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Protection of macaques against simian AIDS by immunization with a recombinant vaccinia virus expressing the envelope glycoproteins of simian type D retrovirus

(virus neutralization/immunodeficiency/vaccine)

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ABSTRACT **Simian AIDS (SAIDS) is an endemic disease of macaques that shares many characteristics with AIDS in humans. SAIDS is etiologically linked to infection by a type D retrovirus, SAIDS retrovirus (SRV). Immunization with an inactivated whole-virus vaccine was shown to protect macaques against infection by SRV serotype 1. To identify the antigen(s) responsible for eliciting protective immunity, we have constructed a recombinant vaccinia virus (v-senv5) that expresses the envelope glycoproteins of SRV serotype 2 (SRV-2/W). Pig-tailed macaques (*Macaca nemestrina*) immunized with v-senv5 showed lymphoproliferative responses to purified SRV-2/W. They also generated antibodies that neutralized SRV-2/W infectivity *in vitro* and mediated antibody-dependent cellular cytotoxicity against SRV-2-infected cells. Four v-senv5-immunized animals, together with four control animals, were challenged intravenously with 5×10^3 tissue culture infectious doses of SRV-2/W. As early as 2 weeks after challenge, three of four control animals became viremic, and two of these three animals also seroconverted. The animal that was viremic but remained antibody negative died of symptoms of SRV infection 6½ weeks after challenge. In contrast, all four v-senv5-immunized animals remained healthy, virus-free, and seropositive against only the immunizing envelope antigens. These results indicate that immunization with a recombinant vaccinia virus expressing the envelope antigens of SRV-2/W protects primates from infection by a retrovirus that causes immunodeficiency diseases.**

Simian AIDS (SAIDS) is a naturally occurring and often fatal disease originally observed among Asian macaques housed at several primate centers in the United States (1–4). This disease closely resembles AIDS in humans. Its symptoms include persistent diarrhea, progressive weight loss, anemia, depletion of B- and T-lymphoid cells, opportunistic infection, and unusual neoplasms. The primary etiologic agent of SAIDS has been identified as a type D retrovirus (5–8), known as SAIDS retrovirus (SRV), or SAIDS D virus. Isolates of SRV can be classified by cross-neutralization into two major serotypes (SRV-1 and -2). These viruses are distinct from the lentiviruses, such as simian and human immunodeficiency viruses (SIV and HIV) (9, 10), which also cause immunodeficiency in macaques and in humans, respectively. However, unlike SIVs, which cause diseases in species other than their natural hosts (11–13), SRV infection is widespread among wild-caught (M. E. Thouless, J. Pamungkas, L.K., and W.R.M., unpublished results) and colony-bred animals (14, 15) and is one of the leading causes of death among macaques in some primate research centers (16).

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Marx *et al.* (17) reported that immunization of rhesus macaques (*Macaca mulatta*) with a Formalin-inactivated type D retrovirus vaccine elicited neutralizing antibodies and protected the animals from experimental infection by SRV-1. However, the antigen(s) responsible for eliciting protective immunity remained to be elucidated. More recently, it was reported that immunization with SRV-1 envelope antigens expressed in yeast did not elicit neutralizing antibodies (18). It is possible that the yeast-produced SRV proteins have different structural and immunogenic properties than the native viral envelope glycoproteins. Alternatively, antigens other than envelope glycoproteins may be responsible for the protective immunity induced by inactivated virions. We report here that immunization with a recombinant vaccinia virus expressing the envelope glycoproteins of SRV-2/W (a Washington isolate) elicited both T-cell-mediated and humoral immune responses against SRV-2/W. Macaques immunized with this recombinant virus were protected from SRV-2/W infection.

MATERIALS AND METHODS

Viruses. SRV-2/W virus was isolated on a canine thymus cell line, Cf2Th, after cocultivation of fibromatous tissue from a rhesus macaque that had died with SAIDS and retroperitoneal fibromatosis (7). Virus was further purified by infecting Cf2Th cells at end-point dilution. A stock of this virus was harvested, passed through 0.45-μm pore diameter filter, adjusted to 20% fetal calf serum (FCS), and stored at −70°C. The *in vitro* titer of the stock, expressed as tissue culture infectious dose (TCID), was determined to be 5×10^5 per ml by reverse transcriptase assays (19) of infected Cf2Th and A549 (a human lung carcinoma line) cells.

Construction of recombinant vaccinia v-senv5 is described in Fig. 1. The *env* gene of SRV-2/W is derived from a full-length proviral genome clone, λSW5, which was obtained from a bacteriophage λ (EMBL3) library constructed from the high molecular weight DNA of Cf2Th cells infected with the virus stock described above. λSW5 is an infectious clone which produces high-titer virus after transfection into Cf2Th cells. Virus produced by these cells has been inoculated into rhesus macaques and has resulted in viremia and seroconversion (G.H. and R.E.B., unpublished data).

Immunization and Challenge Infection of Macaques. Four pig-tailed macaques (*Macaca nemestrina*), all colony-born and 12–24 months of age (animals T85210, F86100, F86103,

Abbreviations: SAIDS, simian AIDS; SRV, SAIDS retrovirus; HIV, human immunodeficiency virus; TCID, tissue culture infectious dose; FCS, fetal calf serum; ADCC, antibody-dependent cell-mediated cytotoxicity; PBL, peripheral blood lymphocytes.

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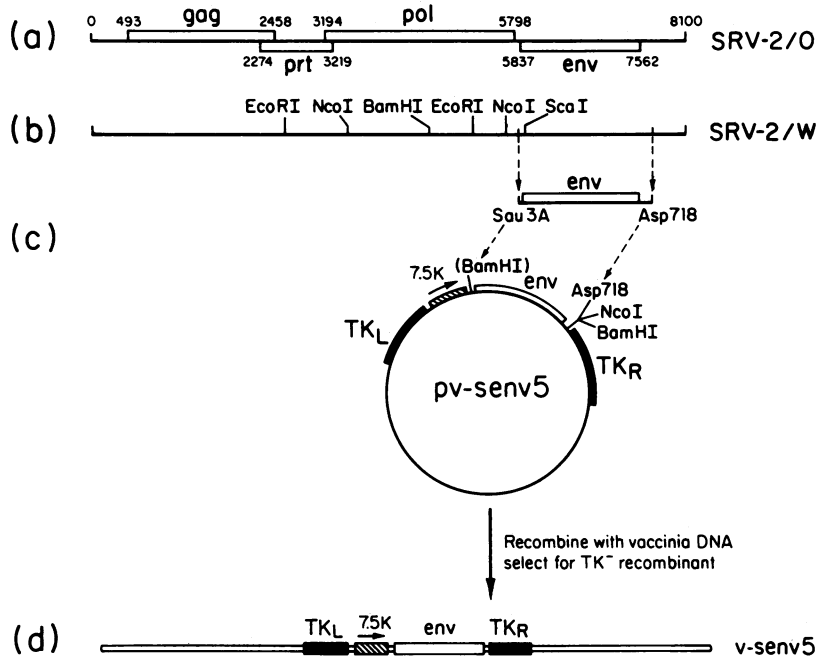

FIG. 1. Construction of recombinant vaccinia virus v-senv5. (*a*) Genetic map of the Oregon isolate of SRV-2, SRV-2/O, which is closely related to the Washington isolate SRV-2/W. Locations of the gag, prt (protease), pol (RNA-directed DNA polymerase), and env coding regions are indicated by their nucleotide sequence numbers according to Thayer *et al.* (10). (*b*) Common restriction sites are used to align the genomes of SRV-2/O and SRV-2/W. The *env* gene of SRV-2/W (from λSW5, see *Materials and Methods*) is located within a *Sau*3A–*Kpn* I fragment corresponding to nucleotides 5811–7673 in the SRV-2/O map. (*c*) The *Sau*3A–*Kpn* I fragment is inserted, via linkers, into plasmid pGS62, which is a derivative of pGS20 (20), at the *Bam*HI site downstream from the vaccinia "7.5K" promoter. (*d*) Genomic structure of recombinant virus v-senv5, showing the insertion of the chimeric gene into the thymidine kinase (TK) gene of vaccinia virus. The parental virus is v-NY (21), which is a plaque-purified isolate of the New York Board of Health strain of vaccinia virus.

and F86051), were immunized twice, at weeks 0 and 16, with 2×10^8 plaque-forming units of v-senv5 by skin scarification. A control animal, F85101, was immunized with the same dose of parental vaccinia virus v-NY (21).

The above five animals and three additional controls (F85073, F85037, and F85077) were inoculated intravenously with 1.0 ml (5×10^3 TCID) of SRV-2/W. All eight macaques were shown to be seronegative for SRV and simian immunodeficiency virus by immunoblot analysis prior to enrollment in this experiment. Throughout the study, the animals were housed in individual cages at the University of Washington Regional Primate Research Center.

Enzyme-Linked Immunosorbent Assay (ELISA). SRV-2/W was grown in Cf2Th cells by Advanced Biotechnologies (Columbia, MD) and harvested by centrifugation. Virus was then purified on sucrose gradients as previously described (19) and resuspended in 0.05 M Tris·HCl, pH 7.8/0.1 M NaCl/1 mM dithiothreitol at 1.0 mg of viral protein per ml. This virus was disrupted in 1% SDS and diluted to 1 μg/ml in sodium carbonate buffer, pH 9.6. Aliquots (0.1 ml per well) were used to coat 96-well flat-bottom plates. Macaque sera were diluted 1:10 in FCS and incubated at 4°C for 16 hr. Twofold dilutions were made in Na^+/K^+ phosphate-buffered saline (PBS) containing 0.1% Tween 20 and gelatin at 1 mg/ml. Diluted serum (0.1 ml) was added to duplicate wells and incubated at 4°C overnight. Wells were washed with PBS/0.1% Tween 20, incubated with goat antibody to macaque immunoglobulin conjugated with horseradish peroxidase, washed again, and treated with tetramethylbenzidine. Absorbance at 490 nm was measured. Endpoint titer was defined as the highest serum dilution that resulted in an absorbance value greater than three standard deviations above the mean absorbance of pooled SRV-negative macaque sera.

Immunoblot Analysis. Gradient-purified SRV-2/W was disrupted in Laemmli's sample buffer and the contents (2.5 μg of protein per lane) were resolved by SDS/PAGE on a 7–15% gradient gel. Proteins were immobilized on nitrocellulose filters by electrotransfer. Strips of filters were allowed to react with serum samples diluted 100-fold in PBS containing 5% nonfat dry milk. Virion proteins recognized by these sera were detected by goat antibodies to macaque immunoglobulin conjugated with alkaline phosphatase followed by treatment with 4-chloro-1-naphthol.

Virus Neutralization Assay. Macaque plasma was heated (56°C, 30 min) and diluted in Dulbecco's modified Eagle's medium (DMEM). SRV-2/W stocks (at 5×10^5 TCID/ml) were diluted 1:1000, mixed with an equal volume of plasma, and incubated at 37°C for 1 hr. Virus–plasma mixtures (0.4 ml) were added to A549 cells in a total volume of 4.0 ml of DMEM/10% FCS for 3 hr at 37°C. Cells were then washed three times with DMEM and cultured. Reverse transcriptase activity was measured at 16 days as described (19).

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Assay. Effector cells used for ADCC were fresh peripheral blood lymphocytes (PBL) pooled from uninfected, nonimmunized macaques and were isolated by Ficoll density gradient sedimentation (87.5% Ficoll/12.5% PBS). Target cells were Cf2Th cells, either uninfected or chronically infected with SRV-2/W. Target cells were detached by treatment with EDTA and labeled with $Na_2{}^{51}CrO_4$ (0.5 mCi per 5×10^6 cells; 1 Ci = 37 GBq) in suspension for 1 hr. After three washes with media, 1×10^4 labeled target cells in 0.1 ml of medium were incubated for 30 min at room temperature in 4 replicate wells of a 96-well V-bottom plate with 0.05 ml of macaque plasma at a final dilution of 1:40. Effector cells were then added to wells at an effector-to-target ratio of 50:1 in DMEM with 10% FCS. After 4 hr of incubation at 37°C, 0.13 ml of supernatant was removed from each well and the ^{51}Cr released was measured with a γ counter. The percent specific ADCC was calculated as the difference in percent ^{51}Cr release in the presence of PBL with or without the addition of plasma. Percent ^{51}Cr release is defined as [(cpm experimental release − cpm spontaneous release)/(cpm maximal release − cpm spontaneous release)] × 100. Maximal release is cpm release from target cells in the presence of detergent and spontaneous release is cpm released from target cells in medium alone.

Lymphoproliferative Assay. Macaque PBL were isolated from heparinized blood by Ficoll density gradient sedimentation (as above). PBL were washed with PBS and resuspended in RPMI 1640 medium containing 10% heat-inactivated, pooled, normal macaque serum. PBL, 1×10^5 in 0.1 ml of medium, were placed in 4 replicate wells of a 96-well round-bottom plate and, to each well, 0.1 ml of medium containing gradient-purified SRV-2/W (1.0 μg/ml) or UV-inactivated vaccinia virus (1×10^6 plaque-forming units/ml prior to inactivation) was added. Six days after stimulation, the cells were labeled with 1 μCi of [^{3}H]thymidine per well for

6 hr and harvested, and the radioactivity incorporated was measured by liquid scintillation counting.

Virus Isolation by Lymphocyte Cocultivation. Macaque PBL were isolated over Histopaque-1077 (Sigma) as previously described (22). PBL were stimulated for 3 days in RPMI 1640 medium containing 10% autologous macaque serum, phytohemagglutinin at 1 μg/ml, and phorbol 12-myristate 13-acetate at 4 ng/ml. A constant number of macaque PBL from all eight animals (usually 0.5×10^6 to 2.0×10^6 cells) were added to A549 cells and cultured in the presence of 10% interleukin 2 (IL-2) and RPMI 1640/10% FCS (Advanced Biotechnologies, Columbia, MD) for 1 week and then in DMEM/10% FCS in the absence of IL-2 until discarded at 8–9 weeks. Reverse transcriptase assays were performed on culture fluids as previously described (19).

RESULTS

Expression of SRV-2 Envelope Glycoproteins by a Recombinant Vaccinia Virus. A recombinant vaccinia virus (v-senv5) was constructed that contains the entire env-coding sequences of SRV-2/W (Fig. 1; ref. 7 and G.H. and R.E.B., unpublished results). Expression of SRV envelope antigens by this recombinant virus was confirmed by radioimmunoprecipitation analysis. Serum from SRV-2/W-infected macaques reacted specifically with two major proteins from v-senv5-infected cells (Fig. 2). The molecular weights of these two proteins correspond to the molecular weight of SRV envelope glycoproteins gp70 and gp20 (23). Results from pulse–chase experiments indicate that the surface protein gp70 and the transmembrane protein gp20 are cleavage products of gp90 and can be detected between 1 and 3 hr after synthesis of the precursor (results not shown). In contrast to a similarly constructed recombinant expressing the envelope antigens of HIV (24), there is little or no shedding of SRV envelope glycoproteins into the culture media of v-senv5-infected cells (Fig. 2).

Immune Responses Elicited by v-senv5. To determine whether T cells were primed to respond to SRV antigens after v-senv5 immunization, we isolated PBL from immunized macaques and stimulated them with either purified SRV or UV-inactivated vaccinia virus as a control, using methods we previously found successful for evaluating T-cell proliferative responses in macaques immunized with a vaccinia–HIV recombinant virus (25). PBL from all immunized macaques proliferated in response to stimulation with vaccinia virus. PBL from three of the four v-senv5-immunized animals demonstrated at least a 2-fold stimulation index after stimulation with SRV 2 weeks after the second immunization (Table 1). Six weeks after the second immunization, PBL from these three animals showed an increase in stimulation index ranging from 4.3 to 6.8. PBL from the control animal immunized with vaccinia virus proliferated upon stimulation with vaccinia virus but not SRV (Table 1).

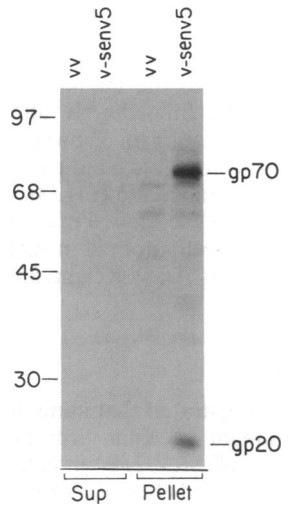

FIG. 2. Radioimmunoprecipitation analysis of the SRV-2/W envelope glycoproteins expressed by recombinant v-senv5. BSC-40 (African green monkey kidney) cells infected with recombinant v-senv5 or parental vaccinia virus (vv) were labeled for 15 min with [^{35}S]methionine and "chased" for 6 hr in nonradioactive medium. Radioactively labeled proteins in the cell lysates (pellet) and from the culture media (Sup) were immunoprecipitated with sera from SRV-2/W-infected macaques and resolved by SDS/PAGE. Molecular masses (kDa) of marker proteins are indicated on the left.

Table 1. SRV-induced proliferative responses of PBL from macaques immunized with recombinant vaccinia virus v-senv5

Macaque	Immunizing virus	Stimulation index: SRV wk 18	SRV wk 24	Vaccinia virus wk 18	Vaccinia virus wk 24
T85210	v-senv5	5.6	6.8	4.7	14.0
F86100	v-senv5	2.0	4.3	3.9	18.9
F86103	v-senv5	2.2	5.1	4.4	11.9
F86051	v-senv5	1.8	1.8	10.1	10.4
F85101	v-NY	1.6	1.0	11.0	16.5

The stimulation index was calculated as (cpm of [^{3}H]thymidine incorporated into virus-stimulated cells)/(cpm [^{3}H]thymidine incorporated into nonstimulated cells). The stimulating antigens were SRV or vaccinia virus, and times given are weeks after primary immunization. Week 18 is 2 weeks after the secondary immunization.

SRV-specific antibody responses in immunized animals were assayed by ELISA and immunoblot. All four v-senv5-immunized animals seroconverted after a single inoculation (Fig. 3). After a second inoculation at week 16, a significant and immediate increase (4- to 8-fold) in antibody titer was observed (Fig. 3). As shown by immunoblot analysis, v-senv5-immunized macaques generated antibodies against the two envelope glycoproteins gp70 and gp20 (Fig. 4). A control animal immunized with parental vaccinia virus v-NY developed the same level of anti-vaccinia antibodies as did the experimental animals (data not shown) but no SRV-specific antibodies (Figs. 3 and 4).

To determine the functions of the SRV-specific antibodies elicited, we assayed sera from v-senv5-immunized animals for virus neutralization and ADCC activities. All four v-senv5-immunized animals generated significant levels of antibodies that neutralized infectivity of SRV-2/W *in vitro* (Table 2). The titers were comparable to those obtained in SRV-infected macaques and did not decrease appreciably in the 12 weeks between the second immunization and the challenge infection (data not shown). Three of the four v-senv5-immunized animals also generated antibodies that mediated ADCC against SRV-2-infected cells (Table 2). Serum from

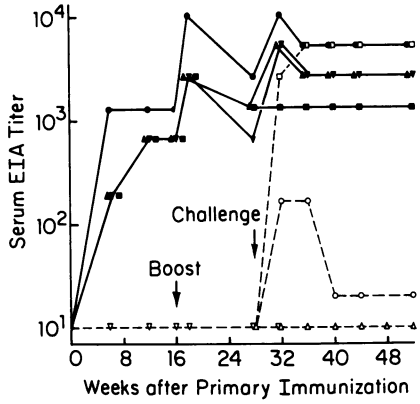

FIG. 3. ELISA of sera from control and immunized animals before and after SRV-2/W challenge. Animals were immunized at 0 and 16 weeks and were challenged at 28 weeks. ELISA (EIA) titer for each serum sample was determined as the highest serum dilution that reacted with SRV-2/W virion proteins. Solid lines and symbols represent v-senv5-immunized animals (●, F86103; ■, F86051; ▼, T85210; ▲, F86100). Broken lines and open symbols represent control animals (□, F85077; ○, F85073; ▽, F85101; △, F85037).

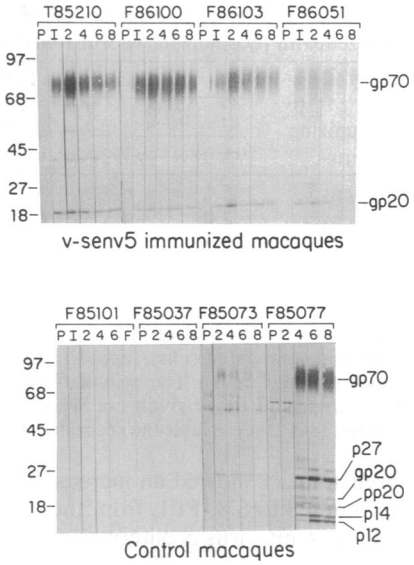

FIG. 4. Immunoblot analysis of sera from control and v-senv5-immunized animals before and after SRV-2/W challenge. Sera were collected prior to immunization (P), after immunization and prior to challenge at week 28 (I), and at biweekly intervals after challenge as indicated. F indicates the final bleed of the animal which died at 6½ weeks after challenge. Control animal F85101 was immunized with parental vaccinia virus. Electrophoretic positions of molecular mass markers (kDa), as well as those of SRV-2 envelope glycoproteins (gp70 and gp20) and core proteins (p27, pp20, p14, and p12) (23) were as indicated.

the animal immunized with vaccinia virus had neither neutralizing nor ADCC activity.

Responses to SRV-2 Challenge Infection. Twelve weeks after the second immunization, four v-senv5-immunized animals, together with four control animals (including one immunized with v-NY), were challenged intravenously with 5×10^3 TCID of SRV-2/W. All animals were monitored for viremia, immune responses, and clinical status.

Three of four control animals developed signs of SRV infection after challenge. One animal (F85077) developed a strong antibody response to SRV. Its ELISA titer increased to $2\text{–}5 \times 10^3$ within 4–8 weeks (Fig. 3). Antibodies against both envelope and core antigens of SRV were detected (Fig. 4). Another control animal (F85073) developed a transient antibody response between 4 and 8 weeks after challenge, with a titer of 160 (Fig. 3). Its serum was weakly reactive with SRV antigens in immunoblots and was directed to both envelope and core (p27) antigens (Fig. 4). The third control animal (F85101) failed to show any ELISA-detectable antibody titer (Fig. 3), but its serum drawn at 2 weeks after challenge reacted weakly with gp70 in immunoblots (Fig. 4). The fourth animal (F85037) did not show any sign of SRV infection, either by antibody assays (Figs. 3 and 4) or by virus isolation (Table 3).

Table 2. Virus neutralization and ADCC activities in v-senv5-immunized macaques

Macaque	Immunizing virus	Neutralization titer	% ADCC
F86103	v-senv5	300	21.6
T85210	v-senv5	150	14.4
F86100	v-senv5	150	14.0
F86051	v-senv5	300	6.2
F85101	v-NY	<25	3.3
F85037	None	<25	5.5

Sera were obtained 2 weeks after the second immunization. Neutralization titer was defined as the highest serum dilution that resulted in complete inhibition of viral infectivity as measured by reverse transcriptase activity. Neutralization titers in sera from SRV-infected animals range between 50 and 400. ADCC activity was assayed in plasma obtained 18 weeks after immunization. Lysis of SRV-infected target cells was measured by percent ^{51}Cr specifically released. Approximately 20% of target cells expressed SRV antigens as determined by cell surface immunofluorescence, accounting for the relatively low level of ADCC. There was less than 5% specific ^{51}Cr release from uninfected target cells in the presence or absence of any of the plasmas.

Table 3. Viremia in SRV-challenged macaques

Macaque	Viremia wk 2	wk 4	wk 8	wk 12	wk 16	wk 32
Vaccinated	–	–	–	–	–	–
T85210	–	–	–	–	–	–
F86103	–	–	–	–	–	–
F86100	–	–	–*	–*	–*	–
F86051	–	–	–	–	–	–
Controls						
F85037	–	–	–	–	–	–
F85101†	+ (2)	+ (2)	NT	NT	NT	NT
F85073	+ (6)	+ (8)	+ (6)	–	–	–
F85077	+ (2)	+ (2)	+ (5)	+ (8)	+ (6)	+ (5)

At the indicated number of weeks after challenge, PBL were cocultivated with A549 cells and the presence of virus was detected by reverse transcriptase assay. Number of input PBL was 1×10^6 per culture, except for week 16 and 32 samples, which contained $3\text{–}11 \times 10^6$ PBL. Numbers in parentheses indicate weeks of culturing before virus was detected. Negative samples were cultured for 8 or 9 weeks.
*A foamy retrovirus was isolated from the PBLs of this animal at the times indicated. The identification of the virus was made by the characteristic vacuolated appearance of the A549 cells, by the preference for manganese instead of magnesium in the reverse transcriptase assay, and by visualization in electron microscopy.
†Animal developed SAIDS and was euthanized at 6½ weeks after challenge. NT, not tested.

Viremia in SRV-challenged animals was determined by reverse transcriptase assay of cocultivations of macaque PBL with A549 cells. SRV was isolated from three of the four control animals as early as 2 weeks after challenge (Table 3). The animal (F85077) that had a strong antibody response became persistently infected and was viremic throughout the course of the experiment (32 weeks after challenge). Animal F85073, which showed a transient antibody response, was also viremic from 2 to 8 weeks after challenge infection. The animal (F85101) that was viremic, but had no detectable SRV antibody titer by ELISA, developed hemorrhagic enteritis and petechial hemorrhages on the extremities and the anterior thorax at 6 weeks after challenge. Within a period of 4 days, this animal developed severe thrombocytopenia and was consequently euthanized at 6 1/2 weeks after challenge.

In contrast, all four v-senv5-immunized animals remained virus-negative throughout the 32-week follow-up period (Table 3) and were antibody-positive only to the immunizing antigens (i.e., gp70 and gp20) (Fig. 4). Immediately after challenge infection, three out of four animals showed a temporary increase in ELISA titers (4- to 8-fold), indicating a boosting effect of the challenge virus inoculum (Fig. 3).

With the exception of animal F85101 described above, all animals remained healthy and were clinically normal in such indicators as blood count, blood chemistry, body weight, and temperature.

DISCUSSION

In this communication, we have demonstrated that immunization of macaques with a recombinant vaccinia virus expressing the envelope glycoproteins of SRV-2/W induced SRV-specific T-cell-mediated immunity and elicited antibod-

ies that neutralized SRV infectivity and mediated lysis of SRV-infected cells by ADCC. Macaques immunized with this recombinant virus were protected against challenge infection with SRV-2/W.

The present study extends the findings of Marx *et al.* (17), who demonstrated that immunization with a Formalin-inactivated whole-virus vaccine protected monkeys from SRV-1 challenge infection. Our results indicate that the envelope glycoproteins of SRV-2/W are sufficient for eliciting protective immunity. The presence of neutralizing antibodies has been associated with better prognosis and survival among SRV-infected macaques (17, 26). Such neutralizing antibodies were produced by v-senv5-immunized animals at levels similar to the level found in SRV-infected convalescent animals, indicating that the envelope glycoproteins contain the major neutralizing epitopes of SRV. In addition, results from the present study indicate that the envelope glycoproteins also contain determinants capable of inducing the T-cell proliferative response and serve as target antigens on SRV-infected cells for ADCC. It is not known whether SRV infection is caused solely by cell-free virus or also by virus-infected cells as has been suspected for HIV. The ability of v-senv5 to induce antibodies capable of lysing SRV-infected cells through ADCC may be important if natural infection can indeed be caused by transfer of infected cells.

It is not clear why animal F85037 failed to show any sign of SRV infection after challenge. However, this is consistent with the observation that a number of animals, for reasons as yet undetermined, are resistant to SRV infection (C. C. Tsai and W.R.M., unpublished observations). It is also possible that the failure to infect F85037 is due to the variability of the *in vivo* infectivity of SRV-2/W at the challenge dose used. Rechallenging this animal may be informative.

A highly conserved peptide sequence present in the envelope transmembrane proteins of several retroviruses has been implicated in the immunosuppressive effects caused by some of these viruses (27). Although such a sequence is present in the envelope glycoprotein of SRV, there is no evidence, either from this or from previous studies (17, 18), that immunization of macaques with SRV envelope antigens results in any immunosuppressive symptoms. Animals immunized with v-senv5 demonstrated lymphoproliferative responses (Table 1) and neutralizing antibodies to vaccinia virus (data not shown) similar to those of the control animal immunized with vaccinia virus alone. These findings indicate that the consensus peptide sequence described above may not be sufficient to induce immunosuppression associated with SRV infections.

The present study also indicates the potential usefulness of the recombinant vaccinia virus we described as a vaccine against SRV-induced AIDS in macaques. Since there are distinct but defined serotypes of SRV, a multivalent vaccine incorporating the envelope glycoproteins of these serotypes might provide the spectrum of protection required. In contrast to whole virion vaccines, immunization with subunit vaccines, such as the recombinant described above, causes seroconversion only to a specific viral antigen, thereby allowing the distinction to be made between infected and immunized animals. This could be of significance in a preventive program to control the spread of SRV infection, not only within a colony, but more importantly, between primate colonies. Because SRV is transmitted horizontally with high rate, it should be possible to determine whether the recombinant virus vaccine, which has been shown to protect animals from experimental challenge of cell-free SRV, can also protect macaques from natural infections.

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