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Prevention of Simian Acquired Immune Deficiency Syndrome with a Formalin-Inactivated Type D Retrovirus Vaccine

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Experimental induction of simian acquired immune deficiency syndrome (SAIDS) by inoculation of juvenile rhesus monkeys with a type D retrovirus was prevented by immunization with Formalin-killed whole SAIDS retrovirus serotype 1 containing the adjuvant threonyl muramyl-dipeptide. All six immunized animals developed neutralizing antibody after three injections, while six age-matched cagemates receiving adjuvant alone were antibody free. All 12 monkeys were challenged intravenously with a potentially lethal dose of SAIDS retrovirus serotype 1. The six immunized animals failed to develop persistent viremia and remained clinically normal 8 months postchallenge. In contrast, five of six nonvaccinates developed persistent viremia, four of six developed clinical SAIDS, and two of six died with SAIDS at 10 weeks and 8 months postchallenge, respectively. These results show that prevention of a common spontaneous retrovirus-induced immunosuppressive disease in macaques is now possible by vaccination.

Simian acquired immune deficiency syndrome (SAIDS) is a spontaneous, often fatal disease observed at four primate research centers in eight species of Asian monkeys belonging to the genus *Macaca* (4, 6, 9, 13, 21, 23). SAIDS is characterized by generalized lymphadenopathy, cell-mediated and humoral immune suppression, severe anemia, neutropenia, and lymphopenia. Findings in fatal SAIDS frequently include one or more of the following: (i) wasting, (ii) severe necrotizing gingivitis (noma), (iii) fatal septicemias due to mixed bacterial species, and (iv) opportunistic infections such as disseminated cytomegalovirus infection, oral-esophageal candidiasis, and cryptosporidiosis (9, 19).

The primary etiological agent of spontaneous SAIDS in Asian monkeys is a group of closely related simian type D retroviruses (16, 17). Two distinct serotypes of SAIDS retroviruses (SRV), SRV-1 and SRV-2, which are not cross-neutralized by homologous antibody, have been found in macaque monkeys at primate centers where SAIDS is endemic (15). Another closely related and immunosuppressive type D retrovirus, Mason-Pfizer monkey virus isolated in 1970, represents a third serotype (2) which has not, however, been isolated from any current outbreak of SAIDS. The divergence of these three serotypes in the *env* region of the external glycoprotein has now been confirmed by nucleotide sequence analysis (20, 22a). These type D retroviruses are not immunologically related to the human AIDS retroviruses (3).

Fatal SAIDS has been repeatedly induced in juvenile rhesus monkeys (*Macaca mulatta*) by intravenous inoculation of SRV-1 (16, 17). Inoculated monkeys become seropositive for SRV-1 and not for other known simian retroviruses such as simian T-cell lymphotropic virus types I and III (STLV-I, STLV-III) (5, 10, 11, 17, 18a; L. J. Lowenstine, N. C. Pedersen, J. Higgins, K. C. Pallis, A. Uyeda, P. A. Marx, N. W. Lerche, and M. B. Gardner, Int. J. Cancer, in press). Serological surveys of captive macaque colonies at two primate centers also show a convincing association between spontaneous SAIDS and type D retrovirus, but not STLV-I and STLV-III (R. Desrosiers, personal communication; N. Lerche, submitted for publication. Lastly, SAIDS has been induced by inoculating juvenile rhesus monkeys with molecularly cloned SRV-1 (G. Heidecker, manuscript in preparation).

The identification of a retrovirus as the etiological agent of an immunosuppressive disease in macaques has focused attention on the potential for immunoprophylaxis of spontaneous retroviral diseases in these animals. Although vaccines have been successful in certain murine, feline, and bovine retroviral model systems (7), retroviral vaccines have not yet been tested for prophylaxis of immunosuppressive disease of primates. Here we describe the testing in juvenile rhesus monkeys of a vaccine containing Formalin-killed SRV-1 plus threonyl muramyl-dipeptide adjuvant. Active immunization with this vaccine induced SRV-1 neutralizing antibody in six of six rhesus monkeys and, following challenge with SRV-1, provided uniform protection against both persistent viremia and disease.

MATERIALS AND METHODS

Rhesus monkeys. Twelve healthy juvenile (8 to 11 months old) rhesus monkeys (Rh) from the SAIDS-free colony at the California Primate Research Center were confirmed free of SRV-1 antibodies by enzyme-linked immunosorbent assay (ELISA) (see Table 1) and Western blot (not shown) (15–17). The presence of viremia was excluded by cocultivating peripheral blood mononuclear cells (PBMC) with Raji cells (16) (see Fig. 1). The 12 monkeys were randomly divided into six pairs. Each pair was housed in a separate HEPA-filtered isolator. By random assignment one member of each pair received a mock vaccine of adjuvant alone, while its partner received complete vaccine. In this way, vaccinates

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TABLE 1. Antibody response to SRV-1 killed virus vaccine and adjuvant alone[a]

Procedure	Time (wk)	Rhesus no., vaccinated group						Procedure	Time (wk)	Rhesus no., adjuvant alone					
		21718	21861	21875	21891	21893	21943			21701	21824	21871	21877	21903	21968
ELISA	Pre-vac (0)	−	−	−	−	−	−	ELISA	Pre- & post-adjuvant alone	−	−	−	−	−	−
ELISA	Post-vac (3)	±	−	±	+	±	+	Neutralization	Post-vac (0–13)	−	−	−	−	−	−
ELISA	Post-vac (9)	+	+	+	+	+	+	ELISA	Post-SRV-1 inoculation (4, 8, 10)	1:1,600	−	−	−	−	1:800
ELISA	Post-vac (13)	1:3,200	1:1,600	1:6,400	1:3,200	1:3,200	1:1,600								
Neutralization antibody	Post-vac (13)	1:40	1:60	1:320	1:40	1:320	1:160								
ELISA	Post-SRV-1 challenge (4)	1:800	1:400	1:400	1:1,600	1:1,600	1:400								

[a] For the ELISA, gradient-purified SRV-1 was used to coat Immulon II plates (Dynatech Laboratories, Inc.) as detailed previously (15). In the ELISA, sera having an optical density reading that was 40% or more of the reading of the positive control serum were scored as positive (+); plus/minus (±) sera were twofold over the negative control sera, and minus (−) sera had optical density readings that were not significantly different from the negative control serum. All ELISAs were done in duplicate and repeated. Serum titers shown are the highest dilution that had either neutralization or ELISA activity. For neutralizations, monkey sera were heated at 56°C for 30 min and used to block infection of Raji cells as detailed before (15). Pre-vac, Prevaccination; post-vac, postvaccination.

and nonvaccinates were exposed to the same environmental and husbandry procedures.

Vaccine preparation. The SRV-1 vaccine was prepared with sucrose gradient-purified, Formalin-inactivated virus combined with a newer generation adjuvant, threonyl muramyl-dipeptide. Each immunized animal received 1 mg of sucrose gradient-purified SRV-1 (16) inactivated in 0.8% formaldehyde (Sigma Chemical Co., St. Louis, Mo.) for 24 h at 4°C. The inactivated virus was not further concentrated. The adjuvant was threonyl muramyl-dipeptide (240 μg per dose) in an emulsion consisting of Pluronic L-121, squalene, and Tween 80 in phosphate-buffered saline (Syntex Corp., Palo Alto, Calif.). The vaccine was stored at 5°C for 7 days before use. Live SRV-1 for challenge of the vaccinated and nonvaccinated monkeys was from a stock of SRV-1 previously used to transmit SAIDS (17). Each animal received 2×10^5 syncytia-inducing units from this same stock of SRV-1.

Vaccine preparations were tested for infectious virus prior to adding the adjuvant. This was done by pelleting the virus from the vaccine preparations, washing the suspended virus free of Formalin, and exposing the Formalin-treated virus to Raji cells (4, 15). The Raji cell cultures were monitored for syncytia induction over a number of blind passages and for viral antigen by immunofluorescence, using monoclonal SRV-1 anti-p27 (15, 17). No infectious SRV-1 was found in vaccine preparations by these procedures.

RESULTS

The antibody responses of individual monkeys to vaccination with Formalin-killed SRV-1 are presented in Table 1. Animals received three intramuscular injections of vaccine or adjuvant alone at 18, 15, and 9 weeks prior to challenge with SRV-1. No local swelling or fevers were observed as a result of inoculation of vaccine or adjuvant alone. However, transient regional lymphadenopathy occurred in both groups, possibly due to the adjuvant properties of threonyl-muramyl dipeptide. Serum and PBMC were obtained before each intramuscular injection and tested for serum antibody to SRV-1 and for infectious virus (viremia), respectively (16). The latter test was included as an in vivo confirmation that the vaccine was free of infectious SRV-1. Two of six vaccinated and none of six nonvaccinated monkeys seroconverted by ELISA at 3 weeks after the first immunization. All six vaccinates and none of six nonvaccinates were ELISA seropositive following the second immunization. At 4 weeks after the third immunization, titers of sera from all 12 animals were determined for SRV-1 antibody by both ELISA and neutralization assay as described before (16). In the vaccinated monkeys titers of ELISA antibodies ranged from 1:1,600 to 1:3,200 and virus-neutralizing antibody ranged from 1:40 to 1:320. The six nonvaccinates were seronegative for SRV-1 in all assays.

At 9 weeks after the last immunization all 12 animals were challenged with a single intravenous injection of virulent SRV-1 (2×10^5 infectious units) (Fig. 1). This dose and route of exposure previously produced persistent viremia in 13 of 15 juvenile rhesus monkeys and death from SAIDS in 8 of 15 animals (16). At weekly intervals for the first 4 weeks and periodically thereafter for 8 months, each animal was evaluated for infectious SRV-1 in PBMC (Fig. 1). Physical examinations for clinical signs of SAIDS including complete blood counts were done every 2 weeks. All six nonvaccinated animals were viremic 7 days postinoculation (p.i.) with SRV-1 (Fig. 1). The level of virus infection in the PBMC increased at 2 weeks p.i. in five of six nonvaccinates. One nonvaccinate was negative for SRV-1 by week 2 p.i. and has remained negative and healthy. A second nonvaccinate was SRV-1 positive for 11 weeks p.i., but was negative at 21 and 33 weeks p.i. This monkey also remains healthy. All four remaining nonvaccinated monkeys were persistently viremic, and two died with SAIDS, one at 10 weeks and one at 8 months p.i. The remaining two are alive with clinical disease and persistent viremia at 33 weeks p.i.

In contrast, viremia was detected in only one vaccinated monkey in one PBMC sample at 7 days p.i. All subsequent samples from this animal were negative (Fig. 1) as were all PBMC samples from the other five vaccinated monkeys. Therefore, five of six immunized monkeys were completely protected from the development of viremia, and all six were protected from persistent viremia. Clinical signs of the

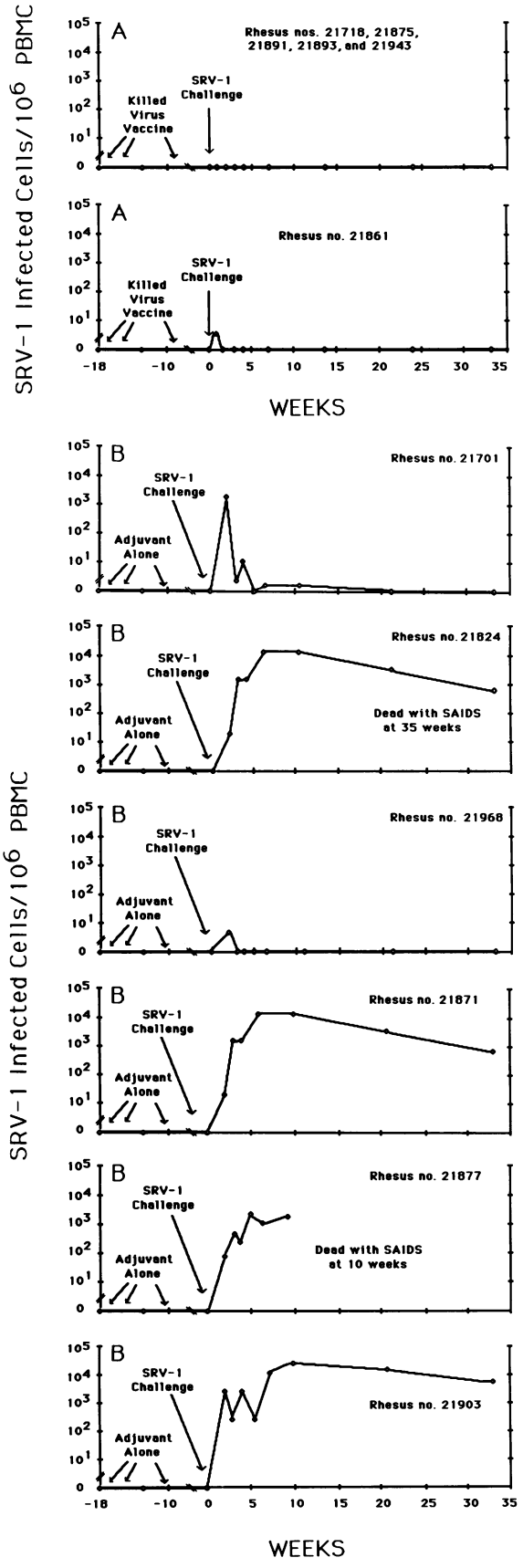

FIG. 1. SRV-1 isolation from PBMC of 12 juvenile rhesus monkeys. Six animals (A) were immunized three times with Formalin-killed whole SRV-1 vaccine containing 1.0 mg of protein in threonyl muramyl-dipeptide adjuvant, and six animals (B) received adjuvant alone. Infectious SRV-1 was isolated by cocultivation of PBMC with Raji cells and confirmed by immunofluorescence as described previously (16, 17). The probability that the distribution of viremia is by chance alone is extremely low ($P = 0.0011$) (12).

disease occurring in vaccinated and nonvaccinated monkeys are compared in Fig. 2. Results from paired animals are shown side by side. Rh 21877 and 21824, both of which died with SAIDS, showed clinical signs early p.i. which persisted until death. At necropsy, both had anemia, lymphopenia, weight loss, generalized lymphoid depletion, and disseminated cytomegalovirus infection. In addition, Rh 21877 had *Escherichia coli* bronchopneumonia and septicemia, while Rh 21824 had chronic gastroenteritis and giant cell bronchiolitis. Their vaccinated partners (Rh 21891 and 21861) have remained clinically healthy. Axillary lymph node biopsies in Rh 21877 and 21824 revealed normal morphology preinoculation and severe lymphoid depletion at death (data not shown). Normal morphology was present both pre- and postinoculation in their vaccinated partners (Rh 21891 and 21861; not shown). In the remaining four pairs, the vaccinated monkeys were not viremic and were clinically healthy except for mild splenomegaly. In contrast, two of the four nonvaccinated partners were persistently viremic and two were transiently viremic. Clinical signs in all four correlated with viremia. In conclusion, all six immunized rhesus monkeys remained in good health 8 months following challenge with a potentially lethal intravenous inoculation of SRV-1. In no case was evidence of life-threatening infections, weight loss, severe anemia, persistent generalized lymphadenopathy, or persistent viremia observed in the vaccinated monkeys. Protection against SAIDS was complete.

DISCUSSION

These experiments show that a killed type D whole-virus vaccine is highly effective in the prevention of a spontaneous, immunosuppressive retroviral disease of macaques. In past studies, killed-virus vaccines were effective in retrovirus-induced murine, feline, equine, and bovine lymphomas and sarcomas (7). This is the first report of successful immunization against an acquired retrovirus in nonhuman primates. Future studies will field test type D vaccines for prevention of spontaneous SAIDS in macaque monkeys in different primate centers where this disease is endemic (9, 13, 16, 23). Since SRV-1 and SRV-2 are naturally occurring distinct serotypes, a polyvalent vaccine will probably be necessary to prevent SAIDS in captive macaques in the United States.

Interestingly, data in Table 1 suggest that the early appearance of antibody in nonvaccinated monkeys may have predicted the ultimate clinical outcome of challenge with SRV-1. In the nonvaccinated group, only two of six nonvaccinated monkeys had mounted an antibody response against the virus at 4, 8, 10, and 16 weeks p.i. In these two, the early clinical signs of SAIDS resolved rapidly and viremia was of limited duration. Among the four nonvaccinated monkeys that failed to develop antibody by 10 weeks p.i., all were persistently viremic and ill with SAIDS, and two died.

The results of the vaccine trial reported here will serve as a guide to future work using newer vaccine strategies and

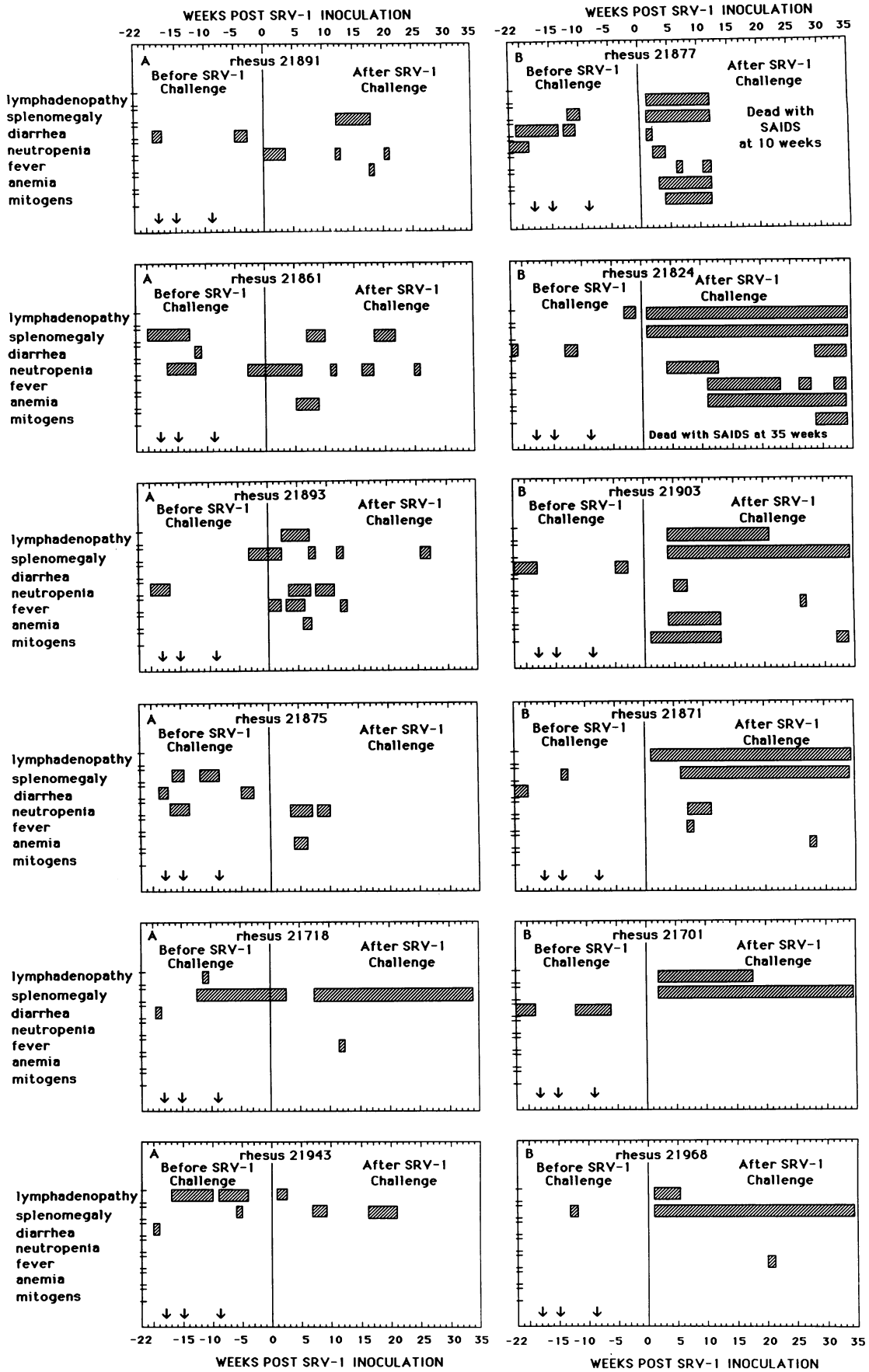

FIG. 2. Presence of clinical signs (hatched bars) in paired (A and B) juvenile rhesus monkeys, shown side by side for comparison. Animals on the left (A) were immunized three times (arrows) with SRV-1 vaccine; cagemates on the right (B) received adjuvant alone. At 0 weeks, each animal was inoculated intravenously with the same dose of SRV-1 from the same frozen stock of virus. Clinical signs of SAIDS were as defined in reference 19. PBMC responses to concanavalin A, phytohemagglutinin, and pokeweed mitogen were performed as reported earlier (17). Animals with depressed mitogen responses were more than fivefold depressed to one or more mitogens compared with normal controls and vaccinated partners.

other adjuvants. Now that a killed-virus vaccine has been shown to be effective, we will determine if other more novel SRV vaccine strategies using recombinant DNA technology are equally effective. In addition, we will compare the effectiveness of various adjuvants. Insofar as the human AIDS viruses are now classified as lentiviruses (8, 18, 22), it will also be of interest to attempt immunoprophylaxis against the experimental infection of rhesus macaques with STLV-III. It remains to be determined, however, whether a killed lentivirus vaccine will work as well in monkeys as did the type D SRV-1 vaccine.

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ADDENDUM IN PROOF

Thirteen months after challenge with a lethal dose of SRV-1, all six vaccinated animals have remained virus-free and clinically normal. An additional animal (Rh 21903) in the unvaccinated control group died with simian acquired immunodeficiency syndrome at 13 months postinoculation. The surviving three monkeys in the unvaccinated group remain as described in Fig. 1 and 2.

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