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* Address correspondence to G.E.M. at the Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, Md. 20205.

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Characterization of Exogenous Type D Retrovirus from a Fibroma of a Macaque with Simian AIDS and Fibromatosis

Abstract. *A novel type D retrovirus was isolated by cocultivation of explants of fibromatous tissue from a rhesus monkey (*Macaca mulatta*) with immunodeficiency and retroperitoneal fibromatosis. This type D virus, isolated from a macaque with simian acquired immunodeficiency syndrome (SAIDS-D/Washington), is exogenous and is partially related to the Mason-Pfizer and the langur monkey type D viruses. The SAIDS-D virus can be distinguished from all other primate retroviruses by antigenicity and molecular hybridization. Nucleic acid hybridization studies reveal that the origin of the SAIDS-D isolate may reside in Old World monkey (subfamily Colobinae) cellular DNA.*

Human acquired immunodeficiency syndrome (AIDS) is characterized pathologically by lymphoid depletion, depressed cellular and humoral immune functions, opportunistic infections, and unusual neoplasms, particularly Kaposi's sarcoma and, less frequently, lymphomas (*1*). An acquired immunodeficiency syndrome similar in certain respects to human AIDS has been observed in macaques at the New England (*2*) and California (*3*) Regional Primate Research Centers (RPRC). The simian AIDS (SAIDS) at the New England RPRC occurs primarily in *Macaca cyclopis* in which some of the affected animals died with lymphoproliferative lesions (*2*). SAIDS in the California facility is epidemic in *M. mulatta* and two cases of cutaneous fibrosarcomas have been reported in rhesus monkeys (*4*). At the RPRC at the University of Washington, various macaques, primarily *M. nemestrina* but also *M. mulatta, M. fuscata*, and *M. fascicularis*, show an immunodeficiency syndrome characterized by persistent diarrhea, progressive weight loss, anemia, lymphocytopenia, unusual chronic infections (noma and cryptosporidiosis), and a peculiar fibromatous tumor termed retroperitoneal fibromatosis (RF) (*5, 6*). Histologically, there is marked thymic atrophy, follicular and paracortical atrophy of lymph nodes, and variable myeloid and lymphoid hyperplasia in bone marrow. Neither RF nor immunodeficiency has been observed among colony-born or feral baboons housed at the Washington RPRC. Retroperitoneal fibromatosis, which is characterized by an aggressive proliferation of highly vascular fibrous tissue, often remains localized to the peritoneum. However, in over one-fourth of the cases it progresses to involve the entire abdominal cavity, inguinal canal, and thoracic cavity. A cutaneous form has been recognized in a small number of RF-affected animals and resembles the cutaneous fibrosarcomas seen in the California colony (*3*). Immunohistochemical studies have shown factor VIII–related antigen in endothelial and scattered fibroblast-like cells throughout the RF lesions (*6*), similar to that described for Kaposi's sarcoma (*7*). Thus, in its progressive form, SAIDS at the RPRC in Washington includes the triad of lymphoid depletion, opportunistic infections, and an unusual neoplasm (RF).

We have investigated the immune status of monkeys with RF. In an experiment involving four *M. nemestrina* monkeys with biopsy-confirmed lesions of RF and three age- and sex-matched controls, peripheral blood mononuclear cells were cultured in the presence of optimal concentrations of phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM) (*8*). Results for the RF-affected animals were expressed as the percentage of the average net [^{3}H]thymidine incorporation by lymphoid cells of the three controls. The results showed that responses of lymphoid cells of RF animals were 2 to 12 percent of the control value for PHA, 1 to 13 percent of the control value for Con A, and 5 to 66 percent of the control value for PWM. Antibody responses to the T-cell–dependent antigen bacteriophage ϕX174 (*9*) were measured in two rhesus monkeys with biopsy-confirmed RF and two age- and sex-matched controls. Control monkeys showed a typical primary immunoglobulin M (IgM) response and a brisk and amplified secondary response consisting mainly of immunoglobulin G (IgG) antibody. In contrast, RF-affected animals had a markedly depressed primary response (both animals less than 1 percent of controls) and failed to amplify and switch from IgM to IgG during the secondary response. Similarly depressed immune responses have been observed in homosexual men with lymphadenopathy and reversed ratios of helper to suppressor lymphocytes (*10*).

To examine the etiology of SAIDS at the Washington RPRC, RF tissue from an immunodeficient rhesus monkey was cocultivated with heterologous mammalian cells known to support the replication of a wide variety of primate viruses (*11*). After only 2 weeks, a Mg^{2+}-dependent reverse transcriptase activity was detected in the conditioned medium from a dog thymus cell line (FCf2Th). Electron microscopic examination of the virus (Fig. 1) revealed typical (*12*) type D retroviral particles, indistinguishable from Mason-Pfizer monkey virus (MPMV), in which intracytoplasmic type A particles were common (Fig. 1A), and budding occurred by envelopment of preformed A particles (Fig. 1B). Budding of virions with incomplete nucleoids was also observed (Fig. 1C) and, characteris-

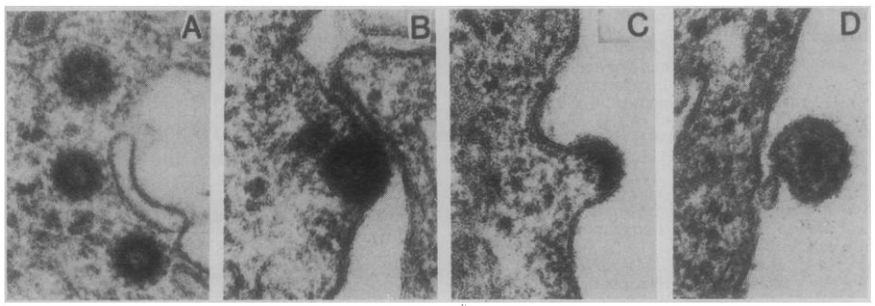

Fig. 1. Morphology of the SAIDS-D viral isolate grown in canine FCf2Th cells. (A) Intracytoplasmic type A particles; (B) budding by envelopment of preformed A particles; (C) budding virions with incomplete nucleoids; and (D) cone or barrel-shaped nucleoids of the "mature" virions. The specimens were prepared as previously described (*12*). (×105,000)

tic of MPMV, nucleoids of the mature virions were cone shaped (Fig. 1D).

This isolate, designated SAIDS-D/Washington, can be grown to high titers in mammalian cell lines of human, canine, rhesus, bat, and mink origin (*13*). These cells that support SAIDS-D virus growth remained fibroblastic and no cytopathic effect or morphological evidence of transformation was observed. However, after infection of several cell lines chosen for their flat morphology and ability to be transformed, foci of proliferating or otherwise abnormal cells were observed in mouse NIH 3T3 cells (*13*).

The antigenic relatedness of SAIDS-D virus to other type D viruses was determined in specific radioimmunoassays (RIA) for the major core protein (p27) and the major envelope glycoprotein (gp70) purified from MPMV. Other type D viruses used as competitors were the endogenous virus of langurs (PO-1-Lu) and the endogenous virus of squirrel monkeys (SMRV). As shown in Fig. 2A, lysed virus pellets of SAIDS-D, MPMV, and PO-1-Lu effectively competed in the MPMV p27 assay. The extent of competition and similarity of the slopes of the competition curves indicate close immunological relatedness of the core proteins of these three viruses. SMRV type D virus, as well as type C retroviruses, did not compete in the MPMV p27 assay. Figure 2B shows that the envelope protein of SAIDS-D appears immunologically distinct from MPMV since, like PO-1-Lu and SMRV, it did not compete in the MPMV gp70 assay. SAIDS-D virus was also found to be negative in a broadly specific RIA for type C virus core antigens as well as in an RIA for human T-cell leukemia virus core antigen (*14*).

To further characterize the SAIDS-D virus from *M. mulatta*, we compared the nucleic acid sequence homology and thermal stability between this isolate and other known primate retroviruses. A retrovirus previously described from the spleen of a *M. nemestrina* with RF (*15*) was not related to MPMV and consisted of a mixture of type C and type D viruses. This isolate was lost during cultivation (*16*) and is thus not available to compare with SAIDS-D virus. Table 1 shows the results obtained after hybridization of a radioactively labeled DNA transcript of SAIDS-D virus to the cellular DNA extracted from tissues of various primate species or from virus-infected cells. The DNA hybrids were digested with S_1 nuclease at low stringency (*17*). Liquid hybridization under these conditions reveals more initial information about overall quantitative nucleic acid homologies than can be obtained by comparison of limited restriction endonuclease digests. The SAIDS-D isolate is distinct from all other primate retroviruses, but is partially related (36 to 38 percent) to MPMV and the endogenous langur virus. No homology was detected to the DNA of the New World type D isolate from squirrel monkeys. Previous hybridization experiments have shown that MPMV and langur endogenous virus are partially related (22 to 33 percent homology) (*18*). In reciprocal experiments not shown here, ^{3}H-labeled DNA transcripts prepared from MPMV and langur virus each hybridize approximately 30 percent to the cellular DNA of SAIDS-D–infected cells.

The thermal stability of nucleic acid hybrids can also be used as an index of

Table 1. Nucleic acid homology and thermal stability between SAIDS-D/Washington viral DNA and the cellular DNA from various species. A ^{3}H-labeled DNA transcript of the SAIDS-D virus grown in the canine cell line FCf2Th was synthesized from detergent-disrupted virus and purified over alkaline sucrose gradients (*18*). ^{3}H-Labeled DNA viral probes consisting of > 13*S* DNA were hybridized to the various cellular DNA's. The percentage hybridization is the saturating normalized value obtained after digestion of the hybrids with nuclease S_1 (*17*). The actual final extent of hybridization to DNA extracted from the SAIDS-D virus–infected cell line varied from 80 to 94 percent; the remainder of the probe hybridized to dog cellular DNA. All hybridizations were carried out to a C_0t value of 3×10^4; the standard deviation of the values varied from ±2.6 to ±4.1 percent, based on five separate hybridization experiments with four cellular DNA samples and viral DNA probes. The T_m is the temperature at which 50 percent of the DNA hybrids dissociated. The various primate viral isolates were grown in canine (FCf2Th), human (A549), and bat (Tb1Lu) cell lines. C_0t is defined as moles of nucleotide per liter times seconds.

Species	Virus	Source of cellular DNA	Hybridization (%)	T_m (°C)
	Cell lines			
Rhesus monkey (*M. mulatta*)	SAIDS-D	Canine	100	93.4
Rhesus monkey (*M. mulatta*)	MPMV	Human	38	81.5
Langur (*P. obscura*)	PO-1-Lu	Bat	36	82.0
Squirrel monkey (*S. sciureus*)	SMRV	Canine	2	
Stump-tailed macaque (*M. arctoides*)	M109	Human	7	
Baboon (*P. papio*)	M7	Canine	8	
Owl monkey (*A. trivirgatus*)	OMC-1	Bat	1	
Gibbon (*H. lar*)	M144	Human	6	
Woolly monkey (*Lagothrix* spp.)	SSV	Human	6	
	Tissue			
Pig-tailed macaque* (*M. nemestrina*)		RF	92	91.6
Pig-tailed macaque* (*M. nemestrina*)		Spleen	48	
Pig-tailed macaque (*M. nemestrina*)		Liver	35	
Rhesus monkey (*M. mulatta*)		Liver	34	
Lion-tailed macaque (*M. silenus*)		Spleen	33	
African green (*C. sabaeus*)		Spleen	38	
Langur (*P. obscura*)		Testes	51	
Colobus (*C. guereza*)		Liver	42	
Gibbon (*H. lar*)		Spleen	2	
Human (*H. sapiens*)		Spleen	1	
Howler monkey (*Alouatta* spp.)		Spleen	3	
Mouse (*Mus caroli*)		Liver	2	
Dog (*C. familiaris*)		Liver	6	
Cow (*B. taurus*)		Thymus	1	

*Spleen and retroperitoneal fibroma cellular DNA were obtained from a pig-tailed macaque (animal T82050) with RF and its associated immunodeficiency.

the degree of base-pair mismatching between DNA strands. As shown in Table 1, the hybrids formed between SAIDS-D viral DNA transcripts and the cellular DNA of MPMV- or langur virus-infected cells dissociate at approximately 12°C lower than the homologous hybrid. Although there may be regions of the SAIDS-D viral genome that are not well represented in our DNA transcripts, the low degree of nucleic acid sequence homology between SAIDS-D virus and the other Old World monkey type D viruses is probably due to an accumulation of base-pair mutations rather than to a recombinational event involving a major portion of the Mason-Pfizer genome.

The cellular DNA of several primate species was tested for nucleic acid sequence homology to the SAIDS-D virus. Table 1 shows that the highest degree of homology was obtained with DNA from the Colobinae subfamily of Old World monkeys, in particular langur DNA (51 percent). The cellular DNA from all Old World monkeys belonging to the subfamily Cercopithecinae (including several macaque species) hybridize 33 to 38 percent to the viral probe. A pig-tailed macaque with RF revealed the presence of SAIDS-D viral sequences in the tumor tissue as shown by the high final extent of hybridization obtained. Spleen cellular DNA from the same animal hybridized 48 percent to the viral probe. This level of hybridization is greater than that observed in normal pig-tailed macaque DNA, and may reflect a low level of SAIDS-D viral sequences present in the spleen of this particular monkey. Thus, SAIDS-D virus from *M. mulatta* is present in the fibromatous tissue and spleen of a second macaque species, *M. nemestrina*, from the same primate colony. Moreover, we have recently obtained multiple viral isolates from different tissues of diseased animals of this species (*13*).

The Old World monkeys of Africa and Eurasia are divided into two distinct subfamilies. The Cercopithecinae include baboon, macaque, African green, patas, and related species belonging to eight genera, while the Colobinae include *Colobus* from Africa and five genera of Southeast Asian monkeys including *Presbytis* (langurs). Previous studies showed that DNA probes prepared from the langur type D virus hybridize to other primate DNA's with the final extent of hybridization and the thermal stability of the hybrids formed correlating with the phylogenetic distance of the primates from langur (*18*). A portion of the MPMV genome can also be detected in the cellular DNA's of all Old World monkeys, particularly in those of the Colobinae subfamily (*18, 19*). The high degree of hybridization of SAIDS-D/Washington viral DNA to langur DNA suggests that this virus, like MPMV, may also have arisen from an endogenous langur virus. The sequence divergence of MPMV and SAIDS-D viruses may reflect the rapid evolution of infectious viruses relative to the genetically transmitted endogenous viral gene sequences.

A type D retrovirus related to MPMV has been isolated from *M. cyclopis* at the New England RPRC (*20*), and another type D virus has been isolated from the blood of rhesus monkeys with SAIDS at the California RPRC. This latter group has also induced SAIDS using tissue culture fluids containing type D retrovirus (*21*). The differences between these two isolates and the one described here from the Washington RPRC remain to be determined. It is possible that this class of type D retroviruses is infectious in macaques and produces the disease spectrum that is defined as simian AIDS.

The gibbon class of type C viruses, which is tumorigenic in primates (*22*), is believed to have been derived by transspecies infection of these apes with endogenous viruses from various species of Southeast Asian rodents, such as *Mus caroli* or *Mus cervicolor* (*23*). It thus appears that MPMV, SAIDS-D viruses, and the gibbon class of infectious primate viruses may have been acquired by infections from the endogenous viruses of other mammalian species that cohabit the same geographic area. Southeast Asia, where both macaques and langurs reside, may thus be a reservoir for the SAIDS-D viruses.

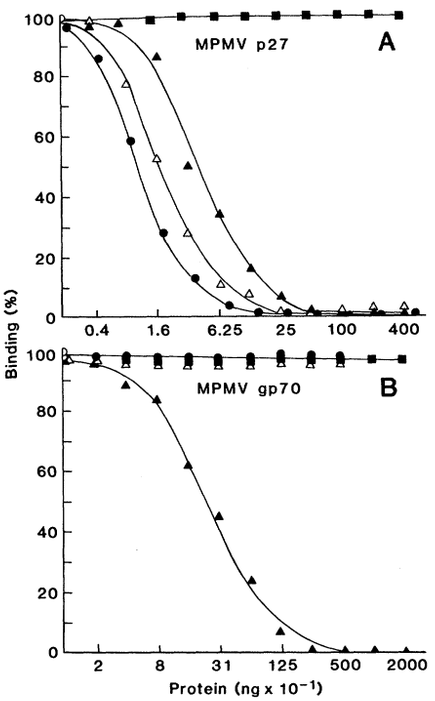

Fig. 2. Competitive RIA for the MPMV major core protein (p27) and envelope glycoprotein (gp70). Detergent-disrupted viruses were tested for their ability to compete with the binding of ^{125}I-labeled viral proteins to limiting amounts of antisera (*24*). All results are normalized to 100 percent binding in the absence of competing antigens. The reagents used were (A) antibody to MPMV p27: ^{125}I-labeled MPMV p27; (B) antibody to MPMV gp70: ^{125}I-labeled MPMV gp70. Viruses include MPMV (▲), SAIDS-D virus (●), PO-1-Lu (△), and SMRV (■). Other retroviruses that were negative in both assays (data not shown) were mouse mammary tumor virus, Rauscher murine leukemia virus, an endogenous type C virus of stump-tailed macaques, and human T-cell leukemia virus.

KURT STROMBERG*
RAOUL E. BENVENISTE
Laboratory of Viral Carcinogenesis, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701
LARRY O. ARTHUR
Biological Products Laboratory, Program Resources, Inc., NCI-Frederick Cancer Research Facility
HARVEY RABIN
LBI-Basic Research Program, NCI-Frederick Cancer Research Facility
W. ELLIS GIDDENS, JR.
Division of Animal Medicine and *Department of Pathology, School of Medicine, University of Washington, Seattle 98195*
HANS D. OCHS
Department of Pediatrics, Division of Immunology and Rheumatology, School of Medicine, University of Washington
WILLIAM R. MORTON
CHE-CHUNG TSAI
Regional Primate Research Center, University of Washington

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* To whom requests for reprints should be sent.

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Spatially Nonuniform Changes in Intracellular Calcium Ion Concentrations

Abstract. *The spatial variation of changes in intracellular calcium ions were studied with a one-dimensional scanning microphotometer. Changes in intracellular calcium were measured with a metallochromic dye, arsenazo III. Both the magnitude and the kinetics of changes in calcium were dramatically different in different regions of a cell. In* Limulus *ventral photoreceptors the maximum change was probably restricted to the rhabdomeric lobe.*

Changes in the concentration of calcium ions in the cytosol $[Ca^{2+}]_i$ are important for the control of physiological and metabolic processes in a wide variety of cells. Changes in $[Ca^{2+}]_i$ can arise from calcium ions that enter through the plasma membrane or that are released from intracellular stores. Any change in $[Ca^{2+}]_i$ might be restricted spatially by diffusion barriers, sequestration mechanisms, or active removal. Such restrictions to the spread of changes in $[Ca^{2+}]_i$ have been indicated by the apparent nonuniformity of Ca^{2+} buffering capacity in the soma of a molluscan neuron (*1*) and of salivary gland cells in larval insects (*2*) and by the radial nonuniformity of changes in $[Ca^{2+}]_i$ demonstrated in squid giant axons (*3*).

We examined the spatial nonuniformity of changes in $[Ca^{2+}]_i$ in single cells. We studied *Limulus* ventral photoreceptors because these cells use Ca^{2+} as an intracellular messenger to signal changes in sensitivity to light (*4*). Our results indicate that $[Ca^{2+}]_i$ does not change uniformly throughout the photoreceptor cell and that measurements of the magnitude and kinetics of changes in $[Ca^{2+}]_i$ can be seriously distorted by spatial nonuniformity.

We used a metallochromic dye, arsenazo III, to measure changes in $[Ca^{2+}]_i$. In most previous studies the absorption of intracellular dye was measured through an aperture that restricts the measuring light to that passing through an entire segment of a tubular cell (*5*) or much or all of the cell soma (*6*). Changes in dye absorption were presumed to indicate the magnitude and kinetics of changes in $[Ca^{2+}]_i$ that could be correlated with physiological events. Results obtained by this method are difficult to interpret because the distribution of changes in

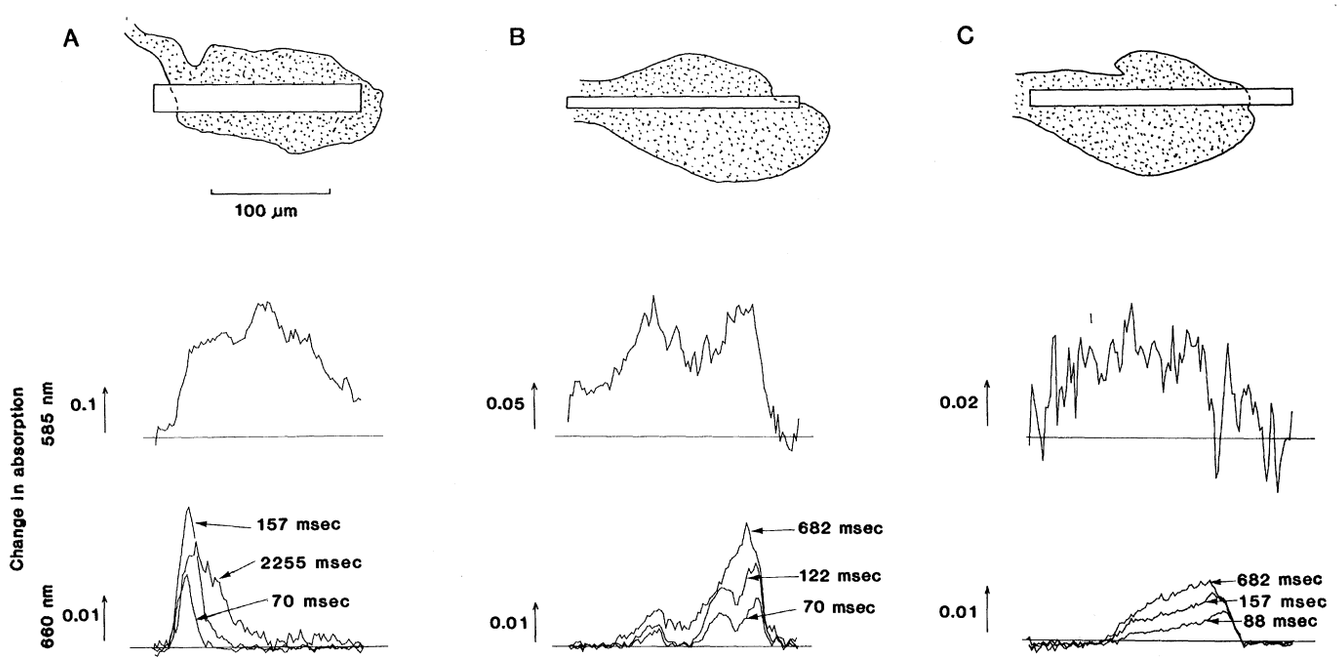

Fig. 1. (A to C) Examples of the spatial distribution of stimulus-induced changes in the absorption of intracellular arsenazo III. (Top) Outlines of the cell and measuring slit as traced from micrographs taken through the measuring optics. In each outline the axon exited from the cell body toward the left. (Middle) Changes in absorption, measured at the isosbestic wavelength (585 nm), as a function of distance across the cell. (Bottom) Changes in absorption, measured at 660 nm, as a function of distance across the cell. The times indicated are the times of the beginning of each scan; scan duration was 18 msec. (A) The change in absorption was localized to the end of the cell nearest the axon. This pattern was observed in two cells. Note that the peak change in absorption at 660 nm rose and fell as a function of time and that at the longest time the absorption profile became broadened. In 13 other cells the change in absorption at 660 nm was localized to the end of the cell opposite the axon. (B) Absorption at 660 nm increased at more than one region of the cell. This is most evident in the 70-msec trace. This pattern was observed in five cells. (C) The change in absorption at 660 nm was broadly distributed across the cell. This pattern was observed in one cell.