Biological Characterization of a Simian Immunodeficiency Virus-Like Retrovirus (HTLV-IV): Evidence for CD4-Associated Molecules Required for Infection

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We have analyzed a number of biological features of HTLV-IV, a retrovirus indistinguishable from a macaque isolate of simian immunodeficiency virus (SIV), and compared this virus with several strains of human immunodeficiency virus type 1 (HIV-1). Although HTLV-IV was found to be similar to HIV-1 in its tropism for CD4⁺ lymphocytes, its effects on CD4 expression and the ability of its externalized envelope molecule to form a complex directly with the CD4 molecule, a number of striking differences were noted. Unlike with HIV-1, the range of cells susceptible to HTLV-IV infection and syncytia formation was restricted to a subset of CD4⁺ cell lines, particularly those that coexpressed CD4 with human leukocyte antigen (HLA) class II antigens. An analysis of the patterns of HTLV-IV infection with B × T somatic cell hybrids indicated that for this virus, molecules in addition to CD4 were probably required to facilitate infection and cell fusion. Additional studies of HTLV-IV infection of Sup-T1 cells, which are exquisitely sensitive to cytopathic effects induced by HIV-1, demonstrated that HTLV-IV infection could occur in the absence of cytopathic effects and, remarkably, with minimal or no downmodulation of the CD4 molecule from the cell surface. The failure of HTLV-IV infection to reduce the expression of several CD4 epitopes suggested that the HTLV-IV envelope produced by Sup-T1 cells was altered in its ability to interact with or bind to CD4. Additional differences were also noted in the size of the transmembrane envelope molecule of HTLV-IV produced by Sup-T1 cells, indicating that cell-specific alterations in processing of the HTLV-IV envelope occurred during the production of virus in this cell line. Understanding the basis for these biological differences between HTLV-IV and the HIV-1 viruses may help to elucidate more general mechanisms for pathogenesis of other members of the SIV and HIV families of retroviruses.

The acquired immunodeficiency syndrome (AIDS) is caused by at least two groups of closely related retroviruses, human immunodeficiency virus type 1 (HIV-1) (3, 31, 42) and type 2 (HIV-2) (2, 6). Although distinct antigenic (2, 6) and molecular (7, 14) features of these viruses have been described, both have been shown to be highly tropic for cells displaying the 56-kilodalton (kDa) glycoprotein CD4 on the cell surface and to induce cytopathic effects on CD4⁺ cells in vitro (6, 9, 28, 33, 46). These human retroviruses are also related to the simian immunodeficiency viruses (SIVs), which include several distinct isolates from captive macaques with an AIDS-like syndrome (4, 9, 25), and isolates from healthy sooty mangabeys (13, 34, 40) and African green monkeys (23, 41). Another retrovirus termed HTLV-IV was reported to have been isolated from healthy prostitutes in West Africa whose sera cross-reacted weakly with HIV-1 but strongly with SIV antigens (2, 24). Recent molecular comparisons have shown that some isolates of HTLV-IV are nearly identical to a macaque isolate (SIV_{mac251}), indicating that they are probably laboratory contaminants (12, 15, 16, 27, 29).

Regardless of the origin of HTLV-IV, biological and molecular comparisons between this virus and other members of the HIV and SIV families will be important in

identifying the viral and cellular factors that contribute to their complex pathogenesis. In the present report, we have analyzed a number of biological features of the HTLV-IV isolate termed HTLV-IV₂₈₉ and compared this virus with several strains of HIV-1 in order to determine whether differences exist with respect to host range, the ability to induce cytopathic effects, and interactions of envelope molecules with CD4. We report that similar to HIV-1, HTLV-IV is tropic for CD4 cells and that its externalized envelope molecule complexes directly with CD4. However, striking differences were noted for HTLV-IV with respect to its host range, suggesting that on T cells, molecules in addition to CD4 are required for infection. In addition, we provide evidence that in some cell lines, cell-specific alterations occur in processing of the HTLV-IV envelope that may alter the ability of this molecule to interact with CD4 and to produce cytopathic effects.

MATERIALS AND METHODS

Materials. Monoclonal antibodies reactive with a variety of cellular antigens were purchased, including OKT3, OKT4, OKT4A, OKT8, OKT9, OKT10, OKT11, OKDR, and OKIa-1 (Ortho Diagnostics, Inc., Raritan, N.J.), Leu-3a (Becton Dickinson, Sunnyvale, Calif.), and B1 (Coulter Immunology, Hialeah, Fla.). The anti-CD4 antibody Cyt-029 was provided by T. McKearn (Cytogen Corp., Princeton,

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N.J.). Anti-human leukocyte antigen (HLA) class I antibodies 20-4E4.11 and 20-3F10.4 and the anti-HLA class II antibodies L227, XD5A.11, 8B9.3, and 10D2.7 were provided by S. Radka (Genetics Systems Inc., Seattle, Wash.). Additional anti-HLA class I antibodies included W6/32 (purchased from Pelfreeze, Rodgers, Ark.) and 131 and 4E (provided by J. Kornbluth, University of Pennsylvania, Philadelphia). A number of other anti-HLA class II-specific monoclonal antibodies from the Tenth International Workshop on Human Histocompatibility Antigens were made available through collaborative studies with K. Zier (Children's Hospital of Philadelphia, Philadelphia, Pa.) and are listed in Table 3. The anti-HLA class II antibody CX2.1 was produced in our laboratory.

Immunofluorescence assays. The expression of cell surface antigens was assessed by immunofluorescence flow cytometry with a FACstar flow cytometer (Becton Dickinson). Cells were labeled in suspension with saturating concentrations of unconjugated monoclonal antibodies followed by F(ab)₂ fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Tago Laboratories, Burlingame, Calif.) as described previously (20). Background levels of fluorescence were determined with the nonreactive murine monoclonal antibody P3X63. HeLa-T4 cells, which grow as an adherent monolayer, were trypsinized for 30 s prior to staining to prepare a cell suspension.

Syncytia induction assays. HUT-78 or H9 cells (10^5 cells) that were productively infected with either HTLV-IV₂₈₉ or the HTLV-IIIB strain of HIV-1 were cocultured with 5×10^5 target cells for 3 days in 24-well plates (Linbro) and evaluated daily for the presence of multinucleated giant cells with a cell diameter greater than four times that of single cells.

Evaluation of HIV-1 or HTLV-IV infection of cell lines. Supernatants from cells lines productively infected with either HTLV-IV₂₈₉ or various strains of HIV-1 (HTLV-IIIB, HTLV-III_{RF}, ARV-2, and WMJ-1) were clarified by centrifugation ($2,000 \times g$ for 10 min) and filtered (0.2 μ m pore size) prior to use. Virus was quantitated by determining reverse transcriptase activity in 4 ml of ultracentrifuged ($100,000 \times g$, 30 min) supernatant as described previously (18) and by inoculating serial log dilutions on HUT-78 cells, which are susceptible to both HTLV-IV and HIV-1 infection. Target cells (10^6) were cultured for 24 h in 24-well plates with supernatants from infected cells, after which time cells were washed and maintained in T-25 flasks (Corning Glass Works, Corning, N.Y.). Infection was monitored by determining reverse transcriptase levels of cell-free supernatant and the percentage of cells positive for viral antigens by immunofluorescence microscopy on methanol-acetone (1:1)-fixed cells with a 1/20 dilution of human serum (21). HIV-1 antigens were detected by using serum from a seropositive patient with AIDS, and HTLV-IV antigens were detected by using serum from an HIV-2-seropositive West African individual. Cell viability was determined by trypan blue dye exclusion.

Radioimmunoprecipitation protocols. Immunoprecipitation of CD4 or viral envelope molecules was performed as described previously (22). Cell lines either uninfected or infected with HIV-1 or HTLV-IV₂₈₉ (50×10^6) were cultured for 1 h in 10 ml of cysteine- and methionine-free RPMI 1640 medium containing 10% dialyzed fetal calf serum, and then [³⁵S]cysteine (100 μ Ci/ml) and [³⁵S]methionine (100 μ Ci/ml) were added. Cells were cultured for 16 h, washed three times, and incubated in lysing buffer (LB) (0.02 M Tris, 0.12 M NaCl, pH 8.0, containing 0.2 mM phenylethylsulfonylethyl fluoride, 0.2 mM EGTA [ethylene glycol (bis-(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid], 0.2 mM NaF, 5 μ g of

aprotinin per ml, 0.2% sodium deoxycholate, and 0.5% Nonidet P-40), 1 ml per 5×10^6 cells, for 30 min at 4°C, and centrifuged at $3,000 \times g$ for 20 min to remove nuclei. Lysates were cleared by incubation with protein A-Sepharose beads for 4 h at 4°C. Portions (200 μ l) of cleared lysates were then added to 20 μ l of protein A-Sepharose beads conjugated to monoclonal antibodies or human serum and rotated for 4 h at 4°C. Beads were washed sequentially in LB, LB containing 0.5 M NaCl, and LB with 0.1% sodium dodecyl sulfate (SDS). The adsorbed material was eluted by heating at 100°C for 5 min in 50 μ l of sample buffer (0.01 M Tris, pH 8.0, containing 2% SDS, 5% 2-mercaptoethanol [by volume], bromophenol blue [25 μ g/ml], and 10% glycerol [by volume]) and analyzed on 7.5% polyacrylamide-SDS gels.

Coprecipitation of CD4 with viral envelope. In order to assess the ability of viral envelope to complex with CD4, lysates were prepared as described above on [³⁵S]cysteine- and [³⁵S]methionine-labeled uninfected CD4-positive cells and on HIV-1- or HTLV-IV-infected cells. CD4 was removed from the lysates of HIV-1- or HTLV-IV-infected cells by adsorbing twice with protein-A Sepharose beads conjugated to OKT4. Lysates from the uninfected cells (used as a source of CD4) and the infected cells (used as a source of viral envelope) were then mixed in equal volumes at 4°C for 2 h and immunoprecipitated with protein A-Sepharose beads conjugated to either OKT4, OKT4A, or OKT3 (used as a negative control) as described above. Complexes of CD4 with HIV-1 or HTLV-IV envelope were demonstrated with the OKT4 antibody by the coprecipitation of high-molecular-weight viral bands with the 56-kDa CD4 molecule (22, 39).

Western blot. The size of the HTLV-IV transmembrane molecule was determined by Western blot (immunoblot) analysis of cell-free virus that was concentrated by pelleting through a 20% sucrose cushion, as described previously (2).

RESULTS

Evaluation of HTLV-IV host range. The ability of HTLV-IV to infect and to induce syncytia on a variety of human lymphoid and nonlymphoid cell lines was evaluated. The panel of cells used in these studies is shown in Table 1 and included CD4-positive and -negative T cells and B cells, as well as the human promyelocytic leukemia cell line HL60, which has been shown to express CD4 and to be susceptible to infection by some strains of HIV-1 (5, 32). In addition, HeLa-T4 cells were provided, which were derived from a human cervical carcinoma cell line that had been transfected with a CD4 cDNA and which expressed CD4 on the cell surface (35). The relative degree of CD4 expression by these lines (Table 1) was determined by flow cytometry with monoclonal antibodies that have been shown either to compete (OKT4A) or not to compete (OKT4) with the HIV-1 envelope for binding to CD4 (38, 39, 44).

In order to evaluate the ability of the HTLV-IV envelope to induce syncytia with these target cells, HUT-78 cells that were productively infected with either HTLV-IV or HTLV-IIIB were cocultured for up to 72 h with the cells shown in Table 1, and the formation of multinucleated cells was evaluated (Table 2). In cocultures with HTLV-IIIB-infected cells, syncytia was observed on all CD4⁺ cells except HL60, although differences were noted in the number and size of the syncytia that formed on these different cell types. However, in the cocultures with HTLV-IV-producing cells, syncytia were induced only on HUT-78, H9, and the CD4⁺ B cell line GM1500-B. No syncytia were observed even on

TABLE 1. Relative expression of CD4 and HLA class-II antigens on cell lines^a

Cell line	% Positive cells				Mean fluorescence channel			
	P3	OKT4	OKT4A	OKDR	P3	OKT4	OKT4A	OKDR
HUT-78	5	93	83	98	22	142	86	476
H9	1	63	64	93	20	61	60	450
Sup-T1	1	100	100	1	5	235	141	7
MOLT-4	1	73	66	3	7	47	33	10
CEM	0	99	99	1	7	194	94	7
Jurkat	1	41	40	1	16	75	71	16
GM1500B	1	96	73	91	15	114	61	427
HL60	3	85	71	2	21	132	76	20
Hela-T4	2	91	89	ND	30	231	215	ND

^a Cell lines were stained with monoclonal antibodies to CD4 (OKT4 and OKT4A) and the HLA-DR class II antigen (OKDR) and analyzed by immunofluorescence flow cytometry. Results are expressed as percent positive cells and the mean channel of fluorescence intensity on a log scale for the entire population. Background fluorescence levels were determined by using the nonreactive antibody P3X63 (P3). ND, Not done.

T-cell lines such as Sup-T1 (45), which have been shown to express high levels of CD4 and to be exquisitely sensitive to syncytia induction by HTLV-III_B (22) (Fig. 1). Interestingly, the cell lines that were susceptible to syncytia induction by HTLV-IV were also the lines that coexpressed HLA class II antigens with CD4 (Tables 1 and 2).

The susceptibility of the cell lines shown in Table 1 to infection by cell-free virus was then evaluated by using filtered supernatant from HTLV-III_B- and HTLV-IV-infected HUT-78 cells. Cultures were inoculated with culture supernatants containing approximately 50,000 cpm of reverse transcriptase activity, and cells from these cultures were evaluated weekly for 28 days by immunofluorescence microscopy for the expression of viral antigens. As shown in Table 2, HTLV-III_B infection occurred in all CD4⁺ cell lines, although in HL60 cells only rare cells were detected that transiently expressed viral antigens. However, similar to the results for syncytia induction, HTLV-IV infection occurred on HUT-78, H9, and GM1500-B cells but not on the majority of other CD4⁺ lines. Interestingly, HTLV-IV was able to infect Sup-T1 cells even though these cells did not form syncytia when cultured with HTLV-IV-infected HUT-78 cells. As described below, however, the time course for Sup-T1 infection by HTLV-IV was markedly prolonged compared with that observed for the other infect-

able cell lines. In additional studies with cells that did not express CD4, neither infection nor syncytia occurred with HTLV-IV on five Epstein-Barr virus-transformed B-cell lines or on AL, an interleukin 2-dependent CD8⁺ line of cloned peripheral blood T cells (10).

Interactions of HTLV-IV envelope with CD4 molecule. (i) Inhibition of syncytia formation by monoclonal antibodies. Several approaches were used to evaluate interactions of the HTLV-IV envelope with the CD4 molecule. A panel of monoclonal antibodies was screened for the ability to inhibit syncytia formation induced by HTLV-IV-infected HUT-78 cells. Uninfected HUT-78 cells were preincubated with saturating concentrations of monoclonal antibodies and were then cocultured with the HTLV-IV-infected cells. As shown in Table 3, and in agreement with the results of Kornfeld and co-workers (29), only anti-CD4 monoclonal antibodies were found to inhibit syncytia formation. Similar to previous studies of HIV-1, inhibition of syncytia formation was only observed for the CD4 antibodies OKT4A, Leu-3a, and Cyt-029, but not with OKT4, suggesting that, as with HIV-1, OKT4 identifies an epitope on CD4 not involved in viral binding (20, 38, 39, 44). No inhibition in the induction of syncytia by HTLV-IV was observed with several monoclonal antibodies that reacted with T-cell, HLA class I, or HLA class II antigens.

(ii) Modulation of surface CD4 following HTLV-IV infection. Previous studies have demonstrated that HIV-1 infection selectively downmodulates CD4 from the cell surface following infection (8, 37), possibly as a result of intracellular complex formation between CD4 and viral envelope gene products (22). Although HUT-78 cells were strongly positive for CD4 prior to HTLV-IV infection, after infection these cells (>99% positive for viral antigens by immunofluorescence microscopy [IFA]) showed no detectable CD4 on the cell surface (Fig. 2). Identical results were obtained with either OKT4 or OKT4A, indicating that, as with HIV-1, the CD4 molecule had been modulated from the cell surface following HTLV-IV infection.

(iii) Coprecipitation of HTLV-IV envelope with CD4. The inhibitory effects of anti-CD4 antibodies on the induction of syncytia and the modulation of CD4 expression by HTLV-IV infection strongly suggested that the HTLV-IV envelope complexed with CD4. In order to demonstrate this directly, we evaluated the binding of the HTLV-IV envelope to CD4 in mixtures of lysates from infected and uninfected cells. Previous studies have shown that the OKT4 antibody can be used to precipitate complexes of CD4 bound to viral envelope glycoproteins (22, 39). Uninfected Sup-T1 and HUT-78

TABLE 2. Susceptibility of cell lines to syncytia formation and infection with HIV-1 (HTLV-III_B strain) and HTLV-IV

Cell line	Syncytia formation ^a		Infection ^b	
	HIV-1	HTLV-IV	HIV-1	HTLV-IV
HUT-78	+	+	+	+
H9	+	+	+	+
Sup-T1	+	—	+	+
MOLT4	+	—	+	—
CEM	+	—	+	—
Jurkat	+	—	+	—
GM1500B	+	+	+	+
HL60	—	—	±	—
HeLa-T4	+	—	+	—

^a Syncytia were determined by the presence of multinucleated giant cells when the cell lines indicated were cultured for 48 h with HUT-78 cells infected with either HIV-1 (HTLV-III_B strain) or HTLV-IV.

^b Cells were inoculated as described in Materials and Methods, and infection was determined by evaluating the expression of viral antigens by using immunofluorescence microscopy. Symbols: +, >80% of cells became positive for viral antigens within 2 weeks, with the exception of HTLV-IV infection of Sup-T1, which is discussed further in the Results section; — or ±, no cells or <1% of cells expressed viral antigens within 4 weeks.

FIG. 1. Comparison of syncytia induction by HIV-1 and HTLV-IV. HTLV-IIIIB-infected HUT-78 cells (top panels) or HTLV-IV-infected HUT-78 cells (bottom panels) were cocultured as described in Materials and Methods for 24 h with uninfected HUT-78 (A and D), Sup-T1 (B and E), or CEM (C and F) cells. Although HTLV-IIIIB induced syncytia on all three cell lines, HTLV-IV produced syncytia only with HUT-78. Although clumps of cells are apparent in the cocultures of HTLV-IV-producing cells with Sup-T1 and CEM cells (E and F, respectively), no syncytia were observed.

cells and HTLV-IV-infected HUT-78 cells (HUT-78/HTLV-IV) and HTLV-IIIIB-infected MOLT-4 cells (MOLT/HTLV-IIIIB) were metabolically labeled with [35 S]methionine and [35 S]cysteine, and cell lysates were prepared. The MOLT/HTLV-IIIIB and HUT-78/HTLV-IV lysates were adsorbed with OKT4 conjugated to Sepharose 4B beads to remove any detectable CD4. As shown in Fig. 3A, when these lysates were precipitated with OKT4, the 56-kDa CD4 band could be demonstrated in the uninfected Sup-T1 and HUT-78 cells, but not in the MOLT/HTLV-IIIIB cells or the HUT-78/HTLV-IV cells. Lysates of HUT-78 and Sup-T1 cell were then mixed with the lysates of either the HTLV-IIIIB- or HTLV-IV-infected cells and incubated for 2 h to allow complex formation between the viral envelope molecules and CD4. As shown in Fig. 3B, OKT4 precipitated CD4 associated with molecules of 160 and 110 kDa (from mixtures containing HTLV-IIIIB proteins) and 140 and 120 kDa (from mixtures containing HTLV-IV proteins). These molecules were clearly shown to represent viral proteins since 160- to 110-kDa bands were precipitated from MOLT/HTLV-IIIIB cells by serum from a patient with AIDS, and 140- to 120-kDa bands were precipitated from the HUT-78/HTLV-IV cells by serum from a West African patient seropositive for HIV-2 (Fig. 2C and D, respectively). Viral bands of these sizes have been shown to correspond to the envelope precursor and externalized envelope glycoproteins for HIV-1 and HTLV-IV, respectively (1, 24). Complexes of CD4 with envelope molecules could not be detected when the OKT4A antibody was used (not shown), similar to results reported for HIV-1 (22, 39). Thus, the HIV envelope gene products were found to complex specifically with CD4 obtained from either HUT-78 or Sup-T1 cells and to bind to an epitope on CD4 that was blocked by OKT4A.

Evaluation of HTLV-IV tropism by using somatic cell hybrids. The findings described above indicated that similar

to HIV-1, the HTLV-IV envelope contained a binding site for the CD4 molecule and that the induction of syncytia by this envelope could be inhibited by some anti-CD4 monoclonal antibodies. However, unlike HTLV-IIIIB and other HIV-1 isolates evaluated in our laboratory, including HTLV-III_{RF} (17), WMJ-1 (18), and ARV-2 (31), which are able to infect all of the CD4-positive lymphoid cell lines shown in Table 1, HTLV-IV₂₈₉ was only able to infect some of these lines, particularly those which coexpressed CD4 and HLA class II gene products.

To investigate the possibility that molecules in addition to CD4 were required for HTLV-IV infection, we evaluated infection of somatic cell hybrids of human B- and T-cell lines. The cells used for this study (kindly provided by Peter Cresswell, Duke University, and Robert DeMars, University of Wisconsin) are shown in Fig. 4A. Hybrids were derived from the T-cell line CEM, which expresses CD4 but not HLA class II molecules, and the B-cell line B721.174, which is both CD4 and HLA class II negative. The B721.174 line was cloned from a B lymphoblastoid cell line that had been mutagenized and selected for the absence of all HLA class II gene products (11). The hybrid of CEM and B721.174, designated CEM $\times$ 721.174, has been shown to express both CD4 and HLA class II, with the class II gene products DR, DP, and DQ produced from the genes located on the CEM 6th chromosomes (43). The relative expression of CD4 and HLA class II molecules is shown in Table 4. When these cell lines were evaluated for susceptibility to HTLV-IV infection, the CEM and B721.174 cells did not form syncytia when cocultured with HTLV-IV-infected HUT-78 cells. In addition, these cells showed no evidence of infection as defined by weekly measurements of reverse transcriptase levels (RT) and viral antigens by IFA for up to 30 days after inoculation with cell-free virus. In contrast, the CEM $\times$ 721.174 hybrid cell line was found to be exquisitely

TABLE 3. Effects of monoclonal antibodies on induction of syncytia by HTLV-IV^a

Group ^b	Antibody	Specificity	Inhibition of syncytia formation
1	OKT4	CD4	—
	OKT4A	CD4	+
	LEU-3a	CD4	+
	Cyt-029	CD4	+
	OKT3	CD3	—
	OKT9	Transferrin receptor	—
	OKT10	Early and activated T cells	—
	OKT11	CD2	—
	3A1	CD7	—
	W6/32	HLA class I	—
	20-4E4.11	HLA class I	—
	20-3F10.4	HLA class I	—
	131	HLA class I	—
	4E	HLA class I	—
2	L227	DR, DP, DQ	—
	XD5.A11	DR, DP, DQ	—
	Tu39	DR, DP, DQ	—
	10D2.7	DR, DQ	—
	8B9.3	DR, DP	—
	Tu35	DR, DP	—
	2D6	DR, DP	—
	OKDR	DR	—
	OKIa1	DR	—
	L234	DR	—
	PL8	DR	—
	CX2.1	DR	—
	Tu22	DQ	—
	1A3	DQ	—
	PL15	DP	—
	B7/21	DP	—
	DP11.1	DP alpha chain	—

^a Monoclonal antibodies were evaluated as described in Materials and Methods for the ability to inhibit syncytia induced by culturing uninfected HUT-78 cells with HTLV-IV-infected HUT-78 cells.

^b Group 1 includes a variety of T-cell and HLA class I antigens expressed on HUT-78 cells. Group 2 includes monoclonal antibodies reactive with nonpolymorphic determinants of HLA class II molecules, including DR, DQ, and DP.

sensitive to both syncytia induction and infection by HTLV-IV. Concentrations of virus that did not infect either of the parental cell lines produced cell killing, syncytia, and viral antigens on >90% of CEM × 721.174 cells within 7 days of inoculation.

In order to determine whether the association of HLA class II gene products with CD4 on the CEM × 721.174 line accounted for the susceptibility of these cells to HTLV-IV infection, we evaluated a clone of this hybrid line, termed CEM × 721.174T.2, that had lost both of the CEM 6th chromosomes and no longer expressed HLA class II antigens (44) (Fig. 4A, Table 4). Although these cells were unreactive with the panel of anti-HLA class II antibodies shown in Table 3, the CEM × 721.174T.2 cells remained highly susceptible to HTLV-IV-induced syncytia formation and infection with cell-free virus. The induction of syncytia by HTLV-IV on both of the hybrid cell lines was completely inhibited by OKT4A, but not by the panel of anti-HLA class II monoclonal antibodies shown in Table 3. These findings suggested that although infection of the hybrid cells by HTLV-IV was dependent on CD4, viral infection could be facilitated by the association of an as yet unidentified molecule provided by or induced by fusion of the B-cell line with CEM. However, despite the association of HTLV-IV infection with the coexpression of CD4 and HLA class II antigens

in the cell lines shown in Table 2, a role for one or more HLA class II products could not be shown from these experiments.

Evaluation of kinetics and cytopathic effects of HTLV-IV and HTLV-IIIB infection. In order to compare the kinetics and cytopathic effects of HTLV-IV and HTLV-IIIB infection on CD4⁺ cell lines, either HUT-78 cells or Sup-T1 cells were inoculated with filtered supernatants from HTLV-IV-infected HUT-78 cells or HTLV-IIIB-infected H9 cells, each containing approximately 50,000 cpm of reverse transcriptase activity (see Materials and Methods section). Cultures were evaluated for viability (by trypan blue dye exclusion), the percentage of cells showing viral antigens (by immunofluorescence microscopy), and the level of reverse transcriptase activity in culture supernatants. As shown in Fig. 5A and B, infection of HUT-78 cells by either HTLV-IIIB or HTLV-IV was detectable within 10 days as determined by rising levels of reverse transcriptase activity and an increase in the percentage of cells expressing viral antigens. By day 10 after inoculation viral antigens could be detected on >99% of the cells in each culture. During the infection of HUT-78 cells, cytopathic effects, including cell death and the formation of large syncytia, were observed for both viruses within the first 20 days, although in three experiments, the degree of cell killing and the size of syncytia were somewhat greater for HTLV-IIIB than for HTLV-IV infection. Interestingly, marked differences were observed between HTLV-IV and HTLV-IIIB during the infection of Sup-T1 cells. As shown in Fig. 5C, productive infection of this line by HTLV-IIIB was detectable within 5 days and produced dramatic cytopathic effects, including the formation of large syncytia and a decrease in cell viability to <1%, for up to 3 weeks after inoculation. Similar results were obtained when other HIV-1 isolates, including HTLV-III_{RF}, WMJ-1, and ARV-2, were used (not shown). In contrast, infection of Sup-T1 cells by HTLV-IV occurred over an extended time period and produced a gradual increase in RT and the percentage of cells expressing viral antigens (Fig. 5D). By day 50 after the inoculation, only 30% of cells were positive for HTLV-IV antigens. Remarkably, no cytopathic effects, including cell killing or syncytia formation, were observed at any time during the infection of Sup-T1 cells by HTLV-IV.

Alterations in HTLV-IV produced by Sup-T1 cells and effects on CD4 expression. In view of the noncytopathic and markedly delayed infection of Sup-T1 cells by HTLV-IV, we attempted to characterize both the virus and the infected cells from these cultures. In order to obtain a cell population that was completely infected, Sup-T1 cells from the HTLV-IV-infected culture shown in Fig. 5D were cloned by limiting dilution 60 days after the start of infection, and clones which expressed viral antigens on the majority of cells were selected by IFA. Four clones were obtained that were 100% positive for viral antigens and that produced >100,000 cpm of RT. CD4 expression on one representative clone, termed Sup-T1/HTLV-IVC.3, is shown in Fig. 6. Remarkably, despite the production of high levels of virus, the expression of the CD4 molecule on the surface of these cells was only minimally reduced, indicating that in contrast to the effects of HTLV-IIIB infection on Sup-T1 cells, productive HTLV-IV infection failed to downmodulate CD4 on this cell line. Moreover, CD4 could also be detected by OKT4A, which, as described above, was found to inhibit syncytia formation induced by HTLV-IV and to compete with envelope for binding to CD4. Identical results (not shown) were also obtained with the Leu-3a monoclonal antibody, which iden-

FIG. 2. Downmodulation of CD4 expression on HUT-78 cells by HTLV-IV infection. CD4 expression with OKT4 and OKT4A was determined by flow cytometry on uninfected HUT-78 cells and HUT-78 cells chronically infected with HTLV-IV (HUT-78/HTLV-IV). In each panel, fluorescence histograms for the anti-CD4 monoclonal antibodies (solid line) are superimposed on the histograms for the nonreactive antibody (P3X63 (dotted line)). HUT-78/HTLV-IV cells were >99% infected as determined by using immunofluorescence microscopy to detect viral antigens.

ties a CD4 epitope closely associated with HIV-1 binding in RT assays (36). The finding that these cells produced high levels of virus yet failed to show a reduction in CD4 expression on the cell surface, particularly of the OKT4A and Leu-3a epitopes, indicated either that viral envelope produced by these cells was not binding to CD4 or that the affinity of binding was markedly reduced. These results were in contrast to the infection of HUT-78 cells by HTLV-IV, which resulted in the complete loss of CD4 from the cell surface (Fig. 2). The apparent alteration in the ability of the HTLV-IV envelope produced by Sup-T1 to interact with CD4 could not be explained by differences in the CD4 molecule on these cells since, as shown in Fig. 3, we demonstrated that CD4 obtained from Sup-T1 cells could complex with the HTLV-IV envelope molecules when this virus was produced by HUT-78 cells.

In order to determine whether the apparent functional differences in the HTLV-IV envelope produced by HUT-78 and Sup-T1 cells corresponded to other detectable differences in envelope processing, viral proteins from HTLV-IV-infected HUT-78 cells and HTLV-IV-infected Sup-T1 cells were analyzed by radioimmunoprecipitation and Western blot. As shown in Fig. 7A, when these cells were metabolically labeled with [35 S]cysteine and [35 S]methionine, no differences were observed in the high-molecular-weight envelope bands (Fig. 7A). However, when cell-free virus from these infected cells was concentrated and analyzed by Western blot, a small but reproducible difference was observed in the size of the envelope transmembrane molecule, which was 32 kDa when HTLV-IV was produced by HUT-78 cells and 34 kDa when this virus was produced by Sup-T1 cells (Fig. 7B, lanes 1 and 2).

The differences observed for HTLV-IV produced by Sup-T1 in its ability to modulate CD4 expression and in the size

of its transmembrane envelope molecule could have resulted from viral mutation or from cell-specific differences in envelope processing. In order to distinguish between these possibilities, cell-free virus obtained from the Sup-T1/HTLV-IVC.3 clone was used to reinfect HUT-78 cells, and a second line of productively-infected HUT-78 cells was established. Although this virus had failed to induce CD4 modulation on Sup-T1 cells, it produced complete loss of CD4 from the cell surface when passaged back into HUT-78 cells. In addition, Western blot analysis of virus produced by these HUT-78 cells again showed a transmembrane molecule of 32 kDa that was identical in size to the band of the original HTLV-IV virus produced in HUT-78 cells (Fig. 7B, lane 3). Restriction enzyme analysis of DNA from HTLV-IV-infected HUT-78 and Sup-T1 cells with an HTLV-IV full-length probe also showed identical patterns for five enzymes (B. Hahn, personal communication). These findings indicate that the difference in size of the HTLV-IV transmembrane envelope molecule produced by Sup-T1 cells most likely resulted from a cell-specific alteration in processing of the viral envelope by Sup-T1 cells rather than mutation in the virus itself.

In additional experiments (not shown), we determined whether biological differences could be detected for HTLV-IV produced by Sup-T1 cells. Similar to our findings described in Tables 1 and 2 for HTLV-IV produced by HUT-78 cells, cell-free virus obtained from Sup-T1/HTLV-IVC.3 were able to infect HUT-78 and Sup-T1 cells but not CEM or MOLT-4 cells. Thus, alterations in host range did not occur for HTLV-IV produced by Sup-T1 cells.

DISCUSSION

In this study, we evaluated biological features of HTLV-IV and determined that this virus shares several character-

FIG. 3. Coprecipitation of CD4 with HIV-1 and HTLV-IV envelope gene products. HUT-78 and Sup-T1 cells were used as a source of CD4, and MOLT 4 cells chronically infected by HTLV-IIIIB (MOLT/HTLV-IIIIB) and HUT-78 cells chronically infected by HTLV-IV (HUT-78/HTLV-IV) were used as a source of HIV-1 and HTLV-IV envelope molecules, respectively. (A) Cell lysates of [³⁵S]cysteine- and [³⁵S]methionine-labeled Sup-T1 (lane 1), HUT-78 (lane 2), MOLT/HTLV-IIIIB (lane 3), and HUT-78/HTLV-IV (lane 4) cells were prepared and immunoprecipitated as described in Materials and Methods with either OKT3 (lanes a), used as a negative control, or OKT4 (lanes b). The MOLT/HTLV-IIIIB cells and HUT-78/HTLV-IV cells were preadsorbed with OKT4 to remove detectable CD4. The 56-kDa CD4 band is seen in Sup-T1 and HUT-78 cells, but not cell lines from which CD4 had been adsorbed. (B) Portions of lysates shown in panel A were mixed together as described and immunoprecipitated with either OKT3 (lanes a) or OKT4 (lanes b). Lysate mixtures shown are Sup-T1 plus MOLT/HTLV-IIIIB (lane 1), Sup-T1 plus HUT-78/HTLV-IV (lane 2), HUT-78 plus MOLT/HTLV-IIIIB (lane 3), and HUT-78 plus HUT-78/HTLV-IV (lane 4). Bands corresponding to the 160- and 110-kDa envelope gene products of HTLV-IIIIB are seen coprecipitating with CD4 in lanes 1b and 3b, and bands corresponding to HTLV-IV envelope gene products are seen coprecipitating with CD4 in lanes 2b and 4b. (C and D) In order to establish the identity of the high-molecular-weight proteins coprecipitating with CD4 in panel B as viral gene products, lysates of Sup-T1 (C, lanes 1, 2, and 3), MOLT/HTLV-IIIIB (C, lanes 4, 5, and 6), HUT-78 (D, lanes 1, 2, and 3) and HUT-78/HTLV-IV (D, lanes 4, 5, and 6) were immunoprecipitated with normal human sera (lanes 1 and 4) or sera from a patient seropositive for either HIV-1 (lanes 2 and 5) or HTLV-IV (lanes 3 and 6). The 160- and 110-kDa bands of HTLV-IIIIB are detectable in the MOLT/HTLV-IIIIB cells with sera with antibodies to HIV-1 (C, lane 5), while the 140-kDa and 120-kDa envelope bands of HTLV-IV are detectable in the HUT-78/HTLV-IV cells with

FIG. 4. Evaluation of HTLV-IV infection of somatic cell hybrids of B and T cells. (A) Cell lines used in this study include the parental T cell line CEM, the parental B-cell line B721.174, and the hybrids CEM × 721.174 and a subclone, CEM × 721.174T.2 as described in the Results section. The surface expression of CD4 (T4), HLA class II (DR), and a B-cell (B1) antigen on each of the cell lines, as determined by monoclonal antibodies OKT4, OKDR, and B1, respectively, is shown. (B) Summary of susceptibility of parental and hybrid cell lines to infection and syncytia induction with either HTLV-IV or HIV-1.

istics with HIV-1. The host range of HTLV-IV is limited to CD4⁺ cells, and infection of HUT-78 and H9 cells was found to produce both cell fusion and cell death. As with HIV-1, both infection and syncytia formation could be completely prevented by anti-CD4 antibodies, and infection of HUT-78 cells by HTLV-IV resulted in the selective loss of CD4 from the cell surface. In addition, the high-molecular-weight externalized viral envelope and envelope precursor molecules of this virus were also shown to complex specifically to and coprecipitate with CD4 on infected cells. These findings clearly demonstrate that a binding site for CD4 exists on the HTLV-IV envelope and that this domain is highly conserved not only among two divergent groups of pathogenic human retroviruses, HIV-1 and HIV-2 (27, 34, 36), but also the simian viruses STLV-III_{agm} (19, 29) and SIV_{mac251}, which are indistinguishable from HTLV-IV (15, 16, 27, 29).

However, despite these similarities, remarkable differences between HTLV-IV and several strains of HIV-1 were observed. Among a panel of CD4⁺ lymphoid cell lines that were all highly susceptible to cell fusion by HIV-1, only HUT-78, H9, and the CD4⁺ B-cell line GM1500B were found to be susceptible to syncytia induction by HTLV-IV.

serum with antibodies to HTLV-IV (D, lane 6). The lack of serological cross-reactivity between the envelope proteins of HIV-1 and HTLV-IV is consistent with previous observations (24).

TABLE 4. Relative expression of CD4 and HLA class-II antigens on somatic cell hybrids^a

Cell line	% Positive cells				Mean fluorescence channel			
	P3	OKT4	OKT4A	OKDR	P3	OKT4	OKT4A	OKDR
CEM	0	99	99	1	7	194	94	7
B721.174	0	1	1	1	11	10	11	9
CEM × 721.174	1	100	97	99	11	168	84	265
CEM × 721.174T2	1	59	37	1	13	44	31	11

^a Cells shown in Fig. 4A were stained with monoclonal antibodies, and the percentage of cells reactive with each antibody and the mean channel of fluorescence intensity were determined by flow cytometry. See also Table 1, footnote a.

Moreover, with the exception of the Sup-T1 cell line discussed below, these were also the only CD4⁺ cell lines that were susceptible to HTLV-IV infection. The restricted host range of HTLV-IV to a minority of CD4⁺ cells indicated that the CD4 molecule was necessary but not sufficient for infection and raised the possibility that molecules in addition to CD4 were required to form a functional receptor for this virus. Interestingly, HUT-78, H9, and GM1500B cells are distinguishable from the HTLV-IV-resistant T-cell lines in that they coexpress CD4 with HLA class II gene products, suggesting that one or more of these molecules could facilitate viral binding, fusion, or penetration.

In order to investigate this possibility, we evaluated HTLV-IV infection of somatic cell hybrids that were produced by the fusion of the T cell line CEM and the B

lymphoblastoid cell line B721.174 (43). Of these parental lines, both CEM (CD4⁺ and HLA class II⁺) and B721.174 (CD4⁺ and HLA class II⁺) were determined to be resistant to HTLV-IV-induced fusion and infection. Remarkably, the hybrid line CEM × 721.174, which coexpressed CD4 and all HLA class II gene products (DR, DP, and DQ), was found to be highly susceptible to HTLV-IV infection and syncytia induction. This finding was consistent with the possibility that one or more HLA class II molecules were associating with CD4 to form a receptor complex for HTLV-IV. However, the finding that a class II⁺ subclone of CEM × 721.174 was also susceptible to HTLV-IV infection and fusion, as well as the failure of several monoclonal antibodies to inhibit fusion induced by this virus, argues against a direct role for the known HLA class II products in HTLV-IV infection. It

FIG. 5. Time course for HIV-1 and HTLV-IV infection of T-cell lines. HUT-78 cells (A and C) and Sup-T1 cells (B and D) were inoculated with cell-free virus containing either the HTLV-IIIIB strain of HIV-1 (A and B) or HTLV-IV (C and D) as described in Materials and Methods. Cultures were monitored at the indicated time points for reverse transcriptase activity (●), the percentage of cells positive for viral antigens by immunofluorescence microscopy (▲), and cell viability (○).

FIG. 6. CD4 expression on Sup-T1 cells infected by HTLV-IV. Fluorescence histograms with the OKT4 monoclonal antibody are shown (solid lines) for uninfected Sup-T1 cells and a cloned line of Sup-T1 cells productively infected by HTLV-IV (Sup-T1/HTLV-IVC.3) and in each panel are superimposed on the histograms for a nonreactive antibody (dotted lines). The Sup-T1/HTLV-IV cells were 100% positive for viral antigens by IFA and produced high levels of reverse transcriptase activity in culture supernatants (see Results). In contrast to the downmodulation of CD4 on HUT-78 cells following HTLV-IV infection (Fig. 2), little change in CD4 expression is observed in the infected Sup-T1 cells with antibodies that compete (OKT4A) and do not compete (OKT4) with the viral envelope for binding to CD4.

appears likely, however, that additional molecules are required for HTLV-IV infection which are either provided or induced by fusion of the B721.174 cell line with CEM, and it is still possible that these molecules are normally coexpressed with HLA class II gene products. Interestingly, in the case of HIV-1, the failure of this virus to penetrate or infect nonhuman cells transfected with a CD4 cDNA has also indicated that cellular factors in addition to CD4 are required for infection by this virus (30, 35). In addition, the report of Mann et al. (D. L. Mann, F. Lesane, W. A. Blattner, and M. Popovic, IIIrd Int. Conf. AIDS, 1987, abstr. 209) showing a reduction in HLA-DR expression following binding of HIV-1 to H9 cells suggested that this HLA determinant, at least for some cells, may participate directly with CD4 during the binding or penetration of HIV-1. Further studies of HTLV-IV infection of hybrid cell lines that appear to reconstitute functional viral receptors may be useful in identifying the putative CD4-associated molecules that are probably required for HTLV-IV infection and by analogy may provide clues to the identity of related cellular molecules associated with HIV-1 infection.

Remarkable differences between HTLV-IV and HIV-1 were also noted during the infection of the Sup-T1 cell line. First, although Sup-T1 is highly susceptible to cytopathic effects induced by HIV-1, including cell fusion and cell death (22), neither fusion nor killing occurred during HTLV-IV infection of this cell line. Second, when the kinetics of infection were compared between HTLV-IV and several strains of HIV-1, nearly all cells became infected by HIV-1 within 10 days, whereas even after 2 months, only a minority of Sup-T1 cells expressed HTLV-IV antigens (Fig. 5). Third, when clones of HTLV-IV-infected Sup-T1 cells were obtained that were 100% infected by IFA and which produced high levels of reverse transcriptase, only minimal reductions

in the expression of CD4 occurred on the cell surface, as determined by monoclonal antibodies that compete (OKT4A and Leu-3a) and do not compete (OKT4) with the viral envelope for binding to CD4. The failure of HTLV-IV to downmodulate CD4 from the cell surface is in marked contrast to the effects of HTLV-IV infection on HUT-78 cells (Fig. 2) and of HIV-1 infection on all T-cell lines evaluated by our laboratory (22). Finally, the size of the HTLV-IV transmembrane envelope molecule was slightly larger for virus produced by Sup-T1 cells than for virus produced by HUT-78 cells. Both the differences in size of the transmembrane molecule and in the lack of an effect on CD4 expression on Sup-T1 were shown not to be due to viral mutation, since when the HTLV-IV virus produced by Sup-T1 cells was passaged back into HUT-78, the infected HUT-78 cells again produced a 32-kDa molecule and expressed no detectable CD4 on the cell surface. The larger size of the HTLV-IV transmembrane molecule produced from Sup-T1 cells could involve differences in glycosylation or possibly the use of an alternative cleavage site within the envelope precursor molecule and will require further biochemical analysis.

Taken together, these observations indicate that HTLV-IV infection of Sup-T1 cells occurs inefficiently compared with that of HUT-78 cells and in a completely noncytopathic manner. In addition, they demonstrate that the inability of the viral envelope to induce cell fusion with Sup-T1 cells probably has profound effects on both the rate of infection and the occurrence of cytopathic effects during infection. Indeed, other CD4-positive lines that were resistant to syncytia formation with HTLV-IV, such as CEM and MOLT-4, could not be infected by this virus. As discussed above, Sup-T1 cells may lack a CD4-associated molecule present on HUT-78, H9, and GM1500B cells that could

FIG. 7. Analysis of HTLV-IV envelope glycoproteins from infected HUT-78 and Sup-T1 cells. (A) Radioimmunoprecipitation. [35 S]cysteine- and [35 S]methionine-labeled cell lysates of HTLV-IV-infected Sup-T1 cells (lanes 1 and 2) and HUT-78 cells (lanes 3 and 4) were prepared and immunoprecipitated with normal human serum (lanes 1 and 3) or serum from a patient with antibodies to HTLV-IV (lanes 2 and 4). Although the intensity of the high-molecular-weight envelope bands is greater for Sup-T1, no apparent differences in molecular weight are apparent. (B) Western blot. Cell-free virus was prepared from cultures of HTLV-IV-infected HUT-78 cells (lane 1) and HTLV-IV-infected Sup-T1 cells (lane 2) and analyzed by Western blot with either serum from a patient with antibodies to HTLV-IV (lanes a) or normal human serum (lanes b). The 32-kDa transmembrane envelope molecule shown for HTLV-IV-infected HUT-78 cells (lane 1a) is increased in size for virus produced by the infected Sup-T1 cells (lane 2a). HTLV-IV produced by Sup-T1 was used to infect fresh HUT-78 cells, and virus produced by these HUT-78 cells was analyzed (lane 3). The size of the transmembrane molecule for this virus is similar to the initial HTLV-IV produced by HUT-78 cells (lane 1). Strips from the same electrophoresis blot are shown.

facilitate viral binding or fusion. It is unclear whether the apparent difference in envelope processing in Sup-T1 cells which produces a slightly larger transmembrane molecule contributes to the functional alteration in the ability of this envelope to bind to and downmodulate CD4. It has clearly been shown for the HIV-1 envelope that the native or recombinant gp110-120 molecule binds directly to CD4 with high affinity and without the need for other cellular or viral molecules (30). However, for some viruses such as HTLV-IV, accessory molecules may play a role in the net interaction of envelope with CD4 on human cells that could effect viral binding, fusion, or penetration during the early phases of infection, as well as the ability of the envelope to modulate CD4 following infection. Thus, the apparent failure of the HTLV-IV envelope to modulate CD4 in Sup-T1 cells could result from the absence of this accessory molecule rather than a difference in processing of the viral envelope. Differences in affinity of the gp120 envelope molecule for CD4 may also exist among different viruses or the same virus produced by different cells that could contribute to these effects. Indeed, the finding that HTLV-IV produced by Sup-T1 cells remained infectious for HUT-78 cells does suggest that the envelope of this virus retained the ability to bind to CD4, and in recent experiments we have demonstrated that the HTLV-IV envelope produced by Sup-T1 cells is able to bind and coprecipitate with CD4 (data not shown).

These results show that although the HIV and SIV retroviruses share a number of characteristics, striking biological

differences exist. Recent studies by Koenig et al. with vaccinia virus constructs which express STLTV-III_{agm} envelope gene products have also shown a limited range of CD4⁺ cells that are susceptible to cell fusion, results which are similar to our observations for HTLV-IV (S. Koenig and P. Johnson, manuscript in preparation). These data are consistent with the findings that HTLV-IV and STLTV-III_{agm} are indistinguishable from each other and from the macaque isolate SIV_{mac251}, noted previously (15, 16, 27, 29). Nonetheless, despite the suspected simian origin of HTLV-IV, the finding that marked biological differences exist among viruses that are all tropic for CD4 may provide clues to the more general viral and cellular factors that are important in the pathogenesis of the members of this diverse family of retroviruses.

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LITERATURE CITED

1. Allan, J. S., J. E. Coligan, F. Barin, M. F. McLane, J. G. Sodroski, C. A. Rosen, W. A. Haseltine, T. H. Lee, and M. Essex. 1985. Major glycoprotein antigens that induce antibodies in AIDS patients are encoded by HTLV-III. *Science* **228**:1091-1094.
2. Barin, F., S. M'Boup, F. Denis, P. Kanki, J. S. Allan, T. H. Lee, and M. Essex. 1985. Serological evidence for virus related to simian T-lymphotropic retrovirus III in residents of west Africa. *Lancet* **ii**:1387-1389.
3. Barré-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Danguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**:868-870.
4. Benveniste, R. E., L. O. Arthur, C. Tsai, R. Sowder, T. D. Copeland, L. E. Henderson, and S. Oroszlan. 1986. Isolation of a lentivirus from a macaque with lymphoma: comparison with HTLV-III/LAV and other lentiviruses. *J. Virol.* **60**:483-490.
5. Clapham, P. R., R. A. Weiss, A. G. Dalgleish, M. Exley, D. Whitby, and N. Hogg. 1987. Human immunodeficiency virus infection of monocytic and T-lymphocytic cells: receptor modulation and differentiation induced by phorbol ester. *Virology* **158**:44-51.
6. Clavel, F., D. Guetard, F. Brun-Vezinet, S. Chamaret, M. A. Rey, M. O. Santos-Ferreira, A. G. Laurent, C. Danguet, C. Katlama, C. Rouzioux, D. Klatzmann, J. L. Champalimaud, and L. Montagnier. 1986. Isolation of a new human retrovirus from West African patients with AIDS. *Science* **233**:343-346.
7. Clavel, F., M. Guyader, D. Guetard, M. Salle, L. Montagnier, and M. Alizon. 1986. Molecular cloning and polymorphism of the human immunodeficiency virus type 2. *Nature (London)* **324**:691-695.
8. Dalgleish, A. G., P. C. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss. 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature (London)* **312**:763-767.

9. Daniel, M. D., N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers. 1985. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* 228:1201-1204.
10. DeFreitis, E. C., B. Dietzschold, and H. Koprowski. 1985. Human T-lymphocyte response in vitro to synthetic peptides of herpes simplex glycoprotein D. *Proc. Natl. Acad. Sci. USA* 82:3425-3429.
11. DeMars, R., C. C. Chang, S. Shaw, P. J. Reitnauer, and P. M. Sondel. 1984. Homozygous deletions that simultaneously eliminate expressions of class I and class II antigens of EBV-transformed B-lymphoblastoid cells. I. Reduced proliferative responses of autologous and allogeneic T cells to mutant cells that have decreased expression of class II antigens. *Hum. Immunol.* 11:77-97.
12. Essex, M., and P. Kanki. 1988. Comparison of simian immunodeficiency isolates. *Nature (London)* 331:621-622.
13. Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan. 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercopithecus atys*). *Proc. Natl. Acad. Sci. USA* 82:5286-5290.
14. Guyader, M., M. Emmerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon. 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature (London)* 326:662-669.
15. Hahn, B. H., L. I. Kong, S.-W. Lee, P. Kumar, M. E. Taylor, S. K. Arya, and G. M. Shaw. 1987. Relation of HTLV-4 to simian and human immunodeficiency-associated viruses. *Nature (London)* 330:184-186.
16. Hahn, B. H., P. Kumar, M. E. Taylor, S. K. Arya, and G. M. Shaw. 1987. Molecular cloning and nucleotide sequence analysis of HTLV-4: comparison to other human and simian immunodeficiency-associated retroviruses, p. 57-74. In D. Bolognesi (ed.), *Human retroviruses, cancer and AIDS: approaches to prevention and therapy*. UCLA Symposia on Molecular and Cellular Biology, vol. 71. Alan R. Liss, Inc., New York.
17. Hahn, B. H., M. A. Gonda, G. M. Shaw, M. Popovic, J. Hoxie, R. C. Gallo, and F. Wong-Staal. 1985. Genomic diversity of the AIDS virus HTLV-III: different viruses exhibit greatest divergence in their envelope genes. *Proc. Natl. Acad. Sci. USA* 82:4813-4817.
18. Hahn, B. H., G. M. Shaw, M. E. Taylor, R. R. Redfield, P. D. Markham, S. Z. Salahuddin, F. Wong-Staal, R. C. Gallo, E. S. Parks, and W. P. Parks. 1986. Genetic variation of HTLV-III/LAV over time in patients with AIDS or at risk of AIDS. *Science* 232:1548-1554.
19. Hirsch, V., N. Riedel, and J. I. Mullins. 1987. The genome organization of STLV-3 is similar to that of AIDS virus except for a truncated transmembrane protein. *Cell* 49:307-319.
20. Hoxie, J. A., L. E. Flaherty, B. S. Haggarty, and J. L. Rackowski. 1986. Infection of T4 lymphocytes by HTLV-III does not require expression of the OKT4 epitope. *J. Immunol.* 136:361-363.
21. Hoxie, J. A., B. S. Haggarty, J. R. Rackowski, N. Pillsbury, and J. A. Levy. 1985. Persistent noncytopathic infection of normal T lymphocytes with AIDS-associated retrovirus. *Science* 229:1400-1402.
22. Hoxie, J. A., J. D. Alpers, J. L. Rackowski, K. Huebner, B. S. Haggarty, A. J. Cedarbaum, and J. C. Reed. 1986. Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. *Science* 234:1123-1127.
23. Kanki, P. J., J. Alroy, and M. Essex. 1985. Isolation of T-lymphotropic retrovirus related to HTLV-III/LAV from wild-caught African green monkeys. *Science* 230:951-954.
24. Kanki, P. J., F. Barin, S. M'Boup, J. S. Allan, J. L. Romet-Lemmonne, R. Marlink, M. F. McLane, T. H. Lee, B. Arbeille, F. Denis, and M. Essex. 1986. New human T-lymphotropic retrovirus related to simian T-lymphotropic virus type III (STLV-IIIAGM). *Science* 232:238-243.
25. Kanki, P. J., M. F. McLane, N. W. King, Jr., N. L. Letvin, R. D. Hunt, P. Sehgal, M. D. Daniel, R. C. Desrosiers, and M. Essex. 1985. Serologic identification and characterization of a macaque T-lymphotropic retrovirus closely related to HTLV-III. *Science* 228:1199-1201.
26. Kannagi, M., J. M. Yetz, and N. L. Letvin. 1986. In vitro growth characteristics of simian T-lymphotropic virus type III. *Proc. Natl. Acad. Sci. USA* 82:7053-7057.
27. Kestler, H. W., III, Y. Li, Y. M. Naidu, C. V. Butler, M. F. Ochs, G. Jaenel, N. W. King, M. D. Daniel, and R. C. Desrosiers. 1988. Comparison of simian immunodeficiency virus isolates. *Nature (London)* 331:619-621.
28. Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier. 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature (London)* 312:767-768.
29. Kornfeld, H., N. Riedel, G. Viglianti, V. Hirsch, and J. I. Mullins. 1987. Molecular cloning and biological activity of human T-lymphotropic virus type 4 and its relation to sub-human primate viruses and human immunodeficiency viruses. *Nature (London)* 326:610-613.
30. Lasky, L. A., G. Nakamura, J. H. Smith, C. Fennie, L. Shimozaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon. 1987. Delineation of a region of the human immunodeficiency virus type 1 (HIV-1) gp120 glycoprotein critical for interaction with the CD4 receptor. *Cell* 50:975-985.
31. Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Lanois, J. M. Shimabukuro, and L. S. Oskiro. 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* 225:840-842.
32. Levy, J. A., J. Shimabukuro, T. McHugh, C. Casavant, D. Stites, and L. Oshiro. 1985. AIDS-associated retroviruses (ARV) can productively infect other cells besides human T helper cells. *Virology* 147:441-448.
33. Lifson, J. D., G. R. Reyes, M. S. McGrath, B. S. Stein, and E. G. Engleman. 1986. AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. *Science* 232:1123-1127.
34. Lowenstein, L. J., N. C. Pederson, J. Higgins, K. C. Pallis, A. Uyeda, P. Marx, N. W. Lerche, R. J. Munn, and M. B. Gardner. 1986. Seroprevalence survey of captive Old World primates for antibodies to human and simian retroviruses, and isolation of a lentivirus from sooty mangabeys (*Cercopithecus atys*). *Int. J. Cancer* 38:563-574.
35. Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* 47:333-348.
36. McClure, M. O., Q. J. Sattentau, P. C. L. Beverley, J. P. Hearn, A. K. Fitzgerald, A. J. Zuckerman, and R. A. Weiss. 1987. HIV infection of primate lymphocytes and conservation of the CD4 receptor. *Nature (London)* 330:487-489.
37. McDougal, J. S., A. Mawle, S. P. Cort, J. K. A. Nicholson, D. G. Cross, J. A. Scheppler-Campbell, D. Hicks, and J. Sligh. 1985. Cellular tropism of the human retrovirus HTLV-III/LAV. I. Role of T cell activation and expression of the T4 antigen. *J. Immunol.* 135:3151-3162.
38. McDougal, J. S., J. K. A. Nicholson, D. G. Cross, S. P. Cort, S. M. Kennedy, and A. C. Mawle. 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *J. Immunol.* 137:2937-2944.
39. McDougal, J. S., M. S. Kennedy, J. N. Sligh, S. P. Cort, A. Mawle, and J. K. A. Nicholson. 1986. Binding of HTLV-III/LAV to T4⁺ cells by a complex of 110K viral protein and the T4 molecule. *Science* 231:382-385.
40. Murphy-Corb, M., L. N. Martin, S. R. S. Rangan, G. B. Baskin, B. J. Gormus, R. H. Wolf, W. A. Andes, M. West, and R. C. Montelaro. 1986. Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. *Nature (London)* 321:435.
41. Ohta, Y., T. Masuda, H. Tsujimoto, K. Ishikawa, T. Kodama, S. Morikawa, M. Nakai, S. Honjo, and M. Hayami. 1988. Isolation of simian immunodeficiency virus from African green monkeys and seroprevalence survey of the virus in various non-human

- primates. *Int. J. Cancer* **41**:115–122.
42. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. A method for detection, isolation, and continuous production of cytopathic human T-lymphotropic retroviruses of the HTLV family (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**:497–500.
43. **Salter, R. D., D. N. Howell, and P. Cresswell.** 1985. Genes regulating HLA class I antigen expression in T-B lymphoblast hybrids. *Immunogenetics* **21**:235–246.
44. **Sattentau, Q. J., A. G. Dalgleish, R. A. Weiss, and P. C. L. Beverley.** 1986. Epitopes of CD4 antigen and HIV infection. *Science* **234**:1120–1122.
45. **Smith, S. D., M. Shatsky, P. S. Cohen, R. Warnke, M. P. Link, and B. E. Glader.** 1984. Monoclonal antibody and enzymatic profiles of human malignant T-lymphoid cells and derived cell lines. *Cancer Res.* **44**:5657–5660.
46. **Sodroski, J., W. C. Goh, C. Rosen, K. Campbell, and W. A. Haseltine.** 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature (London)* **322**:470–474.