gene is not rearranged in either small-cell lung carcinoma or renal cell carcinoma. However, most 3p deletions in small-cell lung carcinoma could result in a hemizygous state for the c-*raf*-1 gene. Whether this alteration reveals the presence of a deleterious recessive gene, as has been proposed for retinoblastoma (*20*), remains to be determined.

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Transmission of Simian Acquired Immunodeficiency Syndrome (SAIDS) with Blood or Filtered Plasma

Abstract. *Simian acquired immunodeficiency syndrome (SAIDS), a disease clinically and pathologically similar to acquired immunodeficiency syndrome in humans, was transmitted from diseased rhesus monkeys* (Macaca mulatta) *to normal monkeys by inoculation with heparinized whole blood or plasma that had been passed through filters of 0.45 micrometer pore size. This suggests that the causative agent is small and most probably a virus. No viruses, however, were isolated by standard cell culture techniques from the blood or filtered plasma which caused SAIDS. Both cellular and humoral immunity were markedly depressed in animals with advanced SAIDS.*

Many different infectious agents have been isolated from patients with acquired immunodeficiency syndrome (AIDS), but none of these has been clearly implicated as the cause of this disease. The difficulty of identifying the cause of AIDS has been compounded by the lack of a susceptible experimental animal. A spontaneous outbreak of a disease clinically and pathologically similar to AIDS in humans was recently described in rhesus monkeys (*Macaca mulatta*) housed in an outdoor corral at the California Primate Research Center of the University of California, Davis (CPRC) (*1*). Affected animals had symptoms similar to those of AIDS victims including profound immunosuppression, lymphadenopathy, splenomegaly, multiple opportunistic infections, persistent diarrhea, chronic wasting, and high mortality. Some animals also had cutaneous fibrosarcomas. A similar immunosuppressive disease was reported to occur in macaque monkeys housed at the New England Primate Research Center in Southborough, Massachusetts (*2*). An understanding of SAIDS is important so that methods can be developed to protect nonhuman primates from this devastating disease. Such studies may also provide clues to the etiology and pathogenesis of AIDS in humans and serve as a useful model for investigation of prophylaxis and therapy.

We recently reported on the experimental transmission of SAIDS from two animals at the CPRC to four rhesus monkeys at the National Institutes of Health (NIH) that were negative for cytomegalovirus (CMV) antibody (*3*). Inocula for these studies were mixtures of unfiltered supernatant fluids from 10 percent homogenates of various organs with or without buffy coat cells from blood. In this report we narrow our focus on the cause of SAIDS by describing transmission of the syndrome to rhesus monkeys using whole blood or filtered plasma from diseased animals.

These studies were carried out at two geographically separated sites, NIH and CPRC, with inocula from different donor animals. The experiments were performed independently but the data were shared.

Four juvenile rhesus monkeys were each inoculated intravenously with 0.9 ml of heparinized whole blood from either of two moribund donor animals with experimentally transmitted SAIDS (Table 1). The clinical history and pathology of the donor animals were described previously (*3*). Monkeys 1 and 2, inoculated at NIH, were 8.5 and 8 months of age, respectively, and monkeys 3 and 4, inoculated at CPRC, were both 11 months of age. All four inoculated animals developed SAIDS; three of them became moribund and died 2 and 3 months after inoculation. Animal 1 remains alive with persistent generalized lymphadenopathy and splenomegaly 5 months after inoculation (Table 1).

In an attempt to characterize the SAIDS agent, plasma from two donor animals with advanced disease was filtered, sequentially, through two 0.45-μm Millipore filters to minimize the chance of filter failure. At CPRC, the integrity of the filters was further verified by retention of a mixture of *Staphylococcus aureus* and *Escherichia coli* by the same filter used to filter the SAIDS plasma. Confluent growth of the bacteria occurred on agar medium prior to filtration of the mixture, but no growth by either organism was seen after filtration.

Four juvenile rhesus monkeys were each inoculated with 3 ml of the filtered plasma from two animals with SAIDS (Table 1). Monkeys 5 and 6, inoculated at NIH, were negative for antibody to rhesus monkey CMV and were 11 and 8 months of age, respectively. At CPRC, animals 7 and 8, both 14 months of age, had antibody to rhesus monkey CMV. Two to four weeks after inoculation with

filtered plasma, all four recipient animals developed SAIDS (Table 1). The disease progressed rapidly in three of the animals, leading to a moribund condition and death 5 to 9 weeks after inoculation. The fourth recipient (monkey 8) remains alive with persistent generalized lymphadenopathy and splenomegaly 3 months after inoculation.

The postmortem findings in the six animals that died with SAIDS were similar (Table 1). The findings paralleled those seen in spontaneous (*1*) and experimental SAIDS (*3*). A characteristic feature was lymphoid depletion of the lymph node cortices, splenic white pulp, and thymic cortex. This was moderate to severe in four of the six cases as evidenced by loss of lymphocytes in both follicular (B cell) and paracortical and periarterial (T cell) zones of the lymph nodes and spleen, respectively. Immunoblasts were sparse and plasma cells virtually absent. In one animal (monkey 6) there was active follicular hyperplasia. The nodes showed sinus histiocytosis and erythrophagocytosis. In four animals examined, the bone marrows were abnormally hypercellular and showed an increase in erythroid and granulocytic precursors, occasional lymphoid nodules, and histiocytic proliferation. Other findings of note were enterocolitis (giardiasis, trichomoniasis, cryptosporidiosis) in five animals, cellulitis and focal suppurative lymphadenitis in two animals, and proliferative glomerulonephritis or interstitial nephritis in single animals. Cytomegalic cells with herpes-like intranuclear inclusions were seen disseminated throughout organs of monkey 5. In monkeys with spontaneous SAIDS, the virus associated with similar inclusion-bearing cells has been identified as monkey CMV by hybridization in situ (*4*).

Studies of the mitogenic responses of lymphocytes from animals in which SAIDS was induced by inoculation with whole blood (monkey 3) or filtered plasma (monkeys 7 and 8) were compared with those of uninoculated healthy controls matched for age and sex. Lymphocytes from SAIDS animals and controls were stimulated with phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) by the standard procedures (*3*). Results of these studies showed that in the early stages of SAIDS, lymphocyte responses to these mitogens were not impaired. However, in animals with advanced disease, lymphocyte stimulation indices were significantly lower than those of controls, with the exception of the PWM response in monkey 3 (Table 2). Concentrations of immunoglobulins M, G, and A (IgM, IgG, and IgA), determined by radial immunodiffusion, were also very low in these animals (Table 2). Regardless of the time after inoculation and severity of disease, there was no evidence in ani-

Table 1. Transmission of SAIDS with blood or filtered plasma.

Animal number and sex	Inoculum and donor numbers	Clinical and laboratory findings	Pathological findings
1 Female (B-923)*	Blood B-784	Lymphadenopathy, splenomegaly, transient lymphopenia	Alive 5 months after inoculation
2 Female (B-925)*	Blood B-784	Lymphadenopathy, splenomegaly, neutropenia, anemia, lymphopenia, weight loss, hypoproteinemia, diarrhea, dehydration, edema of the perineum and lower limbs	Died 10 weeks after inoculation. Moderate lymphoid depletion, glomerulonephritis, cellulitis, suppurative lymphadenitis, bone marrow hyperplasia
3 Female (20141)*	Blood B-883	Lymphadenopathy, splenomegaly, neutropenia, anemia, lymphopenia, weight loss, hypoproteinemia, thrombocytopenia	Died 9 weeks after inoculation. Moderate lymphoid depletion, enterocolitis, hypercellular bone marrow
4 Female (20335)*	Blood B-883	Same as monkey 3, plus diarrhea, but no thrombocytopenia	Died 11 weeks after inoculation. Moderate lymphoid depletion, enterocolitis, interstitial nephritis, bone marrow hyperplasia
5 Female (B-911)*	Filtered plasma B-784	Same as monkey 3, plus diarrhea and polymyostitis, but no thrombocytopenia	Died 5 weeks after inoculation. Moderate lymphoid depletion, disseminated inclusion-bearing cells, enterocolitis (giardiasis and trichomoniasis), lymph node abcess
6 Female (B-920)*	Filtered plasma B-784	Same as monkey 5, but no hypoproteinemia	Died 9 weeks after inoculation. Lymphoid follicular hyperplasia, paracortical depletion, sinus histiocytosis, entercolitis (giardiasis and trichomoniasis)
7 Male (20383)*	Filtered plasma 20265	Same as monkey 3	Died 5 weeks after inoculation. Severe lymphoid depletion, enterocolitis, bone marrow hyperplasia
8 Male (20325)*	Filtered plasma 20265	Lymphadenopathy, splenomegaly, neutropenia	Alive 3 months after inoculation

*Number by which animal was identified in colony.

Table 2. Immunological data from rhesus monkeys with SAIDS. Immunoglobulin concentration (in milligrams per deciliter) from ten normal rhesus monkeys were as follows: IgM, 100 to 310; mean (standard deviation) 226 (68), median, 250; IgG, 560 to 1600; mean (S.D.) 976 (263), median, 950; IgA, 57 to 340; mean (S.D.) 234 (107), median, 240.

Animal number	Disease stage	When tested	Mitogen stimulation index			Helper/suppressor ratio (OKT_4/OKT_8)	Immunoglobulin concentrations (mg/dl)		
			Con A	PHA	PWM		IgM	IgG	IgA
3 (20141)*	Late SAIDS	At death	9.6	9.3	45.1	2.7	< 35	< 283	< 57
7 (20383)*	Late SAIDS	At death	37.0	9.8	2.7	2.7	58	540	125
8 (20325)*	Early SAIDS	At 5 weeks	181.7	93.1	28.4	2.3	58	510	170
9 (19071)*	No SAIDS	Normal control	105.0	58.5	20.4	2.2			
10 (18995)*	No SAIDS	Normal control	262.7	119.2	25.5	3.1			

*Number by which animal was identified in colony.

mals with SAIDS of inversion of helper to suppressor T cell ratios determined with T_4 and T_8 monoclonal antibodies (Table 2). In this regard, the monkeys with SAIDS differed from humans with AIDS.

Numerous attempts were made to isolate viruses from the whole blood or filtered plasma that transmitted SAIDS. A variety of cell cultures were used including the continuous monkey kidney cell lines Vero and MA-104; low-passage Flow 7000 and W138 human fibroblasts, low-passage rhesus monkey kidney and lung fibroblast cells; and low-passage lung fibroblasts from the monkey *Erythrocebus patas*. No viral isolations were made from the blood or filtered plasma that caused SAIDS or from similar samples from recipient monkeys that developed SAIDS.

We previously reported that SAIDS could be experimentally transmitted to rhesus monkeys by inoculation with supernatant fluids from 10 percent homogenates (clarified by centrifugation at low speed) of organs from donor animals with SAIDS (*3*). Rhesus monkey CMV was isolated from the SAIDS-l inoculum used in those studies and from the urine of all four inoculated animals. However, SAIDS did not develop in rhesus monkeys intravenously inoculated with a high-passage laboratory strain of rhesus monkey CMV (283T) or with a recent isolate from a normal healthy animal, passed only three times in vitro (*3*). To determine whether a unique strain of CMV might be the cause of SAIDS, we inoculated two rhesus monkeys with an isolate of rhesus monkey CMV made from the SAIDS-l inoculum and passed four times in vitro in Flow 7000 human fibroblasts (*3*). These animals also did not develop SAIDS. In addition, rhesus monkeys with preexisting high titers of antibody to CMV have become infected naturally and experimentally with SAIDS. Rhesus monkey CMV was not isolated from the filtered plasma or whole blood used to transmit SAIDS, nor has it been isolated from CMV antibody negative animals receiving these inocula. These data suggest that rhesus monkey CMV is not the etiologic agent of SAIDS.

Since human T-cell leukemia virus (HTLV) has been associated with human AIDS (*5*), evidence was sought for the presence of a similar agent in SAIDS. Antibody to the p24 polypeptide of HTLV was not found by radioimmunoassay in infectious plasma from monkeys with SAIDS or in healthy rhesus monkeys housed with diseased monkeys and thus at risk of acquiring the immunodeficiency syndrome (*6*). Also, a significant reverse transcriptase activity was not detected in infectious plasma (*6*). However, these results only rule out a marked retroviremia and further studies with in vitro culture techniques are required (*6*). Type C retrovirus particles were not seen by electron microscopy in thin sections of lymph nodes or bone marrow or in cultured T cells of animals with SAIDS (*7*).

Although simian adenoviruses were not isolated from the filtered plasma or whole blood used to produce SAIDS, they have been isolated from many animals with SAIDS. Adenoviruses were isolated from all four experimentally inoculated monkeys (B-784, B-649, B-883, and B-884) from our previous study (*3*). The isolate from B-784 was typed as adenovirus type 11 (*8*). Filtered plasma from B-784 was the inoculum for monkeys 5 and 6 described in this report. An adenovirus was also isolated from the urine, feces, and kidney of monkeys 5 and 6 and also from the mesenteric node of monkey 6. Typing of these isolates has not been completed. Simian adenovirus type 23 was isolated from the feces of two rhesus monkeys that received urine from monkey B-784 (*7*). Adenovirus type 11 was also isolated from a healthy uninoculated normal control animal randomly selected from the CPRC colony, suggesting that these adenovirus isolates are opportunistic agents not etiologically linked to SAIDS.

The present studies demonstrate the experimental transmission of SAIDS with whole blood or filtered plasma. All recipients (eight of eight rhesus monkeys) developed signs of SAIDS within 2 to 4 weeks after inoculation, and (six of the eight recipients became moribund and died between 5 and 11 weeks after inoculation. We have also succeeded in transmitting SAIDS to two rhesus monkeys inoculated with pooled serum from diseased animals (data not shown). The transmission of SAIDS with infectious plasma that was passed through a 0.45-μm pore size filter provides evidence that the causative agent is small and probably a virus. These results are consistent with a recent report on transmission of a similar disease to macaque monkeys with cell-free material. However, in that study, a filtrate of lymphoma tissue was used as the inoculum (*2*). Efforts must now be focused on identifying and characterizing the etiologic agent of SAIDS. Such studies will contribute to the understanding and control of both SAIDS and human AIDS.

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Analyzing Nonlinear Scatchard Plots

The report by Paul *et. al.* (*1*) is a significant contribution to the field of receptor pharmacology. The authors described saturable and specific binding of (+)-[^{3}H]amphetamine in rat brain. However, the resolution of their Scatchard plot (figure 1A) into two apparent binding sites was done incorrectly, and consequently the kinetic constants determined are inaccurate.

The advent of radioligand receptor binding techniques and the rapid increase in the application and sophistication of receptor studies has led to a propensity for inappropriate interpretation of Scatchard-type data. Norby *et.*