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Simian Retrovirus D Serogroup 1 Has a Broad Cellular Tropism for Lymphoid and Nonlymphoid Cells

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Simian acquired immunodeficiency syndrome is a fatal immunosuppressive disease caused by type D retroviruses such as simian acquired immunodeficiency syndrome retrovirus type 1 (SRV-1). The disease is characterized by generalized lymphadenopathy, opportunistic infections, and lymphoid depletion with defects in both humoral and cell-mediated immunity. To understand how SRV-1 infection relates to the immune defect, we studied in vivo-infected lymphocytes from SRV-1-positive macaques with and without clinical signs of immunosuppressive disease. B and T helper/inducer and T suppressor/cytotoxic lymphocytes were purified by panning or by flow cytometry. Neutrophils were purified by dextran sedimentation, and platelets were purified by low-speed centrifugation. In vitro infection studies were also done with HUT78, H9, K562, rhesus lung fibroblast, rhesus monkey kidney, and bat lung cells. SRV-1 in lymphocytes or culture supernatants was detected by the induction of syncytia in cocultivated Raji cells and was confirmed by immunofluorescence, electron microscopy, or reverse transcriptase assay. We found that B and T helper/inducer lymphocytes were infected in all animals tested. The number of infected T suppressor/cytotoxic cells was generally lower than that of the other cell subsets, and not all animals in this subset had SRV-1 infections. All other cells exposed in vitro to SRV-1, except bat lung cells, were able to be infected. These findings show that SRV-1 has a broad cell tropism for lymphoid and nonlymphoid cell types.

Simian acquired immunodeficiency syndrome (SAIDS) in rhesus macaques is caused by a type D retrovirus, SAIDS retrovirus type 1 (SRV-1). The etiology of type D retrovirus-induced SAIDS has been proven by induction of disease with molecularly cloned SRV-1 (10). It is characterized clinically by generalized lymphadenopathy, opportunistic infections, lymphoid depletion with an associated decrease in serum immunoglobulins, and decreased in vitro lymphocyte blastogenesis to both B- and T-cell lectins (22). The T helper/suppressor ratio is not consistently reversed, and the reversal does not correlate with the stage of the clinical disease as described in the human acquired immunodeficiency syndrome (23). The unchanged ratio in animals with SAIDS appears to be due to a progressive decline in the total number of circulating $CD4^+$ and $CD8^+$ lymphocytes that occurs throughout the course of the disease. Immunoperoxidase staining with gp20 monoclonal antibody (12) shows a low prevalence of infected cells except in germinal centers of lymph nodes and spleen and in salivary gland acini (A. A. Lackner, M. H. Rodriguez, C. E. Bush, R. J. Munn, H.-S. Kwang, P. F. Moore, K. J. Osborn, P. A. Marx, M. B. Gardner, and L. J. Lowenstine, J. Virol., in press). Histologic sections of lymph nodes from macaques with fatal disease show an absence of follicular germinal centers, as well as a loss of paracortical lymphocytes, suggesting that both T and B lymphocytes may be infected and eliminated (25).

In addition to SRV-1 infected animals with clinical SAIDS, we have identified persistently infected, healthy carriers of SRV-1 (16). In these monkeys, serum immunoglobulins are normal, as is the in vitro immune response to mitogens. Understanding the differences between healthy carriers and clinically diseased animals could enhance our knowledge of the pathogenesis of the virus-induced immunosuppression. A first step is to determine whether there are differences in the lymphocyte subsets which are infected in healthy carriers and clinically ill animals.

Human immunodeficiency virus (HIV) has been shown to have a narrow cell tropism, predominantly infecting cells expressing the CD4 cell surface antigen. HIV uses this epitope as the cell surface receptor, providing a means for entrance of virus into the cell (2, 11, 24). The infected subpopulation is then susceptible to the direct (7, 35) and indirect (18) cytopathic effects of the virus and possibly to an antiself immune response (5, 36) resulting in elimination of lymphocytes and reversal of the T helper/suppressor ratio. Functions related to T helper lymphocytes are eliminated, including B-cell help, production of lymphokines, decreased expression of interleukin-2 receptors, and decreased alloreactivity (13, 31).

Simian immunodeficiency virus (SIV), also a T-cell tropic retrovirus, causes a clinical syndrome in macaques which is very similar to acquired immunodeficiency syndrome in humans, including lymphoma and encephalitis (3, 4, 17, 27). SIV preferentially infects lymphocytes bearing the CD4 epitope, and infection can be blocked in vitro by a monoclonal antibody to this epitope. Immune defects related to loss of T helper cell function are also found in SIV-infected animals.

In both HIV and SIV infections, the immune defect relates to the cell type infected. Macaques with type D SRV-1 infection have immune defects related to both T- and B-cell deficiencies; thus, SRV-1 may have a broader cell tropism than SIV or HIV. In vitro studies by Yetz and Letvin (34) have shown that human B-cell lines could be infected with SRV-1 but that interleukin-2-dependent T-cell lines from macaques could not be infected in vitro. LeGrande et al. (14) showed that monocytes from SRV-1-infected macaques

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were infected with SRV-1. However, the in vivo distribution of SRV-1 in lymphocytes from infected macaques is unknown.

In this study, we show that SRV-1 has an in vivo tropism for both macaque T helper cells and B cells in moderately high titers. T suppressor cells are also infected, but in lesser numbers than T helper and B lymphocytes. SRV-1 is not restricted to lymphoid cells from nonhuman primates but can infect leukocyte lines of human origin such as the Raji B lymphoma line and the HUT78 $CD4^+$ lymphocyte line. Other continuous cell lines which are susceptible to productive infection by SRV-1 include rhesus fetal lung fibroblasts and rhesus monkey kidney cells.

MATERIALS AND METHODS

Animals. Nine rhesus macaques (six male and three female) were studied. Six were between 1 and 2.5 years of age, and three were between 4.5 and 8.5 years of age. Six had been experimentally inoculated intravenously with the type D retrovirus SRV-1 as described elsewhere (10), and three were spontaneously infected while living in a 0.5-acre outdoor cage (15). Two of the spontaneously infected macaques (17636 and 19729) had no clinical signs of SAIDS but were healthy carriers of SRV-1 (16). The remaining seven animals had clinical signs meeting the case definition of SAIDS caused by SRV-1 (10). Before macaques were selected for this study, the peripheral blood mononuclear cells (PBMC) were shown to be infected with SRV-1 by cocultivation with Raji cells (3, 10).

The animals were fed Purina monkey chow (Ralston Purina Co., Checkerboard Square, St. Louis, Mo.), and water was provided ad libitum.

Cell separation by panning. (i) Plate preparation. For purification of the B-lymphocyte subpopulation, 15 ml of 0.05 M Tris buffer (ph 9.4) with 10 μg of polyvalent goat anti-monkey immunoglobulin (Nordic Laboratories, Capistrano Beach, Calif.) per ml was added to sterile polystyrene tissue culture plates (100 by 20 mm; Optilux; Becton Dickinson Labware, Oxnard, Calif.). Plates used to enrich for the T-lymphocyte subpopulations were each incubated for 16 to 24 h at 4°C with 160 μg of goat anti-mouse immunoglobulin (Organon Teknika, Malvern, Pa.) diluted in 15 ml of sterile 0.05 M Tris buffer (pH 8.5; Sigma Chemical Co., St. Louis, Mo). All plates were gently washed with sterile Ca^{2+}-Mg^{2+}-free phosphate-buffered saline (PBS), followed by addition of 15 ml of 5% bovine serum albumin in PBS. The plates were allowed to stand at room temperature for 1 h, washed with PBS, and then rinsed with 1% heat-inactivated fetal bovine serum in PBS just before use.

(ii) Lymphocyte separation. The panning procedure was done essentially as reported by Tsoi et al. (33), with the methods briefly described here. Rhesus PBMC were isolated from heparinized whole blood by using a modification of the Ficoll-Hypaque density gradient centrifugation technique described by Böyum (1). Isolated cells were suspended and cultured in Eagle minimum essential medium (MEM; GIBCO Life Technologies Inc.) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories), 2 mM L-glutamine, 10^{-5}M β-mercaptoethanol, penicillin (50 U/ml), and streptomycin (50 μg/ml). This will be referred to as complete MEM.

Adherent monocytes and macrophages were removed by adding 3×10^7 cells suspended in 5 ml of MEM to a T25 tissue culture flask and allowed to incubate for 2 h at 37°C in 5% CO_2 and room air. The nonadherent cells were removed, pelleted, and suspended in 3 ml of RPMI 1640, and they were then added to the plates coated with the polyvalent goat anti-monkey immunoglobulin for removal of the B cells. Plates were incubated on a rocker platform for 1 h at room temperature. Nonadherent cells were removed by gentle washing with cold RPMI 1640 (GIBCO Life Technologies) and then pelleted at $800 \times g$, suspended in complete MEM, and allowed to incubate overnight at 37°C. The nonadherent cells were again pelleted at $800 \times g$ and suspended in 3 ml of RPMI 1640. This cell suspension was divided into two aliquots. One aliquot was incubated with OKT4 (Ortho Diagnostics, Raritan, N.J.), while the second aliquot was incubated with OKT8f, graciously supplied by Gideon Goldstein, Ortho Diagnostics. A technique described previously was used to label the cells with the monoclonal antibodies (23). The OKT4 and OKT8 antibody-labeled cells were then pipetted onto the goat anti-mouse immunoglobulin-coated plates and incubated on a shaker for 1 h at room temperature. Unbound cells were removed from the plates by gentle washing with 5-ml aliquots of RPMI 1640 and by aspirating and saving each wash. Cells were pelleted from the wash and suspended in 5 ml of complete MEM. Cells adherent to antibody-coated plates were removed by vigorous pipetting and pressure washing of the plate surface with three 5-ml volumes of RPMI 1640. The three washes were combined, and the cells were pelleted. A portion of each cell population was then labeled for surface immunoglobulin, OKT4, or OKT8, and the purity of each sample was determined by flow cytometry as previously described (23).

Differential counts were done on cytocentrifuged cell samples after the panning purification. At least 100 cells were counted by light microscopy, and the percentages of lymphocytes, monocyte-macrophages, and neutrophils were determined.

Virus isolation. Nonadherent cells or supernatant from adherent cells was cultured for the presence of virus by cocultivation with Raji cells as described elsewhere (3, 10). Raji cells are a human Burkitt's B-cell lymphoma cell line (6). The presence of SRV-1 was determined by visual detection of characteristic syncytium formation in the Raji cells. The number of infected cells was determined by endpoint dilution of lymphocytes, beginning with 80,000 lymphocytes per well and cocultivating the diluted lymphocytes with 10^4 Raji cells per well. All type D retrovirus isolates were confirmed by electron microscopy, reverse transcriptase assay, or immunofluorescence as described elsewhere (12, 19, 26).

The following cells were cocultivated with PBMC and were tested for the ability to support replication of SRV-1 in vitro: two human T-lymphocyte cell lines, HUT78 (8) and H9 (30), a human myelogenous leukemia cell line, K562 (20), rhesus monkey lung fibroblasts and kidney cells, and bat lung cells. Supernatant fluid from these cells was harvested and assayed for the presence of SRV-1 by induction of syncytia in Raji cells as described above.

Cell sorting. Staining of the PBMC was performed by using sterile reagents. The PBMC (5×10^6 to 10×10^6 cells) were suspended at 2×10^6 cells per ml in wash buffer consisting of RPMI 1640 and 5% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). Monoclonal anti-Leu2a or anti-Leu3a (5 μl/10^6 cells; Becton Dickinson) was added. After 30 min of incubation on ice, the cells were washed three times with wash buffer and resuspended in the original volume of wash buffer. Fluoresceinated goat F(ab')2 anti-mouse immunoglobulin G (Tago, Inc.) was added to bring the fluorescent reagent concentration to 1:200. After an

additional 30 min of incubation on ice, the cells were washed three times with wash buffer and suspended to 10^6 cells per ml.

The stained cells were sorted on an EPICS C flow cytometer (Coulter Electronics, Inc., Hialeah, Fla.) under sterile conditions. The flow tip (76-μm orifice) and cell injection system were sterilized by allowing 70% ethanol to pass through them for 30 min. The PBS sheath fluid was sterilized by passing it through a sterile filter (pore size, 0.45 μm) before the fluid entered the flow tip. The 488-nm wavelength was used at 300 mW. The bimorph crystal was set at 32 kHz, the flow rate was about 1,000 cells per s, and 2 drops were sorted. The sort was gated on the PBMC and on the fluorescent stained cells. After being sorted, the cells were centrifuged, washed once with culture medium, counted by using a Coulter counter (Coulter Electronics), and assayed for virus-infected cells by the Raji coculture assay. A sample of the cells was analyzed on the EPICS C to determine the efficacy of the sorting.

Neutrophil purification. Neutrophil populations were obtained from heparinized whole-blood samples by using a modified method of Böyum (1). Briefly, the erythrocyte-granulocyte soft pellet obtained was suspended in 2 volumes of a balanced salt solution, D-PBS (Pharmacia Fine Chemicals, Piscataway, N.J.). To this cell suspension was added an equal volume of 3% dextran T500.

The resulting mix was allowed to stand undisturbed (1 × *g*) for 2 h at 4°C. The neutrophil-enriched fraction was aspirated, and the cells were pelleted at 800 × *g* for 10 min at 4°C. Contaminating erythrocytes were removed by hypotonic shock by brief exposure to sterile, glass-distilled, deionized water, and isotonicity was reestablished by using hypertonic saline. The remaining intact cells were pelleted at 800 × *g* for 10 min at 4°C and suspended in MEM. The cell suspension was then examined microscopically for viability by erythrocin exclusion and homogeneity by a differential count. A sample of cells was cocultured with Raji cells to determine whether SRV-1 was present.

Platelet purification. Isolation of platelets was accomplished by producing a platelet-rich plasma fraction by low-speed centrifugation of a heparinized whole-blood sample at 15 × *g* for 15 min at room temperature. The platelet-rich plasma supernatant above the cell suspension was aspirated and centrifuged at 800 × *g* to pellet the platelets. The pelleted platelets were suspended in 4.0 ml of PBS and again pelleted at 800 × *g*. This wash procedure was repeated twice, after which the pellet was suspended in complete MEM. A differential count was done to determine platelet purity, and a sample was assayed for SRV-1 by cocultivation with Raji cells.

RESULTS

Nine rhesus macaques were studied, all of which were persistently infected with SRV-1. Five had clinical signs meeting the case definition of SAIDS (10) (animals 21083, 21092, 19494, 21472, and 19496), one had mild clinical signs (animal 20331), one animal was naturally infected (animal 19215) while living in an outdoor cage where SAIDS was endemic and was near death with SAIDS at the time of testing, and two macaques (animals 19729 and 17636) were proven healthy carriers of SRV-1 with no clinical signs (16).

In the first experiment, PBMCs from seven animals were isolated and separated into B lymphocytes (surface immunoglobulin positive), T helper lymphocytes ($OKT4^+$), and T suppressor lymphocytes ($OKT8^+$) by the panning technique described above. Samples of unseparated cells and each panning-purified cell subset were cocultivated with Raji cells in endpoint dilutions to determine the number of SRV-1-infected cells. Results are shown in Table 1.

TABLE 1. Infected-cell titers in rhesus macaque lymphocyte subsets purified by panning [a]

Animal and cell group	Infected-cell titer[b]	% Positive by IFA[c]			Cell count (%)		
		SIg^+	$T4^+$	$T8^+$	Lymphocytes	Monocytes	Other cells
21083							
PBMC	1:128						
B	1:128	79			92	3	5
$T4^+$	1:32		81	0	96	2	2
$T8^+$	1:4		0	74	95	3	2
21092							
PBMC	1:64						
B	1:64	97			86	10	4
$T4^+$	1:32		93	1	97	3	
$T8^+$	1:4		0	95	90	10	
19494							
PBMC	ND						
B	1:128	82			94	6	
$T4^+$	1:256		93	1	95	5	
$T8^+$	1:128		0	90	96	4	
19729							
PBMC	1:2						
B	1:8	59			78	22	
$T4^+$	1:4	2	90	20	98	2	
$T8^+$	<1:2	0	0	84	98	2	
21472							
PBMC	1:16						
B	1:32	71			67	33	
$T4^+$	1:2	0	78	1	97	3	
$T8^+$	<1:2	1	22	82	ND	ND	
19215							
PBMC	1:128						
B	1:64	77			ND	ND	
$T4^+$	1:128		88	0	ND	ND	
$T8^+$	1:64		0	75	ND	ND	
19496							
PBMC	ND						
B	1:16	85			ND	ND	
$T4^+$	1:64		94	2	96	4	
$T8^+$	1:16		9	88	99	1	

[a] PBMCs were divided by panning into surface immunoglobulin-positive (SIg^+) B lymphocytes and $OKT4^+$ or $OKT8^+$ T lymphocytes. The purity of each population was determined by flow cytometry and a differential count of cytocentrifuged cells. The SRV-1 titer was determined by cocultivation with Raji cells in endpoint dilutions as described elsewhere (10). ND, Not done.

[b] The number of infected cells was determined by endpoint dilution of lymphocytes, beginning with 80,000 lymphocytes per well and cocultivating the diluted lymphocytes with 10^4 Raji cells per well.

[c] IFA, Immunofluorescence assay by flow cytometry.

The B lymphocytes and $OKT4^+$ subsets from all seven animals were infected. Titers of the $OKT4^+$ and B lymphocytes ranged from 1:2 to 1:256. In the $OKT8^+$ subset, virus could be detected in only 5 of 7 animals. However, the infected-cell titers were generally lower than those of the B cells or the $OKT4^+$ T cells, which had titers ranging from 1:4 to 1:64. There was no correlation between clinical status and infected-cell number.

In a second experiment, we looked more specifically at the

TABLE 2. Infected-cell titers in rhesus macaque T-cell subsets purified by flow cytometry[a]

Animal no. and clinical status	Purified-cell population	Infected-cell titer	% Leu3a	% Leu2a	% Non-Leu3a or -Leu2a
20331, Clinical SAIDS	Unsorted	1:3,280	49	44	17
	Leu3a$^+$	1:1,640	98	2	0
	Leu2a$^+$	1:100	2	98	0
19729, Healthy carrier	Unsorted	1:200	58	27	15
	Leu3a$^+$	1:800	98	4	0
	Leu2a$^+$	1:100	5	98	0
17636, Healthy carrier	Unsorted	1:16,200	30	46	24
	Leu3a$^+$	1:2,840	95	1	4
	Leu2a$^+$	1:1,440	2	99	0

[a] Peripheral blood lymphocytes were labeled with either Leu3a or Leu2a monoclonal antibodies (Becton Dickinson) and a fluoresceinated goat anti-mouse secondary antibody. Cells were then sorted on a Beckton Dickinson fluorescence-activated cell sorter to select positively for Leu3a$^+$ or Leu2a$^+$ cell populations. Non-Leu3a or Leu2a populations remained after positively selected cells were removed. SRV-1 titers were determined by cocultivation with Raji cells in endpoint dilutions as described elsewhere (10).

T-cell subset infections in two macaques (animals 17636 and 19729) which were healthy carriers of SRV-1 and one macaque (animal 20331) chronically infected with SRV-1 with mild clinical signs. Lymphocytes from these animals were labeled with either Leu3a or Leu2a monoclonal antibodies and positively selected for either Leu3a$^+$ (T helper/inducer) or Leu2a$^+$ (T suppressor/cytotoxic) cell subsets by cell sorting. Purified cell populations were cocultivated with Raji cells to determine the number of SRV-1-infected T lymphocytes.

Table 2 shows the results of this experiment. One healthy carrier, macaque 17636, had the highest infected-lymphocyte titer of any macaque studied. Both purified T helper cells (95% Leu3a$^+$) and T suppressor cells (99% Leu2a$^+$) were infected at titers of 1:2,800 and 1:1,400, respectively. The Leu3a$^-$, Leu2a$^-$, and unsorted populations had 5- to 11-fold-higher titers, ranging from 1:7,000 to 1:16,200. The ratio of T helper to T suppressor cells was 0.65.

Macaque 19729, also a healthy carrier, had the highest infected-lymphocyte titer (1:800) in the T helper cells (98% Leu3a$^+$) and the lowest titer (1:25) in the Leu3a$^-$ population depleted of the T helper cells (4% Leu3a$^+$). The other cell populations, including the T suppressor/cytotoxic cells (98% Leu2a$^+$) were also infected, with titers ranging from 1:100 to 1:200. The ratio of T helper to T suppressor cells was 2.15.

Macaque 20331 had an infected-lymphocyte titer of 1:1,640 in the T helper lymphocytes (98% Leu3a$^+$). The T suppressor/cytotoxic (98% Leu2a$^+$) and the Leu3a$^-$ groups enriched for T suppressor/cytotoxic lymphocytes had a 16-fold-lower titer of 1:100. The other cell populations had a titer of 1:1,640 or 1:3,280. The ratio of T helper to T suppressor cells was 0.89.

Polymorphonuclear leukocytes from two animals were purified by dextran sedimentation. More than 94% of the cells were polymorphonuclear leukocytes, as determined by differential counts of cytoprep smears. These cells were free of detectable infectious SRV-1 by the Raji cell assay.

Platelets were isolated from heparinized blood from four rhesus macaques with clinical SAIDS. No contaminating leukocytes or erythrocytes were detected in cytoprep smears of the purified platelets. Platelets from all four macaques were negative for infectious SRV-1.

We previously reported isolation of SRV-1 in rhesus monkey kidney cells (21) and macrophages from SRV-1-infected macaques (14). Subsequently, we attempted infection of several other cells or cell lines: HUT78, H-9, K-562, Raji, rhesus monkey lung fibroblasts, and bat lung cells. Results in Table 3 show that all but the bat lung cells could be infected with SRV-1 as detected by electron microscopy, immunofluorescence assay using monoclonal antibody to gp20 transmembrane protein of SRV-1, Raji syncytial assay, or reverse transcriptase activity in cell culture supernatant fluid. These results confirm the apparent broad cell tropism of SRV-1 in the lymphocyte subsets we examined. By light microscopy there was no detectable cytopathology in any cell line other than Raji cells which formed syncytia.

DISCUSSION

We have shown that SRV-1 has a broad in vivo lymphocyte tropism in SRV-1-infected macaques. In vitro it also infects lymphoid and nonlymphoid cells of human origin. There is no apparent in vitro cytopathology, except syncytial formation, in human Raji cells. No correlation is apparent between the clinical stage of disease or the healthy carrier state and the type of lymphoid cell subset that is infected. We found that B cells and T helper/inducer (OKT4$^+$ or Leu3a$^+$) cells were infected in nine of nine macaques, and T suppressor/cytotoxic (OKT8$^+$ or Leu2a$^+$) cells were infected in seven of nine macaques.

In four macaques whose cells were purified by panning (Table 1), the titer of SRV-1-infected B cells was equal to or higher than that in the unseparated PBMC or T-cell subset. In three animals, the infected-cell titer of the OKT4$^+$ subset was higher or equal to the titer in the unseparated PBMC or the B-cell subset. In general, the infected-cell titer in the T suppressor/cytotoxic subset was lower than in the B-cell or T helper/inducer cell subset. Of nine macaques tested, none had an SRV-1-infected-cell titer in the OKT8$^+$ subset that was higher than titers in the other subsets. These results suggest that the T suppressor/cytotoxic subset is not as easily infected by SRV-1 or possibly that only a subset of this cell population is susceptible to infection. It is also possible that the OKT8$^+$ subset is infected in vivo but is rapidly removed by cytotoxicity or immune-mediated elimination and thus is not detectable in vitro.

TABLE 3. Cell tropism of SRV-1[a]

Cell group tested	Presence of virus	Infection confirmed by[b]:
HUT78 (human T helper cell line from sezary leukemia)	+	Raji assay, EM, IFA, RT
H9 (human T-cell line)	+	Raji assay, IFA
K-562 (human chronic myelogenous leukemia cell line)	+	Raji assay, IFA
Raji (human Burkitt's B-cell lymphoma)	+	DNA hybridization, EM, IFA, RT
Rhesus lung fibroblast	+	Raji assay, EM
Rhesus monkey kidney	+	Raji assay, EM
Bat lung	−	

[a] All lymphoid and nonlymphoid cell lines tested from human and nonhuman primates were found to support replication of SRV-1 in vitro. In vitro cytopathology was present only in Raji cells (syncytia) and was absent in all other cells tested. Bat lung cells were unable to be infected in these experiments.

[b] Abbreviations: IFA, immunofluorescence assay; EM, electron microscopy; RT, reverse transcriptase.

These results were confirmed in the second experiment by using a different method in which T-cell subsets were purified by cell sorting. Again, all T-cell subsets were infected, but the T suppressor/cytotoxic cell-enriched subsets ($Leu2a^+$) had the lowest numbers of infected cells in two of three of the animals, compared with those in the other cell groups. There did not appear to be a correlation between clinical status and the cell subset infected. In addition, as reported earlier, there was no clear pattern between the T helper/suppressor ratio and the clinical stage of disease (23).

Macaque 17636 has been identified as a healthy carrier of SRV-1 (16). We have previously shown that this animal has a high number of infected cells in the total population of PBMCs, as well as cell-free virus in saliva. Virus isolated from this animal is pathogenic in susceptible rhesus macaques (16). Our results here show that the purified T helper/inducer and T suppressor/cytotoxic cell subsets have the lowest number of infected cells compared with those of other cell populations from that animal. This indicates that infected cells are in higher titers in other lymphoid cell subsets, such as B cells or macrophages, than in T-cell subsets.

In past in vitro studies, we have observed a presence of this extremely high titer of virus-infected cells without apparent cytotoxicity (data not shown). This is in contrast to HIV-infected $CD4^+$ lymphocytes, in which in vitro cytotoxicity occurs. The mechanism of SRV-1-induced lymphoid cell depletion is unknown, but an effector mechanism independent of virus infection and replication must explain cytotoxicity in vivo.

The pathogenesis of several viral diseases appears to be related to cell-mediated immunity directed against virus-infected cells (9, 28, 29). In several of these models, immunosuppressive therapy has eliminated or delayed expression of disease by preventing immune elimination of virus-infected cells (28). Cell-mediated lymphoid depletion is a potential mechanism for lymphoid depletion in SRV-1-infected animals. The lack of virus-specific immunity is an attractive hypothesis in the case of macaque 17636, the healthy carrier studied here. This animal was born to an SRV-1-infected mother and may have been infected in utero. Early exposure to SRV-1 antigen in utero could have resulted in immunological tolerance to the virus and thus a lack of virus-specific immunity, which could explain the very high virus titer in the PBMC and saliva without clinical disease. This animal lacks detectable antibody to SRV-1 in serum.

In addition to detection of SRV-1 in rhesus PBMC, we and others have shown that SRV-1 is amphotropic and can infect rhesus lung fibroblasts, rhesus monkey kidney cells, and several human T- and B-cell lines (Table 3), as well as canine thymus cells, mink cells, NIH 3T3 mouse cells, and bat lung cells (3, 32). However, we were unsuccessful in infecting bat lung cells with SRV-1. In related studies SRV-1 has been found in salivary gland, mammary gland, pancreas, oral mucosa, and choroid plexus by immunohistochemistry and in situ hybridization or electron microscopy (A. A. Lackner, et al., in press).

This broad cellular tropism appears to be important in the natural transmission of SAIDS. Biting and transfer of saliva-borne virus is probably a major route of transmission of SRV-1, and SAIDS has been induced by intravenous injection of saliva from healthy carrier macaque 17636 into susceptible rhesus macaques (16). Transmission by breast milk has not been experimentally demonstrated, but virus has been isolated from breast milk of SRV-1-infected rhesus macaques (P. A. Marx, personal communication). SRV-1 has also been isolated from urine (16), and this is supported by our finding that rhesus monkey kidney cells can be infected in vitro.

The apparent broad cell tropism of SRV-1 indicates that the cell surface viral receptor is a structure common to many cell types. This is in contrast to HIV and SIV, which apparently primarily use the CD4 cell surface protein recognized specifically by the OKT4a monoclonal antibody (2, 11, 24). The cellular tropism of HIV and SIV correlates with the clinical signs and immune defects observed in the infected individuals. In SRV-1 infection, the spectrum of clinical signs, histopathological findings, and immune defects can be explained by a broad cell tropism if it is accepted that, except in healthy carriers, virus-infected cells are eliminated by some cytotoxic mechanism. In contrast to acquired immunodeficiency syndrome, we have found no reversal of the T helper/T suppressor cell ratio in SRV-1-infected animals (23). Instead, there was a decline in both T helper and T suppressor cells. In addition, there was a progressive decline in serum immunoglobulins throughout the disease course. Lymph node histopathology confirmed that both T and B cells were eliminated, since both interfollicular and follicular cells, respectively, were depleted. This uniform lymphoid depletion can be explained by our findings that both B and T lymphocytes could be infected with SRV-1.

Yetz and Letvin (34) reported that neither T nor B lymphocytes from macaques could be infected in vitro with SRV-1, but several human B-cell lines could be infected in vitro. Our studies here used cells only from infected macaques and therefore detected an infection which began in vivo. Perhaps another cell type not present in vitro must be infected in vivo and is then capable of transferring virus to lymphocytes, (i.e., lymph node dendritic cells). Alternatively, lymphoid cells may be susceptible to infection only while they are multipotential progenitors and cannot be infected as easily after differentiation. Further studies are needed to understand the mechanism of infection and immunosuppression induced by SRV-1.

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