$-immunized monkeys that were not challenged were healthy and antibody positive 22 months after the initial immunization. Thus, although live $SIV_{MAC-1A11}$ was immunogenic and did not induce any disease, it failed to protect rhesus macaques against infection with a moderately high dose of pathogenic virus. However, immunization prevented severe, early disease and prolonged the lives of monkeys subsequently infected with pathogenic SIV.

Prevention of retrovirus infections and diseases in humans and animals is the most recent challenge for vaccinologists. Successful vaccines against type C and type D retroviruses have been made (6, 10, 11, 17). Induction of protective immunity by vaccination was predicted for these viruses because a substantial proportion of naturally infected animals mount a vigorous immune response and recover spontaneously. Vaccines against the lentivirus subfamily of retroviruses have been more difficult to develop, in part because naturally acquired lentivirus infections persist in virtually all infected individuals in spite of host immune response (16). Of the various animal lentivirus infections, only equine infectious anemia virus appears to be strongly inhibited by natural host immunity, and it may be amenable to vaccination (16).

Attempts to develop vaccines for human lentiviruses associated with immunodeficiency (human immunodeficiency virus type 1 [HIV-1] and HIV-2) have been unsuccessful (1, 5). Recently, however, whole, inactivated simian immunodeficiency virus (SIV) vaccines have provided some protection against infection, mainly against low challenge doses of virulent virus (4, 13). Even in SIV-immunized animals that were not protected against infection, clinical signs of immunosuppressive disease were apparently delayed (4, 13, 20).

It is generally accepted that live, virulence-attenuated viral vaccines are more immunogenic than inactivated or subunit viral vaccines (15). Attenuated vaccines have rarely been used against retrovirus infections but have been successfully used to prevent feline leukemia virus in cats (17). It is unknown whether attenuated, live virus vaccines would protect against lentivirus infection or disease. SIV infection of rhesus macaques provides a nonhuman primate model system for evaluating the efficacy of an attenuated, live SIV vaccine to prevent either SIV infection or SIV-induced disease (3).

Certain infectious molecular clones derived from pathogenic biological isolates of SIV have features desirable for a live, attenuated vaccine. They induce transient subclinical infection of rhesus macaques and a persistent antiviral immune response that includes virus-neutralizing antibodies (9, 14). The ability of such clones to prevent infection or disease by virulent SIV isolates has not been determined. Therefore, we assessed the potential of an attenuated, molecularly cloned SIV, $SIV_{MAC-1A11}$, to elicit protection against subsequent infection or disease caused by pathogenic SIV.

MATERIALS AND METHODS

Animals. Animals used were colony-bred, juvenile rhesus macaques (*Macaca mulatta*) from the type D retrovirus-free and SIV-free colony at the California Primate Research Center. All of the animals were negative for antibodies for HIV-2, SIV, simian type D retrovirus, and simian T-cell lymphotropic virus type 1. When necessary, animals were immobilized with 1 mg of ketamine hydrochloride (Parke,

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TABLE 1. Intravenous immunization of rhesus macaques with virulence-attenuated $SIV_{MAC\text{-}1A11}$ and challenge with pathogenic SIV_{MAC}

Group	Animal no.	Age[a]	Sex	Type of $SIV_{MAC\text{-}1A11}$ used at immunization		Challenge dose (AID_{100})[b]
				First	Second	
Immunized (controls)	23704	18	Male	Cell associated	Cell free	None
	23833	17	Female	Cell associated	Cell free	None
Immunized and challenged	23668	18	Female	Cell free	Cell free	10^2–10^3
	23671	20	Female	Cell free	Cell free	10^2–10^3
	23747	18	Female	Cell associated	Cell free	10^2–10^3
Unimmunized (controls)	22852	27	Male	None	None	10^2–10^3
	23217	30	Female	None	None	10^2–10^3
	23219	27	Male	None	None	10^2–10^3
	23393	21	Female	None	None	10^2–10^3

[a] Age in months at time of challenge.
[b] As determined by in vivo titration with intravenous inoculation of 1-ml portions of serial 10-fold dilutions of the cell-free, pathogenic virus stock into naive rhesus macaques (20).

Davis & Co., Morris Plains, N.J.) per kg of body weight injected intramuscularly.

Virus stocks. $SIV_{MAC\text{-}1A11}$ used for immunization was prepared by transfection of proviral DNA into HUT78 cells (from the National Institutes of Health repository) and passage of recovered virus into human or rhesus peripheral blood mononuclear cells (PBMC) (9). Tissue culture supernatants from $SIV_{MAC\text{-}1A11}$-infected human PBMC containing reverse transcriptase activity of 75,000 cpm/ml were filtered (0.4 μm-pore size), divided into 2-ml portions, and cryopreserved at −70°C till used for inoculations (12). This cell-free stock contained approximately 128 50% tissue culture infectious doses per ml (12). The cell-associated virus stock was prepared from $SIV_{MAC\text{-}1A11}$-infected rhesus PBMC with culture supernatant reverse transcriptase activity of >40,000 cpm/ml. The infected cells were pelleted, washed twice with phosphate-buffered saline to remove free virus, and suspended in phosphate-buffered saline at 5×10^6 viable cells per ml.

The preparation of the cell-free pathogenic SIV_{MAC} challenge stock has been described previously (20). The challenge doses of pathogenic SIV_{MAC} used were between 10^2 and 10^3 100% animal infectious doses (AID_{100}), as determined by in vivo titration with intravenous inoculation of 1-ml portions of serial 10-fold dilutions of the cell-free virus stock into naive rhesus macaques (20).

Inoculation of rhesus macaques with $SIV_{MAC\text{-}1A11}$ and pathogenic SIV_{MAC}. Table 1 gives the animal number, sex, age at the time of challenge, and intravenous inoculations administered for each animal used in this experiment. Five juvenile rhesus macaques were each given two intravenous inoculations of $SIV_{MAC\text{-}1A11}$ 7 months apart. Thawed cell-free and fresh cell-associated virus were used for the first immunization. Animals 23668 and 23671 were inoculated with 2 ml of cell-free $SIV_{MAC\text{-}1A11}$, and the remaining three animals (23747, 23704, and 23833) each received 10^7 infected rhesus PBMC. For the second immunization, each of the five animals was inoculated with 2 ml of cell-free $SIV_{MAC\text{-}1A11}$. Three of the $SIV_{MAC\text{-}1A11}$-immunized animals (23668, 23671, and 23747) were later challenged intravenously with 10^2 to 10^3 AID_{100} of uncloned, pathogenic SIV_{MAC} (20). (Because of a minor bacterial infection which resolved with treatment, animal 23671 was challenged 37 days after the other immunized animals.) The two remaining $SIV_{MAC\text{-}1A11}$-immunized animals (23704 and 23833) served as immunized, unchallenged controls. Four naive juvenile rhesus macaques that were given the same challenge dose served as unimmunized controls (20).

Virus isolation and detection of antigenemia. SIV was isolated from PBMC that were collected from macaques at various intervals after immunizations and challenge. The PBMC were cocultivated with CEMX174 cells and monitored for SIV, as described elsewhere (12).

Serum and plasma from animals inoculated with live, attenuated and pathogenic strains of SIV were analyzed for the presence of the SIV major core protein (p27) by cross-reaction with anti-HIV-1 p24 polyclonal antibody by using a commercial antigen capture enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, Ill.) according to the instructions of the manufacturer. Samples were defined as positive for SIV antigen when the amount of p27 detected was greater than minimal values obtained from standards provided by the manufacturer.

Determinations of SIV antibodies. Antibodies to whole SIV were measured by enzyme-linked immunosorbent assay (19) or immunoblot (12, 20). A 1:100 dilution of serum or plasma was used in both of these assays. Virus-neutralizing antibodies in serum from infected macaques were determined by a colorimetric assay that measured the prevention of CEMX174 cytolysis by a known amount of SIV (12).

Measurement of $CD4^+$ T lymphocytes. The proportion of $CD4^+$ cells in samples of PBMC was measured by fluorescence-activated flow cytometry (7) with the Leu3A monoclonal antibody (Becton-Dickinson, Mountain View, Calif.). The absolute numbers of $CD4^+$ T-lymphocytes were calculated by multiplying the proportion of $CD4^+$ cells determined from fluorescence-activated flow cytometry analysis by the total number of lymphocytes obtained from complete blood cell counts.

RESULTS

For clarity, all rhesus macaques inoculated with $SIV_{MAC\text{-}1A11}$ are referred to as immunized and all animals inoculated with pathogenic SIV_{MAC} are referred to as challenged. There were three groups of experimentally inoculated macaques in this study (Table 1): animals that received $SIV_{MAC\text{-}1A11}$ first and then pathogenic SIV_{MAC} (immunized and challenged), animals that received only $SIV_{MAC\text{-}1A11}$ (immunized controls), and animals that received pathogenic SIV_{MAC} only (challenged controls).

Immunization of rhesus macaques with live, virulence-

TABLE 2. Virus isolations from PMBC of rhesus macaques immunized with virulence-attenuated $SIV_{MAC-1A11}$ and either not challenged or challenged with pathogenic SIV_{MAC}

Group	Animal no.	SIV detection at week[a]:														
		1	2	4	6	8	16	24	28[b]	29	31	39 (0)	41 (2)	45 (6)	53 (14)	69–74 (25–30)
Challenged	23668	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+
	23671	+	+	−	−	−	−	−	−	−	−	−	+	+	ND	+
	23747	+	ND	−	−	−	−	−	−	−	−	−	+	+	+	+
Not challenged	23704	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−
	23833	+	+	−	+	−	−	−	−	−	+	+	−	−	−	−

[a] Number of weeks after first immunization with live, attenuated $SIV_{MAC-1A11}$. Numbers in parentheses indicate number of weeks postchallenge with pathogenic SIV_{MAC}. ND, Not done; +, SIV detected by cocultivation of rhesus PBMC with CEMX174 cells; −, no SIV detected by cocultivation.
[b] All animals were given the second immunization with $SIV_{MAC-1A11}$ at this time.

attenuated $SIV_{MAC-1A11}$. Virus was recovered from PBMC of all five rhesus macaques at 1 to 2 weeks after the first $SIV_{MAC-1A11}$ immunization (Table 2). However, virus could not be recovered from PBMC of these animals by 8 weeks after inoculation. Three to thirteen weeks after a second immunization 7 months later, SIV was isolated transiently from two of five animals (23704 and 23833) (Table 2). However, it could not be determined whether this virus resulted from the first or the second immunization.

There were no hematological abnormalities in any of the five immunized monkeys, and lymphadenopathy and splenomegaly were equivocal and mild. Three months after the initial immunization, one animal (23704) developed a transient maculopapular rash typical of SIV infection that resolved in 4 weeks. The two immunized monkeys that were not challenged with pathogenic virus (23704 and 23833) remained clinically normal over a 22-month observation period.

The numbers of $CD4^+$ T lymphocytes for the immunized monkeys were comparable to those for unimmunized animals prior to challenge (see Fig. 4). SIV antigen was also undetectable in serum or plasma following immunization and preceding challenge (data not shown).

$SIV_{MAC-1A11}$-immunized animals developed antibodies to whole SIV within 6 weeks, and these antibodies increased over the next 2 to 9 months (Fig. 1 and 2). This response was directed primarily against viral envelope proteins (Fig. 1, lanes d and e) (9). There was no detectable anamnestic response following the second immunization. None of the four unimmunized control monkeys produced SIV antibodies prior to challenge (Fig. 1 and 2) (20).

All immunized animals produced serum-neutralizing antibodies by 2 months after inoculation, with 50% endpoint titers ranging from 1:25 to 1:512 (Fig. 3). These antibody titers were maintained or increased slightly in all animals after the second immunization. The two immunized monkeys that were not challenged with pathogenic SIV remained whole-virus, immunoblot, and virus-neutralizing-antibody positive for 22 months after initial immunization (data not shown).

Although virus isolation was negative in all five animals at the time of the second immunization, an attempt was made

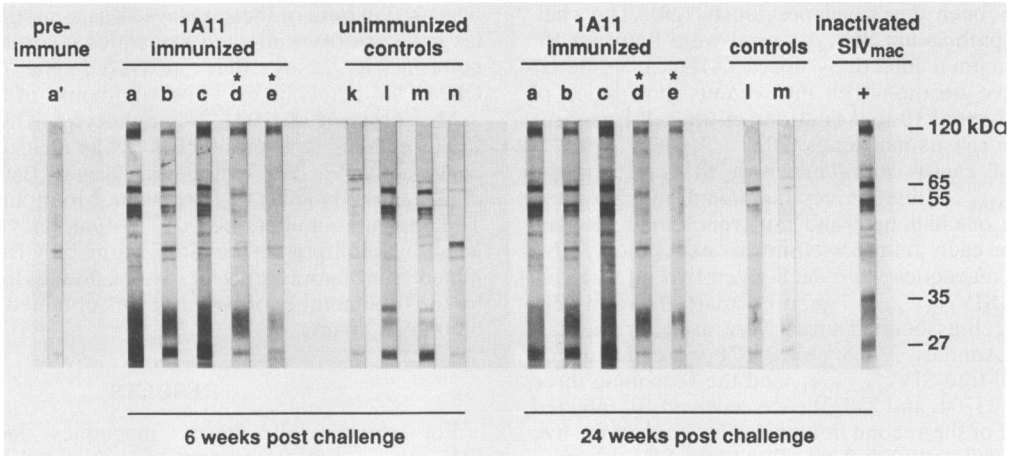

FIG. 1. Immunoblot analysis of antibodies to viral proteins in sera of rhesus macaques after intravenous challenge with pathogenic SIV_{MAC}. The antigen was whole virus from a biological isolate grown on HUT78 cells and prepared for immunoblotting as described elsewhere (12, 19). Each lane contains 10 μg of viral protein. All sera were diluted 1:100. Lanes a through c, Sera from immunized and challenged animals 23747, 23668, and 23671, respectively; lanes d and e, sera from immunized but unchallenged (*) controls 23833 and 23704, respectively (their antibody reactivities are representative of those of all animals immunized with $SIV_{MAC-1A11}$ prior to challenge); lanes k through n, sera from unimmunized controls 22852, 23219, 23217, and 23393, respectively; lane + (control), typical reactivity of an animal infected with SIV_{MAC}. Approximate molecular masses of major viral proteins, in kilodaltons (kDa), are shown in the right margin. Envelope glycoproteins are 120 and 32 kDa, the *gag* precursor is 55 kDa, the major *gag* protein is 27 kDa, and the 65-kDa protein may represent SIV_{MAC} reverse transcriptase.

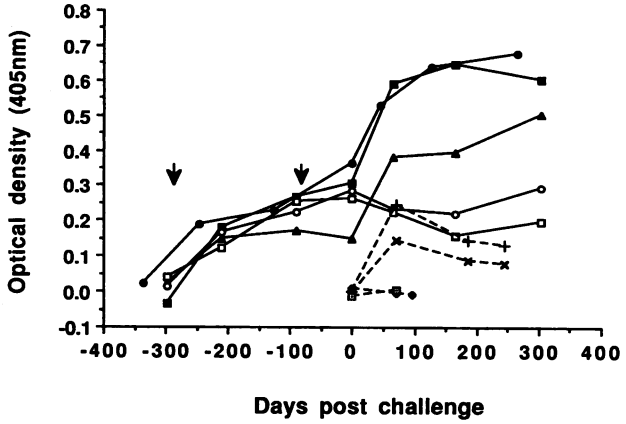

FIG. 2. Enzyme-linked immunosorbent assay of anti-SIV serum antibodies in immunized and challenged macaques. Values represent the means of two determinations for 1:100 dilution of serum. ——, Macaques immunized with $SIV_{MAC\text{-}1A11}$ and challenged (closed symbols) or not challenged (open symbols) with pathogenic SIV_{MAC}; – – – –, unimmunized controls after challenge. Values are shown for the following animals: 23747 (■), 23668 (▲), 23671 (●), 23833 (○), 23704 (□), 22852 (◆), 23217 (×), 23219 (+), and 23393 (⊡). Animal 23671 was challenged 37 days after the other immunized animals, as described in Materials and Methods. Arrows indicate times of immunization with $SIV_{MAC\text{-}1A11}$.

to detect infectious $SIV_{MAC\text{-}1A11}$ by transfusion of whole blood into naive animals. A 2-ml blood sample was collected from each of the five immunized animals and pooled, and half of the total was inoculated intravenously into each of two naive rhesus monkeys. Only one of these animals was transiently viremic at 3 weeks after transfusion and developed serum antibodies to SIV; the other animal remained seronegative, and virus was never isolated from its PBMC (data not shown).

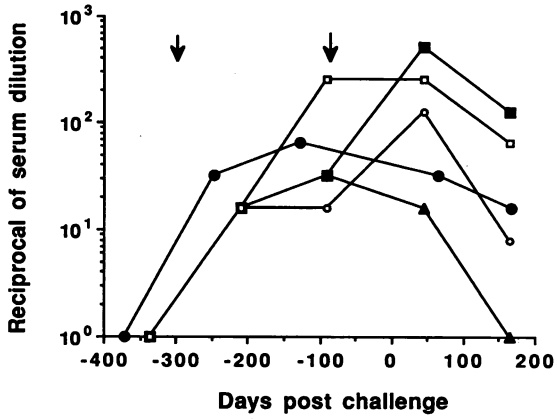

FIG. 3. Titers of SIV-neutralizing antibodies for macaques immunized with $SIV_{MAC\text{-}1A11}$ before and after challenge. Values are the reciprocals of the serum dilutions which decreased death of SIV-infected indicator cells by more than 50% of that observed for infected cells without the addition of serum. Cell viability was measured by an MTT [3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (12, 20); each point represents the mean of three determinations. Closed symbols represent immunized and challenged animals; open symbols represent immunized but unchallenged controls. Values are shown for the following animals: 23747 (■), 23668 (▲), 23671 (●), 23833 (○), and 23704 (□). Animal 23671 was challenged 37 days after the other immunized animals, as described in Materials and Methods. Arrows indicate times of immunization with $SIV_{MAC\text{-}1A11}$.

Effects of challenge with pathogenic SIV on immunized and nonimmunized rhesus macaques. Virus was isolated from PBMC of the three immunized (Table 2) and the four unimmunized animals at 2 weeks after challenge and throughout the experiment (20). At 1 to 2 weeks after challenge, SIV antigen was detected in serum or plasma of all unimmunized animals but not in serum or plasma of any $SIV_{MAC\text{-}1A11}$-immunized animals (data not shown). Because the assay used detects the SIV core antigen which cross-reacts with a monoclonal antibody to HIV-1 p24, these data indicate only that after challenge, less SIV antigen was present in the serum of immunized than of unimmunized animals.

All four of the monkeys that were not immunized prior to challenge with pathogenic SIV developed signs of simian acquired immune deficiency syndrome (AIDS) (diarrhea, weight loss, hematological abnormalities, and opportunistic infections) from days 38 to 227 postchallenge (Table 3). The last survivor of this group died 260 days after challenge. The most severe disease was observed in the two unimmunized animals (22852 and 23393) that failed to develop measurable antiviral antibodies after challenge (Fig. 1, lanes k and n; Fig. 2). The onset of clinical signs in the other two unimmunized animals (23217 and 23219) was associated with a decrease in antiviral antibodies (Fig. 1, lanes l and m, and Fig. 2). The numbers of $CD4^+$ T-lymphocytes were low in two unimmunized animals at the time of death, but were within normal ranges in the other two (Fig. 4b). There was no difference in the numbers of $CD4^+$ cells among immunized animals until 264 days after challenge. At that time, the numbers of $CD4^+$ cells for two of the three immunized animals (23671 and 23747) decreased substantially (Fig. 4a), and both animals became lymphopenic and thrombocytopenic; one of these animals (23747) died with clinical signs of simian AIDS at 445 days after challenge (Table 3). The numbers of $CD4^+$ cells for the third immunized animal (23668) remained within the normal range for 365 days after challenge. Both of the immunized animals that were not challenged with virulent SIV maintained normal numbers of $CD4^+$ lymphocytes for 22 months after the initial immunization.

Following challenge, antibodies to all SIV proteins increased rapidly for the three immunized monkeys (Fig. 1, lanes a through c; Fig. 2). The most prominent change was the increase in antibodies to SIV major core (p27) protein (Fig. 1, lanes a through c). The two immunized animals that were not challenged with virulent SIV retained essentially the same patterns and levels of antibody response (Fig. 1, lanes d and e, and Fig. 2). The two unimmunized control animals (23217 and 23219) developed measurable antibodies to the SIV major core protein following challenge, but not to envelope glycoproteins (gp120 and gp32). However, these antibodies decreased by 6 months after challenge (Fig. 1, lanes l and m, and Fig. 2).

Levels of virus-neutralizing antibodies did not change appreciably for two of the three immunized animals (23671 and 23747) after challenge but were undetectable in one animal (23668) by 6 months following challenge (Fig. 3). Unimmunized animals made no detectable virus-neutralizing antibodies following challenge with virulent SIV (data not shown).

TABLE 3. Clinical outcome after intravenous immunization of rhesus macaques with virulence-attenuated $SIV_{MAC-1A11}$ and challenge with pathogenic SIV_{MAC}[a]

Group[b]	Animal no.	Prominent clinical signs of disease (time of onset [days PC])	Clinical outcome of infection (day)
Immunized (controls)	23704	None	Clinically normal (758 PI)
	23833	None	Clinically normal (758 PI)
Immunized and challenged	23668	None	Clinically normal (445 PC)
	23671	Thrombocytopenia, lymphopenia (267)	Mild disease signs (408 PC)[c]
	23747	Thrombocytopenia, lymphopenia (304)	Died (445 PC)
Unimmunized (controls)	22852	Facial edema, splenomegaly, pneumonia (90)	Died (127 PC)
	23217	Diarrhea, weight loss, dehydration (227)	Died (260 PC)
	23219	Thrombocytopenia, diarrhea, weight loss (188)	Died (248 PC)
	23393	Diarrhea, lymphoid depletion, candidiasis (38)	Died (91 PC)

[a] PI, Since first immunization; PC, since challenge.
[b] For description, see Table 1.
[c] Animal 23671 was challenged 37 days after the other immunized animals, as described in Materials and Methods.

DISCUSSION

Virulence-attenuated virus strains have usually been obtained by screening tissue-culture-propagated stocks of pathogenic viruses for spontaneous mutants with altered phenotypes (2). Here, we report one of the first isolations of an infectious attenuated lentivirus by using molecular cloning techniques. Like many other virulence-attenuated viruses, $SIV_{MAC-1A11}$ was probably present as a subpopulation in the SIV_{MAC} stock used for cloning. Serial passage of the parent virus in human T-lymphoblastoid cells (HUT78) for many generations prior to molecular cloning may have increased the chances of isolating a proviral clone that produced attenuated virus (9). Long-term passage of virus in cells of a heterologous species has often been used to select virulence-attenuated viruses for vaccines (2). Recently, it has been reported that long-term propagation of primate lentiviruses in transformed lymphoid cell lines may select viral strains with altered biological properties, including attenuated replication and virulence (8, 18).

The molecular genetic basis for the altered in vivo behavior of the $SIV_{MAC-1A11}$ virus was not determined. $SIV_{MAC-1A11}$ replicated to high titers in cultures of human lymphoid cells and rhesus PBMC (9). In these respects, it was indistinguishable from the parent stock of pathogenic SIV_{MAC} (12). $SIV_{MAC-1A11}$ was also able to replicate in vivo, but only for several weeks at easily detectable levels. After that time, it replicated at either very low levels or not at all. The fact that the attenuated virus replicated at relatively high levels for a brief period suggests that acquired host defense mechanisms influenced its ultimate expression in the animals.

In this experiment, it is not certain whether $SIV_{MAC-1A11}$ persisted in immunized macaques. Virus could not be isolated from blood by 6 weeks after inoculation. However, pooled blood from the immunized monkeys taken 7 months after the initial immunization contained sufficient infectious virus to induce a transient viremia in one of two naive monkeys transfused with that blood. Thus, at least one

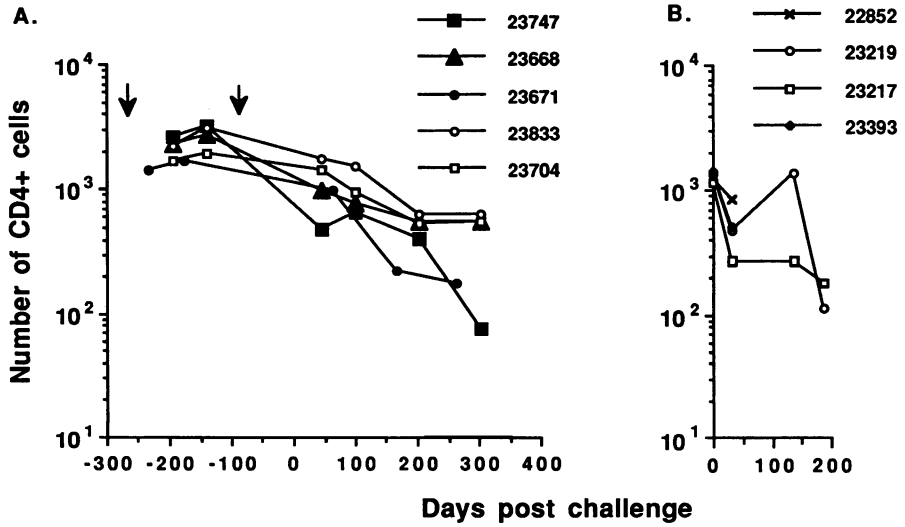

FIG. 4. Number of $CD4^+$ lymphocytes in macaques per microliter of whole blood. Symbols for individual animal numbers are indicated. Animal 23671 was challenged 37 days after the other immunized animals, as described in Materials and Methods. (A) Animals immunized with $SIV_{MAC-1A11}$. Closed symbols represent animals challenged with pathogenic SIV_{MAC} and open symbols represent unchallenged controls. Arrows indicate the times of immunization with $SIV_{MAC-1A11}$. (B) Unimmunized controls after challenge with pathogenic SIV_{MAC}.

animal harbored infectious virus or latently infected cells in peripheral blood for 7 months following immunization. The fact that antibodies persisted for 22 months in the two monkeys that were immunized but not challenged supports the idea that $SIV_{MAC\text{-}1A11}$ was not eliminated from immunized animals. Antibody responses to whole, inactivated SIV tend to decrease over time (13).

The failure of attenuated, live $SIV_{MAC\text{-}1A11}$ to induce high levels of antibodies to the SIV core proteins was unusual. Whole, inactivated SIV induces strong antibody responses to these proteins when given in high doses repeatedly (4, 13, 20). The failure of $SIV_{MAC\text{-}1A11}$ to induce high levels of antibodies to core proteins indicates that such antibody production requires a high level of antigenic stimulation. The appearance of antibodies to core proteins within a few weeks after challenge of immunized animals and coincident with virus isolation from PBMC supports this hypothesis.

Immunization with virulence-attenuated $SIV_{MAC\text{-}1A11}$ failed to prevent infection with a moderate dose of virulent SIV, despite the presence of virus-neutralizing antibodies in serum at the time of challenge. This result was unexpected because live virus vaccines generally produce greater immunity than do inactivated vaccines (2). Two recent whole, inactivated SIV vaccinees have evoked protection against infection with virulent SIV with vaccine-induced virus-neutralizing titers no greater than those in the present study (4, 13). There are several possible explanations for these discrepant results. The whole, inactivated virus vaccines induced antibodies to both envelope and core proteins, while the present attenuated, live virus vaccine induced antibodies primarily to viral envelope proteins. Thus, antibodies to core proteins may be involved in protection. A second difference in the successful studies with live and inactivated virus vaccines is the combination of route of challenge and amount of virus used for the challenge. Recent experiments at the Delta Primate Center have demonstrated protection in eight of nine macaques challenged intravenously with 10 AID_{50}; this challenge dose is approximately 1/10 to 1/100 the dose used herein (13). Thus, the challenge dose may be a significant factor in the success of whole, killed SIV vaccines. Studies of inactivated vaccines at the New England Regional Primate Research Center have shown protection of two of six macaques given 200 to 1,000 AID_{100} intramuscularly (4). Though the intramuscular challenge dose does not differ from that used in the present study, it is possible that the level of vaccine protection is significantly affected by the specific route of challenge. Thus, the efficacy of a virulence-attenuated SIV vaccine needs to be evaluated with both lower challenge doses and different routes of challenge.

Although immunization of rhesus macaques with live, attenuated SIV did not prevent infection in this study, it did delay the onset of clinical disease. By the end of the study, one of the three immunized animals died with clinical signs of simian AIDS at 445 days after challenge, one developed mild clinical signs of simian AIDS, and one remained clinically healthy. In contrast, all unimmunized animals were dead by 260 days following challenge.

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