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Isolation of a New Serotype of Simian Acquired Immune Deficiency Syndrome Type D Retrovirus from Celebes Black Macaques (*Macaca nigra*) with Immune Deficiency and Retroperitoneal Fibromatosis

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A new serotype of simian acquired immune deficiency syndrome (SAIDS) retrovirus (type 2) belonging to the D genus of retroviruses is associated with a SAIDS occurring spontaneously in a colony of Celebes macaques (*Macaca nigra*) and rhesus macaques (*Macaca mulatta*) at the Oregon Regional Primate Research Center. This syndrome resembles SAIDS in *M. mulatta* at the California Primate Research Center, which is associated with a similar type D retrovirus (type 1). However, at the Oregon Center, SAIDS is distinguished by the occurrence of retroperitoneal fibromatosis in some of the affected monkeys. Type 2 virus was isolated from seven of seven macaques with SAIDS, retroperitoneal fibromatosis, or both and from one of six healthy macaques. The new strain is closely related to SAIDS retrovirus type 1 and Mason-Pfizer monkey virus but can be distinguished by competitive radioimmunoassay for minor core (p10) antigen and by genomic restriction endonuclease cleavage patterns. Neutralization tests indicate that type 1 and type 2 SAIDS retroviruses are distinct serotypes. Therefore, separate vaccines may be necessary to control these infections in colonies of captive macaques.

Simian acquired immune deficiency syndrome (SAIDS) is a spontaneous disease of macaque monkeys that resembles acquired immune deficiency syndrome (AIDS) in humans (10, 18, 22, 26). SAIDS has been reported in rhesus monkeys (*Macaca mulatta*) (10, 18, 26), Taiwanese rock macaques (*M. cyclopis*) (18), crab-eating macaques (*M. fascicularis*), (18) and pigtailed macaques (*M. nemestrina*) (26). Recent studies have shown that type D retroviruses, related to but distinct from Mason-Pfizer monkey virus (MPMV), are strongly associated with SAIDS at the California and Washington Regional Primate Research Centers (9, 20, 26). At the New England Regional Primate Research Center, a new retrovirus named simian T-cell leukemia virus type III has been described that is not a member of the type D group and is, instead, related to the AIDS retrovirus lymphadenopathy-associated virus–human T-cell leukemia virus type III (6, 13; R. Desrosiers, personal communication). This virus was isolated from animals negative for type D virus and is associated with immunodeficiency disease in some of the macaques at the New England Primate Center. In contrast, at the California primate center, 100% of SAIDS cases tested to date have yielded a type D virus isolate (9, 20; P. A. Marx, unpublished data). Furthermore, at the California primate center, rhesus monkey tissue culture fluids containing SAIDS retrovirus (SRV) have successfully induced the disease in nine of nine juvenile (10 to 30 months old) rhesus monkeys (20; D. Maul, submitted for publication). Four of this group have since died of fulminant SAIDS with opportunistic infections such as noma, *Klebsiella pneumoniae* and *Streptococcus canis* septicemia, diarrhea, disseminated cytomegalovirus infection, and pneumonia, all unresponsive to therapy. At the Washington Primate Research Center, SAIDS frequently occurs in association with retroperitoneal fibromatosis (RF), a syndrome characterized by an aggressive proliferation of fibrous tissue in the abdominal cavity (8). Because this lesion was not found at the California center and only rarely occurs at the New England center, it signifies a major difference in the presentation of SAIDS at the various primate centers (10, 18, 23, 26).

A colony of Celebes black macaques (*M. nigra*) housed at the Oregon primate center has experienced chronic health problems for many years. Over a 6-year period (1978 to 1983), 51.9% (67 of 129) of these animals died. The affected monkeys have shown lymphadenopathy, recurrent diarrhea, hypoproteinemia, loss of peripheral blood mononuclear cell (PBMC) response to T-cell mitogens, anemia, lymphopenia, severe weight loss, noma, and RF (24). These findings suggested that the Celebes monkeys might have a SAIDS-like disease similar to that described at the other primate centers (10, 18, 23, 26) and prompted us to determine whether a type D retrovirus was associated with the syndrome in the Celebes monkeys. We report here that both the Celebes and rhesus monkeys at the Oregon center harbor a variant of the SRV found at the California primate center (20). This new virus can be distinguished from MPMV and the original isolate of SRV, now designated as the type 1 strain (SRV-1), by competitive radioimmunoassay (RIA),

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restriction endonuclease mapping, and serum neutralization. This new strain of SRV is designated SRV-2 in accordance with published guidelines (21).

MATERIALS AND METHODS

Animals. Before August 1982, the Celebes macaque colony was housed in three inside-outside runs at the Oregon primate center. Other Celebes were housed in individual hanging cages inside the same building. Males and females were rotated between the runs and the cages, depending on their breeding cycle and age. In August 1982, all of the animals were moved into individual hanging cages inside two rooms of a different building. During 1983 and 1984, 39 monkeys were added to the colony by acquisition. Eighty-two animals are currently alive in this colony.

At the Oregon primate center, the Celebes macaques were under close clinical surveillance because of the susceptibility to spontaneous diabetes mellitus (12). Intra-abdominal examination was done on 30 of these animals during the course of open pancreatic biopsy.

Animal inoculations. For virus isolation studies at the California primate center, two juvenile rhesus monkeys were used for experimental tranmission of the virus from the Oregon Celebes macaques. Before intravenous inoculation of plasma from ill Celebes macaques, each rhesus was clinically healthy by physical examination and complete blood count and was free of serum antibody to SRV. Throughout the study, the two inoculated monkeys were housed together in a negative-pressure, HEPA-filtered isolator cage and fed Purina monkey chow ad lib. No immunosuppressive drugs were given. Physical examinations and complete blood counts were done, and serum was frozen at 2- to 4-week intervals postinoculation. The last virus isolation was done 14 months postinoculation.

Virus isolation. Infectious virus in PBMC was determined by coculture with Raji cells as described by Daniel et al. (5). Raji cells (1×10^4) were cocultivated with serially diluted PBMC (8×10^4 to about 4×10^1 cells). The lower limit of sensitivity was 1 type D virus-infected cell per 8×10^4 PBMC. The cultures were fed on days 3 and 6 with 100 μl of complete medium and examined for syncytia formation on day 9. If results on day 9 were equivocal, the cultures were transferred into 24-well plates in 1 ml of complete medium and reexamined on days 12 and 15. The identification of isolates was confirmed by restriction endonuclease mapping, RIA, and electron microscopy (EM).

Serological assays. Macaque sera reactive with virus-induced membrane antigens were detected by a flow cytometric-based fluorescence assay. Raji cells infected with the Celebes macaque isolate of the type D retrovirus were used as the positive target, while uninfected Raji cells were used as the negative target. Positive control sera were selected from animals who had recovered from spontaneous SRV infection at both primate centers. A negative control serum from unexposed animals was also selected.

The staining procedure was as previously described (24). Two sets of a 1:5 dilution of the control and test serum samples (25 μl/well) were placed in wells of a U-bottomed 96-well plate (Linbro, Van Nuys, Calif.). Infected and uninfected Raji cells (5×10^4) were placed into corresponding sets of wells. The final dilution of the serum samples was 1:10. After a 30-min incubation on ice, 150 μl of wash medium (RPMI 1640, 5% newborn calf serum, 0.02% sodium azide) was added to the wells, and the plate was centrifuged at $320 \times g$ for 5 min. The wash medium was flicked out from the wells. The cells in the wells were then mixed with a Maxi Mix II (Thermolyne Corp., Dubuque, Iowa) and washed twice with 200 μl of wash medium. After the last wash, 25 μl of a 1:200 dilution of fluoresceinated $F(ab')_2$ goat anti-monkey immunoglobulin G (Cooperbiomedical, Inc., Malvern, Pa.) was added to each well. After a 30-min incubation on ice, the cells were washed as described above. A 200-μl sample of 1% methanol-free formaldehyde in phosphate-buffered saline (Polyscience, Inc., Warrington, Pa.) was added to each well.

Fluorescent staining of the cell targets was measured by flow cytometry on an EPICS C (Coulter Electronics, Inc., Hialeah, Fla.). The instrument was adjusted so that analysis of cells stained with the negative control serum resulted in 2 to 5% positive cells. Under these conditions, the positive control serum stained 2 to 5% of the uninfected Raji cells and 95% of the infected cells. The percent-specific staining was calculated as percent staining of infected cells minus percent staining of uninfected cells. Serum samples that gave percent-specific staining of >10% at a 1:10 serum dilution were designated as seropositive for the type D retrovirus.

For the enzyme-linked immunosorbent assay, sucrose gradient-purified SRV was prepared at the Frederick Cancer Research Facility, Frederick, Md. Briefly, SRV, characterized and described in reference 20, was grown in human rhabdomyosarcoma cells (American Type Culture Collection HTB82 cells) and centrifuged to equilibrium in a continuous flow rotor at 5°C. Banded virus was pelleted and suspended in TEN (0.01 M Tris [pH 7.4], 0.01 M EDTA, 0.1 M NaCl) at 2.0 mg/ml. Virus was diluted to 10 μg/ml in coating buffer (0.5 M CO_3-HCO_3 [pH 9.6]), and 50 μl was used to coat 96-well microtiter plates (Dynatech Immulon II plates; Dynatech Laboratories, Inc., Alexandria, Va.) at 5°C for 16 h. Plates were washed twice with saline–Tween 20 solution (0.05% Tween 20, 0.15 M NaCl). A 50-μl sample of diluted sera (1:20) in TEN–0.1% bovine serum albumin–0.05% Tween 20 was added for 1 h at 37°C. After three washes with saline–Tween solution, 50 μl of 1:200 peroxidase-conjugated anti-macaque immunoglobulin G (Cooperbiomedical, Inc.) was added for 30 min at 37°C. After three washes with saline–Tween 20 solution, 100 μl of freshly prepared substrate solution (0.05 M citric acid [pH 4.0], 0.15 mM 2,2′-azinobis(3-ethylbenzthiazoline sulfonic acid), 0.008% H_2O_2) was added to each well. Color development was stopped with 100 μl of 0.2 M HF (pH 3.3). Optical density was read at 405 nm in an enzyme-linked immunosorbent assay reader. Also, all sera were negative with fetal bovine serum as the target antigen in the enzyme-linked immunosorbent assay (data not shown). All sera scored as positive were four- to fivefold more reactive than negative control rhesus sera.

EM. Thin sections for EM were stained with uranyl acetate and lead citrate as described previously (20).

Mitogenic assay. The mitogen reactivity of PBMC obtained from the monkeys was measured as previously described (24).

Neutralization assay. In the neutralization assay, macaque sera were tested for neutralizing activity against the two isolates of SRV. Stocks of virus were frozen at −70°C, and serum titers were determined for inhibition of syncytia formation in Raji cells. Diluted sera were added to wells of microtiter plates, and 8 to 10 syncytia-inducing units were added to each serum dilution. The cultures were observed for syncytia formation for 10 days as described above for the Raji cell assay. The reported titer was the highest dilution

TABLE 1. Association of RF and SAIDS with type D retrovirus infection of macaques[a] at the Oregon Primate Research Center

Animal no.	Age (years)	Abdominal mass[b]	Clinical signs of SAIDS[c]	Mitogen response[d]	SAIDS membrane fluorescence	Neutralizing[e] antibody
Virus positive[f]						
1560	19	+	Diarrhea, lymphadenopathy, anemia	D/D	+	−
2255	18	−	Normal	N/D	+	−
10867	4	+	Died, diarrhea, TP, lymphadenopathy, splenomegaly, anemia, weight loss, RF	N/D	+	−
12700	13	+	TP, lymphadenopathy, RF	D/D	+	−
12703	13	+	Died, RF, TP, lymphadenopathy, emaciation, lymphopenia	ND[g]	+	−
12704	8	−	Died, lymphadenopathy, anemia	N/N	+	−
11180	1	−	Noma, lymphoid depletion	ND	−	−
3464[h]	16	+	Died, diarrhea, weight loss, anemia, TP, RF	ND	ND	−
Virus negative						
5220	15	−	Splenomegaly	N/N	+	+
7707	10	−	Normal	N/N	+	+
10757	5	−	Normal	N/N	+	+
11401	4	−	Normal	N/N	+	+
11720	3	−	Normal	D/N	+	+

[a] Macaque 11180 is a rhesus monkey (*M. mulatta*). All others are Celebes black macaques (*M. nigra*).

[b] A firm, palpable, irregular mass on the right side between the upper and lower abdominal quadrants. RF is palpated on the right side because the RF lesion typically arises at the ileocecal junction mesenteric attachment or adjacent lymph nodes or both (8).

[c] Determined by reviewing the clinical records of each animal for the previous 12 months. Diarrhea, At least three episodes; anemia, hemoglobin concentration of <10 g/dl; lymphopenia, <800/μl for males and <1,800/μl for females; lymphadenopathy, >10 mm at one or more sites; RF, confirmed at necropsy or surgically; splenomegaly, enlarged spleen being below the costal margin; TP, hypoproteinemia (total protein concentration of <7.6 g/dl); weight loss, >10% weight reduction.

[d] Peripheral lymphocytes tested for blastogenesis against phytohemagglutinin and concanavalin A as previously described (24). N, Normal values ranging from 3.0×10^4 to 1.10×10^5 cpm for concanavalin A and 2.0×10^4 cpm to 7.0×10^4 cpm for phytohemagglutinin. D, Below normal values for one mitogen. D/D, Below normal values for both mitogens.

[e] Symbols: −, no neutralizing activity at 1:10 dilution of test serum; +, complete inhibition of syncytia formation at 1:10 dilution of test serum.

[f] PBMC were separated and assayed for induction of syncytia in Raji cells as previously described (5). All virus assays were done on coded samples. Virus negative is <1 syncytia-inducing unit per 8.0×10^4 lymphocytes.

[g] ND, Not determined.

[h] Celebes monkey 3464 died with RF. Type D retrovirus was isolated indirectly from no. 3464 by inoculation of frozen plasma into a juvenile rhesus monkey (at the California primate center) not previously exposed to SAIDS. Virus was then isolated from PBMC of this rhesus monkey.

that completely inhibited syncytia formation. Negative control sera were included in each assay.

Molecular probes and analysis of DNA. Full-length SRV-1 (8.2 kilobases) originally cloned into the *Bam*HI site of lambda phage L47.1 (4) was subcloned into the *Bam*HI site of the Sp65 expression vector (Promega Biotec, Madison, Wis.). MPMV (8.1 kilobases)-cloned DNA in the plasmid PAT153 was as previously reported (1). Detailed restriction maps of the SRV-1 and MPMV genome have recently been published (1, 4). For analysis of the SRV-2 virus genome, DNA was extracted from Raji cells productively infected with SRV-1, SRV-2, or MPMV. High-molecular-weight DNA was purified from cells grown in vitro and quick frozen in liquid nitrogen or from tissues pulverized in liquid nitrogen. The frozen samples were thawed and suspended in 100 mM NaCl–50 mM Tris–10 mM EDTA (pH 7.8) containing 100 μg of proteinase K per ml. Sodium dodecyl sulfate was added to a final concentration of 0.5%, and the samples were incubated at 65°C for 60 min. DNA was extracted with phenol and dialyzed exhaustively against extraction buffer without sodium dodecyl sulfate. Each DNA sample (10 μg) was digested with restriction endonuclease by using conditions specified by the suppliers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The resulting restriction fragments were separated on 0.8% agarose gels in Tris-borate–EDTA buffer (19) and transferred to nitrocellulose paper as described by Southern (25). Virus-specific restriction fragments were detected by moderate-stringency hybridization with [^{32}P]dATP-labeled DNA probes in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt solution (25), and 40% formamide at 37°C for 36 to 48 h. The posthybridization wash was done in 1× SSC at 65°C for 10 min.

RESULTS

Retrovirus isolations from unhealthy macaques. Based on retrospective health reviews at the Oregon primate center, PBMC were obtained from 13 animals, 12 Celebes and 1 rhesus macaque, with and without signs of a SAIDS-like disease. Ficoll-purified PBMC samples were coded at the Oregon primate center and sent to the California primate center for cocultivation with Raji cells. PBMC from 8 of the 13 monkeys induced syncytia in the Raji cell assay. After culture, the samples were decoded, and the clinical history of each animal was compared as summarized in Table 1. During the previous 12 months, each of the seven virus-positive animals had shown clinical signs often associated with SAIDS in previous studies (10, 23). These signs included diarrhea, anemia, lymphopenia, lymphadenopathy, RF, splenomegaly, hypoproteinemia, and weight loss. Five of these animals developed abdominal masses which were microscopically confirmed as RF in three animals. PBMC from all three of these animals yielded a type D retrovirus. Two of the monkeys which died during the study had RF and SAIDS, while one monkey died during the study with

lymphadenopathy, severe anemia, and trypanosomiasis (L. C. Olson, unpublished data), an unexpected finding whose relationship to SAIDS is not known. One infected monkey is currently alive with a depressed mitogen response but no other clinical signs of SAIDS. One rhesus macaque was found to have a retrovirus infection. At the time of culture, this animal was moribund with lymphoid depletion and noma. None of the five virus-negative animals had abdominal masses, and among this group only one had splenomegaly and one had a depressed response to phytohemagglutinin. Also listed in Table 1 is a virus-positive Celebes macaque, which had died with RF before this study was done. A retrovirus was isolated indirectly from this monkey by inoculation of once frozen-thawed plasma, collected 6 months before death, into a juvenile rhesus monkey (animal no. 21336 at the California Primate Research Center) and by subsequent cultivation of PBMC from this infected rhesus monkey (details presented below). Thus, over a 12-month period, clinical signs of SAIDS were found much more frequently among the eight virus-positive animals than among the group of five virus-negative monkeys. RF was found exclusively in the virus-positive group.

Serum from each animal shown in Table 1 was tested for membrane-binding antibody. Animals in both the healthy and unhealthy group were found to have antibody that reacted with the membranes of homologous virus-infected fixed Raji cells. However, when sera from each group were tested in the neutralization test, only the healthy group contained positive activity. This result indicates that the healthy group, while exposed to the retrovirus, have developed an effective immune response against the agent.

Characterization of a new type D retrovirus. The following four separate isolates were chosen for characterization: (i) isolated directly from Celebes 10867, a fatal RF case; (ii) isolated from rhesus 21337, which had been inoculated with plasma from Celebes 10867; (iii) isolated from rhesus 21336 which had been inoculated with plasma from Celebes 3464, a fatal RF case; and (iv) isolated directly from Celebes 12703, a fatal RF case. All four isolates were the same by all criteria applied. Both rhesus monkeys (no. 21336 and 21337) developed persistent lymphadenopathy by 3 weeks postinoculation, but neither progressed to clinical SAIDS or RF as described for rhesus macaques (23). However, one animal (no. 21336) that was viremic throughout the course died at 15 months postinfection with anemia, streptococcal arthritis, and septicemia, but without evidence of typical opportunistic infections or extensive lymphoid depletion seen in terminal SAIDS (23). The other (no. 21337) was clinically normal at 18 months postinfection, not viremic, and had neutralizing antibody (described below). More definitive clinical studies are in progress, which are using a rhesus-derived SRV-2 isolate obtained from a cage at the Oregon primate center that is experiencing SAIDS and RF.

Thin sections of no. 10867 and 21337 virus-infected Raji cells showed large numbers of extracellular type D virions ranging in size from 90 to 170 nm by EM. Figure 1 shows a 170-nm extracellular particle (panel A) and a budding intracytoplasmic type A particle (panel B) typical of type D retroviruses. In several specimens, type D virions with an electron-dense core situated outside the cylindrical core were found (Fig. 1C, arrow). The significance of this morphology is unknown and has only been previously reported for the lentivirus genus of retroviruses and retroviruses associated with AIDS (22a).

The four viral isolates (virus from animals no. 10867 and 21336 are shown; Fig. 2) were next assayed for antigens related to MPMV, the prototype exogenous type D retrovirus of the macaques. Viruses concentrated by ultracentrifugation showed complete competition in an RIA for the core antigen p27 of MPMV (Fig. 2A). The slopes of the competition curves for all virus isolates were similar, indicating a close p27 antigenic relationship to each other and to MPMV. In contrast, when the same viral pellets were tested in a homologous assay for MPMV p10 antigen, statistically significant differences ($P < 0.01$) (14) in the slopes of the competition curves were found (Fig. 2B). SRV derived from the Celebes monkeys did not completely compete for antibody to MPMV p10, whereas SRV-1 completely competed. Thus, the p10 RIA showed a significant difference between SRV-2 and both SRV-1 virus and MPMV. The reliability of the p10 assay was demonstrated when we were able to distinguish five SRV-1s from five SRV-2s in a p10 antigen RIA with coded samples (data not shown). The probability of attaining this perfect assignment by chance alone is extremely low ($P = 0.004$; Fisher Exact Test) (15).

The SRV-2 obtained from Celebes 10867 was also compared with SRV-1 and MPMV by restriction endonuclease digestion and Southern blot analysis (25) of integrated proviral DNA obtained from infected Raji cells (Fig. 3). Full-length DNA clones (8.2 kilobases) of SRV-1 (4) and MPMV (1) were used as molecular probes. Under the moderately stringent conditions used, the complete 8.2-kilobase genome of SRV-2 was detected (Fig. 3, lane A). However, the restriction enzyme cleavage pattern of SRV-2 was clearly different from either SRV-1 or MPMV. Three additional SRV-2 isolates, as described above, gave identical restriction patterns (data not shown). The extent of homology between SRV-2 and either SRV-1 or MPMV is reduced, since SRV-2 sequences were not detected under conditions of high stringency (data not shown). Figure 4 shows a preliminary restriction endonuclease map of SRV-2, obtained from Celebes 10867. Orientation of the genome, with the long terminal repeats at each end, was established by subgenomic fragment analysis and comparison to SRV-1 (4) and MPMV (1). The restriction sites shown for SRV-1 were verified from the nucleotide sequence of SRV-1 (P. Luciw, personal communication). Digestion with a total of nine enzymes shows many differences between SRV-1 and SRV-2. Conserved sites appear to be *Sph*I in the long terminal repeats, and there is one site each for *Hin*dIII and *Eco*RI. Sequence analysis of SRV-2 is in progress and will allow a more detailed comparison of these three distinct retroviruses.

SRV-1 and SRV-2 are distinct serotypes. SRV-1 and SRV-2 are closely related as shown by cross-reactions of the core antigen p27 and molecular probing (Fig. 2 and 3). To determine whether they are distinct serotypes, neutralization tests based on syncytial inhibition were done with sera of infected monkeys that showed both clinical and virological evidence of recovery (i.e., absence of clinical signs and viremia). Table 2 shows that serum from an SRV-1-recovered animal (no. 21315) had high neutralizing activity against SRV-1 but no such activity against SRV-2. With serum from the animal recovered from the type 2 infection, the opposite result was obtained. Serum from a rhesus monkey exposed to the noma case listed in Table 1 was also tested and found to neutralize type 2 virus but not type 1. Therefore, these two different isolates of SRV represent distinct serotypes and indicate that recovery from infection with one type may not confer immunity against the other type. Lastly, sera from all Celebes macaques were tested for neutralizing activity against type 1 and type 2 viruses. Only

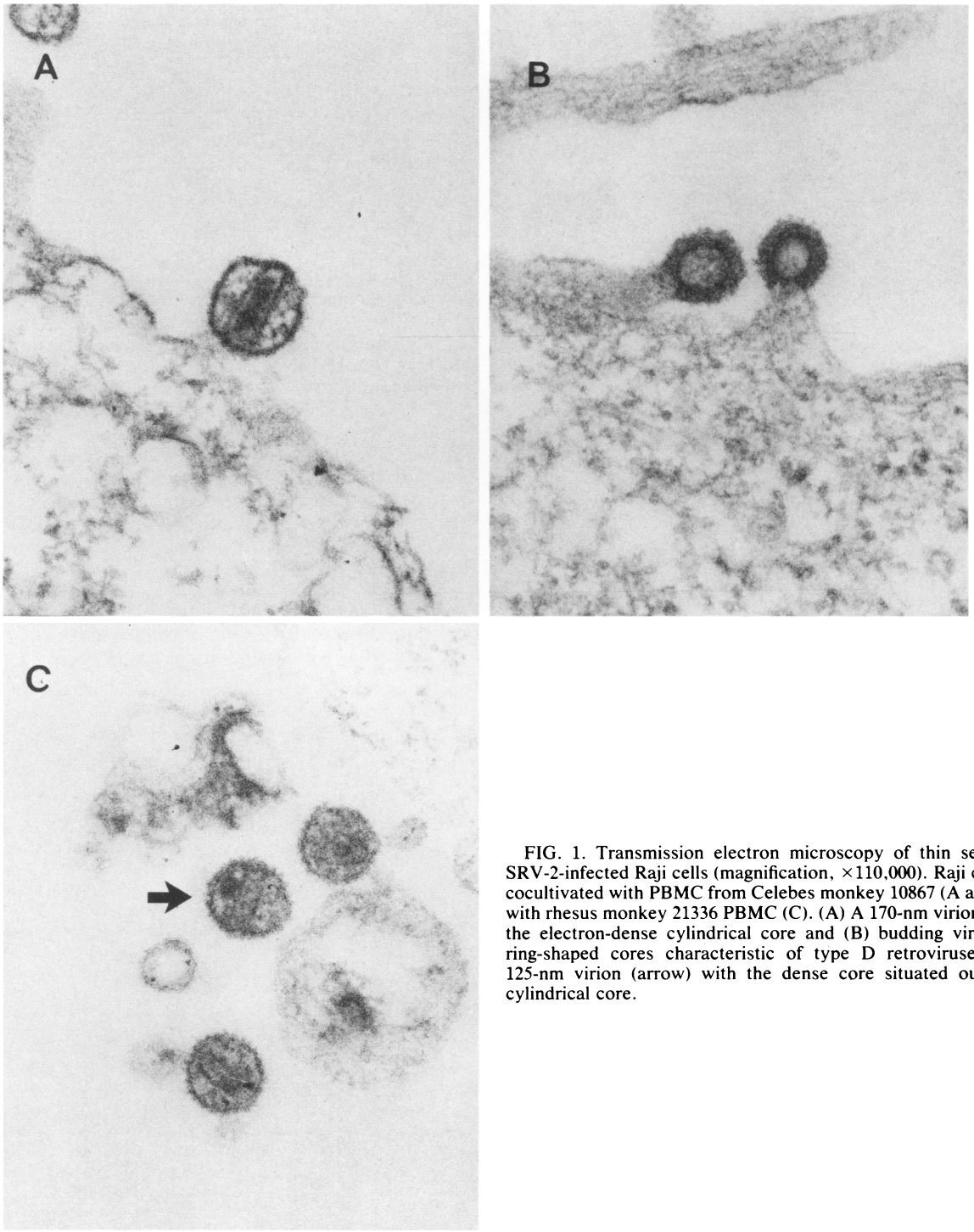

FIG. 1. Transmission electron microscopy of thin sections of SRV-2-infected Raji cells (magnification, ×110,000). Raji cells were cocultivated with PBMC from Celebes monkey 10867 (A and B) and with rhesus monkey 21336 PBMC (C). (A) A 170-nm virion showing the electron-dense cylindrical core and (B) budding virions with ring-shaped cores characteristic of type D retroviruses. (C) A 125-nm virion (arrow) with the dense core situated outside the cylindrical core.

activity against the type 2 virus was found (S. Shiigi, manuscript in preparation).

DISCUSSION

SRV-2 represents a new member of a family of horizontally acquired type D retroviruses of the genus *Macaca*. This is also the first report of an exogenous type D retrovirus in *M. nigra*, the Celebes black macaque.

In this study, viremia was present in every animal which had shown multiple clinical signs previously associated with SAIDS in macaques at other primate facilities (8, 10, 23, 26). In contrast, only one clinically healthy animal was viremic. Whether that animal is a clinically healthy carrier as occurs with SRV-1 (N. Lerche, manuscript in preparation) or is incubating the disease can only be determined with more time. RF occurs frequently in the infected Celebes monkeys at the Oregon primate center. Recently, we have found SRV-2-specific DNA in the RF lesion. Such sequences are not present in normal muscle tissue taken from the same animal (M. Bryant, manuscript in preparation). A similar

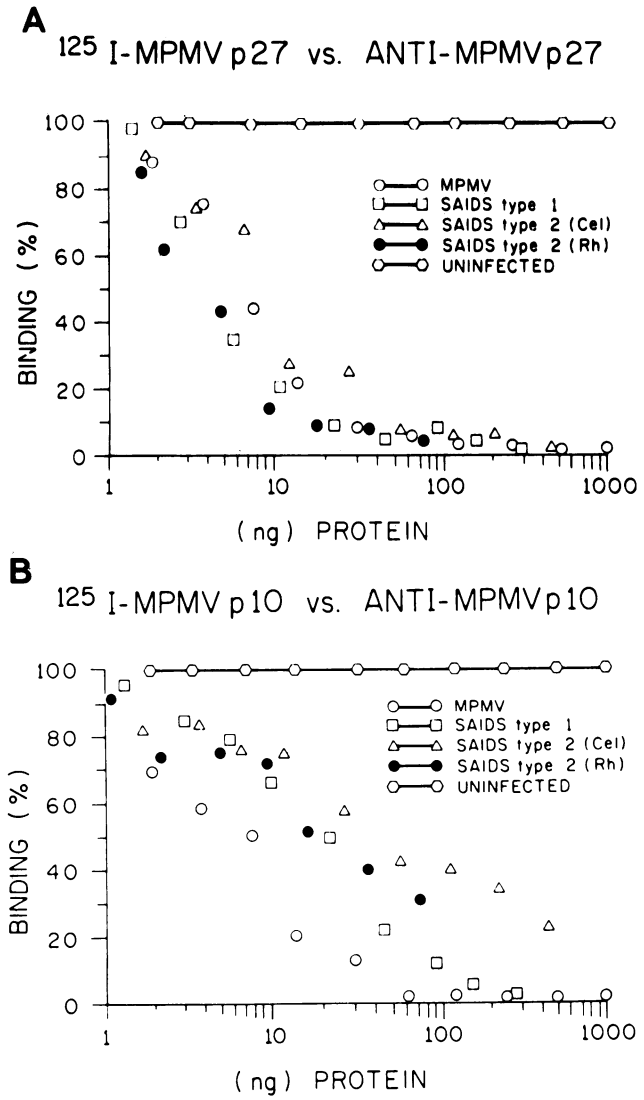

FIG. 2. Homologous RIA for MPMV p27 (A) and MPMV p10 (B). Pellets obtained by ultracentrifugation of cultures were disrupted by 1 h of incubation in 0.01 M Tris hydrochloride (pH 7.8)–0.1 M NaCl–0.001 M EDTA–0.1% Triton X-100–0.05% sodium desoxycholate and then tested in twofold serial dilutions for the ability to compete with ^{125}I-labeled MPMV proteins for binding limiting amounts of antisera. Antisera and unlabeled antigen were incubated for 1 h at 37°C, and then ^{125}I-labeled antigen was added (20,000 cpm). After incubation for 1 h at 37°C and overnight at 4°C, antigen-antibody complexes were precipitated by the addition of 20 μl of *Staphylococcus aureus* (10%) and centrifugation at 2,500 × *g* for 30 min. Radioactivity in the pellets was determined in a gamma counter. All of the results were normalized to 100% binding in the absence of competing antigens.

association of a type D retrovirus (SAIDS/D-Washington) infection and RF in *M. nemestrina*, the pigtailed macaque, was reported from the Washington Primate Research Center (26). Recently, in vitro-cultured SAIDS/D-Washington retrovirus has transmitted RF to a susceptible pigtailed macaque (R. Benveniste, K. Stromberg, L. O. Arthur, W. E. Giddens, Jr., H. D. Ochs, W. R. Morton, and J. A. Clagett, Lab. Invest. **52**:7A, 1985). However, proof for the etiologic role of SRV-2 in RF and SAIDS-like diseases will require induction of disease from molecular or biologically cloned virus. In contrast, another study (17) reported that inoculation of a macaque-derived type D retrovirus into macaques at the New England primate center did not induce a SAIDS-like syndrome; however, many differences exist between studies at the California center (9, 20) and the New England center. Specifically, these differences included age of animals used, routes of inoculation, species (*M. cyclopis*) source of the type D virus, and lastly, the species origin and cell type used for culture of the type D virus. In four animals, conditions more closely matched those of our studies, and of these four, two deaths resulted from the inoculations (17). Lastly, a new retrovirus, unrelated to the type D viruses, has been recently isolated from animals with acquired immunodeficiencies, lymphomas, and encephalopathies at the New England center (6, 13). Some of the differences in interpretation of clinical syndromes may be attributed to different viruses being present in the two primate colonies. In other studies (6a), a detailed restriction endonuclease map of D-398 virus, the SRV isolate at the New England Regional Primate Research Center, shows a close relationship to SRV-1 (compare restriction maps). However, several differences have been found (e.g., *Pst*I site in the long terminal repeat; Fig. 4). Direct comparisons are needed to determine the relationship of SAIDS-D/Washington, D-398 virus, SRV-1, and SRV-2. Arrangements for a collaborative study are in progress.

Serological assays show that at least two types of antibody are found in sera of infected macaques, neutralizing and nonneutralizing cell membrane-binding antibody. Neutralizing antibodies were found in the healthy animals that were

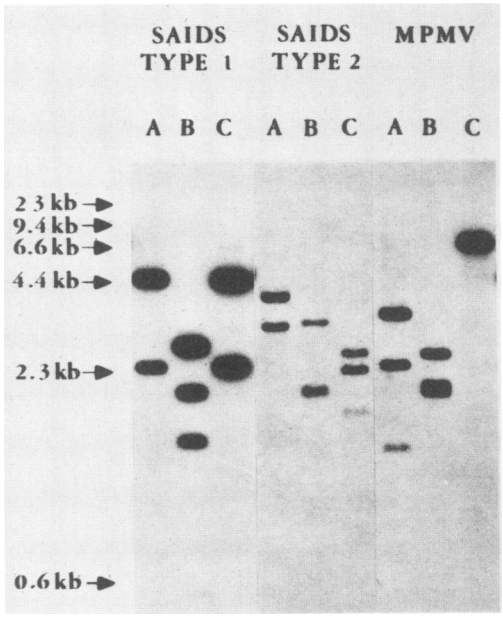

FIG. 3. Comparison of SRV and MPMV by restriction endonuclease cleavage pattern. High-molecular-weight DNA obtained from productively infected Raji cells (SRV-2C from Celebes macaque 10867) was digested with restriction endonucleases and analyzed by agarose gel electrophoresis and Southern blotting (25) with ^{32}P-labeled nick translated SRV-1 (shown here) and MPMV-cloned DNA probes. Shown are double digests with *Sph*I-*Bam*HI (lanes A), *Sph*I-*Hin*dIII (lanes B), and *Sph*I-*Eco*RI (lanes C). Uninfected Raji cell DNA does not hybridize under the conditions used here. Size markers are displayed in kilobases (kb).

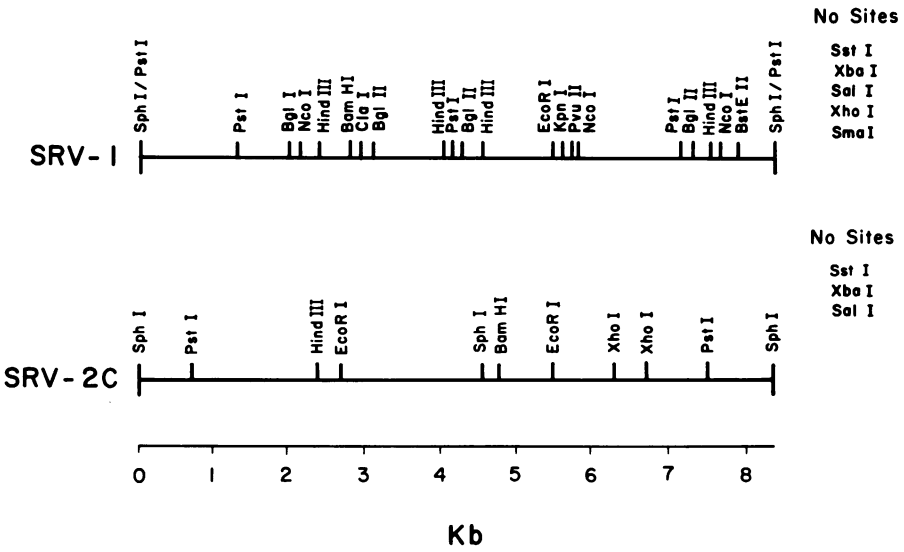

FIG. 4. Restriction map of SRV-2C compared with SRV-1. Unintegrated SRV-2 (8.2 kb) from Celebes 10867 (a fatal RF case) virus-infected Raji cells was characterized by restriction enzyme mapping. Orientation of the genome was established by subgenomic fragment analysis and comparison of SRV-1 and MPMV. The derivation of the SRV-1 detailed restriction map has been presented elsewhere (4).

clinically normal and appeared to have recovered from infection. These healthy macaques were exposed to the type 2 virus presumably when all were housed together in the outdoor runs. Animals that were viremic had specific antibody reactive with cell surface viral antigens but were not neutralizing. These results emphasize the importance of neutralizing antibodies as evidence of recovery from spontaneous retrovirus infections. The additional finding that the two SRVs are distinct serotypes has important implications for attempts to control these viruses by immunization. Studies are in progress to test whether or not rhesus monkeys immune to one virus have protection against the other.

Spontaneous diabetes mellitus associated with serum antibody against pancreatic islet cells also occurs in Oregon Celebes macaques (12). In addition, some of these Celebes monkeys also have shown antibody against human T-cell leukemia virus type 1 as determined by fixed-cell immunofluorescence (3) (J. Blakeslee, personal communication). A review of these data indicated the presence of such antibodies did not correlate with the presence of SRV-2 or RF in the Celebes monkeys reported here. Furthermore, SRV-2 was isolated from both diabetic and nondiabetic animals.

TABLE 2. Neutralization of SRV with sera from monkeys recovered from SAIDS

Animal no.[a]	Neutralization titer[b]	Challenge virus
21315	1:2,580	Type 1
21315	<1:20	Type 2
21337	<1:20	Type 1
21337	1:640	Type 2
19837	<1:20	Type 1
19837	<1:20	Type 2
10443	<1:20	Type 1
10443	1:1,500	Type 2

[a] All animals are clinically normal with no signs of SAIDS and have been naturally exposed to or inoculated with SRV. Animal 21315 has apparently recovered from inoculation with SRV-1. Animals 21337 and 10443 are a Celebes black macaque and a rhesus monkey, respectively, that have apparently recovered from SRV-2 infections. Animal 19837 is an unexposed, normal rhesus control.

[b] Neutralization titer is expressed as the highest dilution of serum to inhibit syncytia formation in Raji cells.

Three exogenous retroviruses are now associated with macaque immunosuppressive disorders, SRV-1 (9, 20), SRV-2, and simian T-cell leukemia virus type III (6, 13). The syndromes associated with each virus also differ. With SRV-1 at the California center, SAIDS occurs in the absence of RF (23), lymphomas (16), and encephalopathies (23). With SRV-2 at the Oregon center, a SAIDS-like disease occurs in association with RF but also in the absence of lymphomas and encephalopathies (W. McNulty, unpublished data). A similar disease is found at the Washington primate center (26). Lastly, simian T-cell leukemia virus type III occurs in association with macaque AIDS, lymphomas, and encephalopathies, but without RF. We speculate that three different retroviruses may be responsible for the three somewhat different syndromes at each primate center. Another exogenous retrovirus, simian T-cell leukemia virus type I, a human T-cell leukemia virus type I-like agent, has been linked serologically to the macaque lymphomas at the New England center (11). The exact role of all of these agents in these various disease syndromes will require much additional work. The spontaneous disease associated with SRV-2 serves as one of several animal models for understanding horizontally acquired retrovirus diseases in humans (2, 7). More studies are needed to unequivocally demonstrate the etiologic relationship and pathogenesis of SAIDS and RF associated with this new type D retrovirus strain.

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