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Specific Cell Surface Requirements for the Infection of CD4-Positive Cells by Human Immunodeficiency Virus Types 1 and 2 and by Simian Immunodeficiency Virus

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Human CD4 was expressed on a range of mammalian cell lines. CD4$^+$ non-primate cells, derived from rat, hamster, mink, cat, and rabbit, bind recombinant gp120 of human immunodeficiency virus type 1 (HIV-1) but are resistant to HIV-1 infection. CD4 expression on various human, rhesus, and African green monkey cell lines confers differential susceptibilities for HIV-1, HIV-2, and simian immunodeficiency (SIV) strains. For example, CD4$^+$ TE671 rhabdomyosarcoma cells are sensitive to HIV-1 and HIV-2 but resistant to SIV, whereas CD4$^+$ U87 glioma cells are resistant to HIV-1 infection but sensitive to HIV-2 and SIV. HIV-1 infection was not dependent on human major histocompatibility class I expression. Studies of cell fusion and of infection by vesicular stomatitis virus pseudotypes bearing HIV-1 and HIV-2 envelopes showed that the differential cell tropisms of HIV-1, HIV-2, and SIV are determined at the cell surface. © 1991 Academic Press, Inc.

INTRODUCTION

Human and simian immunodeficiency viruses (HIV-1, HIV-2, and SIV) utilize the CD4 antigen as a receptor for binding to the cell surface (Dalgleish *et al.*, 1984; Klatzmann *et al.*, 1984; Sattentau *et al.*, 1988). The events following binding to CD4 that effect membrane fusion and internalization remain obscure. Many enveloped viruses such as influenza virus, Semliki forest virus, and vesicular stomatitis virus are pH-dependent for entry, whereas others such as paramyxoviruses are pH-independent (Marsh and Helenius, 1989). HIVs, like most mammalian retroviruses, are pH-independent (Stein *et al.*, 1987; McClure *et al.*, 1988, 1990), and this is reflected in their capacity to induce cell-to-cell fusion resulting in multinucleated syncytia.

The hemagglutinin (HA) spike glycoprotein of influenza virus determines its pH dependence and requires the acidic environment of the endosome to trigger a conformational change in its structure. This alteration is essential for the HA fusion domain to interact with the endosomal membrane and induce fusion of viral and cell membranes (White *et al.*, 1983). HIV may require a similar change in structure of the gp120/gp41 spikes to position the putative gp41 fusion domain (Gallagher, 1987; Kowalsky *et al.*, 1987) within reach of the cell membrane. Although little is understood about the sequence of events occurring between CD4 binding and membrane fusion, several observations suggest that at least one event is needed at this stage:

(1) Mouse cells of different lineage expressing human CD4 bind virus but infection is blocked before fusion (Maddon *et al.*, 1986). CD4 constructs with truncated cytoplasmic sequences, with substituted murine CD4 cytoplasmic domains, or with the two CD4 N-terminal domains linked to the cell membrane by a glycophosphotidyl inositol tail confer susceptibility to human HeLa cells but not to mouse cells (Bedinger *et al.*, 1988; Maddon *et al.*, 1988; Diamond *et al.*, 1990).

(2) Antibodies to the major type-specific neutralization epitope on gp120 (the V3 loop, amino acid residues 306–325) efficiently inhibit infection after adsorption to cells, and do not affect the virion/CD4 interaction (Linsley *et al.*, 1988; Skinner *et al.*, 1988).

(3) Single amino acid changes at residues 266, 267, and 268 in gp120 lead to viral mutants that bind to CD4 but are not infectious (Willey *et al.*, 1988). Antibodies raised to synthetic peptides spanning residues 254–274 of gp120 neutralize HIV-1 strains without affecting CD4 binding (Ho *et al.*, 1988).

(4) Regions of CD4 not required for gp120 binding may play a role in postbinding events leading to membrane fusion (Camerini and Seed, 1990; Healey *et al.*, 1990).

We expressed human CD4 on cell lines derived from rabbit, rat, hamster, cat, and mink to ascertain whether the restriction to infection of CD4$^+$ mouse cells was widespread among different species. Further CD4$^+$ cell lines derived from human, rhesus macaque and African green monkey cells were constructed to investigate their susceptibility to HIV-1, HIV-2, and SIV strains. While none of the non-primate cell lines was

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permissive for HIV or SIV, we observed differential susceptibilities of human and simian cells expressing human CD4.

MATERIALS AND METHODS

Cell lines

$CD4^+$ cell lines constructed included human glioma U87.MG (Westermark *et al.*, 1973), human rhabdomyosarcoma TE671/RD (McAllister *et al.*, 1977; Stratton *et al.*, 1989), human (class I negative, EBV transformed) B cell line Daudi (Ploegh *et al.*, 1979) rhesus macaque kidney LLCMK2 (Hull *et al.*, 1962), and FRhK/4T (Wallace *et al.*, 1973), African green monkey BGM (Barron *et al.*, 1970), rabbit corneal SIRC (Leerhoy, 1965), cat kidney CCC (Crandel *et al.*, 1973), mink lung Mv-1-lu (CCL64, Henderson *et al.*, 1974), Chinese hamster ovary (CHO) cells (Stephens *et al.*, 1990), rat XC cells (Simkovic *et al.*, 1963), rat leukemic basophil 2H3 (Siragarian *et al.*, 1982), and rat sarcoma HSN (Currie and Gage, 1973). Human HeLa-$CD4^+$ cells were provided by P. Maddon (Maddon *et al.*, 1986). All these cell lines except Daudi-$CD4^+$ were maintained in Dulbecco's modified Eagle's medium with 5% fetal calf serum (FCS). Human lymphoid cell lines, C8166 (Salahuddin *et al.*, 1983), H9 (Popovic *et al.*, 1984), MOLT 4 (clone 8) (Ohta *et al.*, 1988), and Daudi were maintained in RPMI 1640 and 10% FCS.

Viruses

HIV-1 strains used in this study were HTLV-IIIB (IIIB) and HTLV-IIIRF (RF) (Popovic *et al.*, 1984), SF-2 (Levy *et al.*, 1984), NY5 (Benn *et al.*, 1985), CBL-4, Z84, and Z129 (Weiss *et al.*, 1986), U455 (Oram *et al.*, 1990), and Z34 (Srinivasan *et al.*, 1987). NDK (Spire *et al.*, 1989) was provided by B. Spire.

HIV-2 strains used were $LAV\text{-}2_{ROD}$ (Clavel *et al.*, 1986), SBL6669 (Albert *et al.*, 1987), CBL-20, CBL-21, CBL-22, and CBL-23 (Schulz *et al.*, 1990).

SIV_{AGM} strain TYO-2 (Ohta *et al.*, 1988) was obtained from M. Hayami. SIV_{MAC} strain 251 (Daniel *et al.*, 1985) and its derivative 32H (Cranage *et al.*, 1989) were obtained from H. Holmes. SIV_{SM} strain B690 was provided by M. Murphey-Corb (Murphey-Corb *et al.*, 1986).

Monoclonal antibodies (mAbs)

OKT4 A (Ortho Diagnostics), Leu 3a (Becton Dickinson), and 318/4120 (Evans and Co.) were used for surface CD4 labeling. These antibodies bind to the V1 domain of CD4 and sensitively inhibit HIV infection of $CD4^+$ T-cells.

B2.62.2 (Immunotech) and HC11.151.1 (a gift from C. Devaux) are monoclonal antibodies to human β-2-microglobulin. W6/32 (Serolabs) reacts with a conserved epitope expressed on all human class I heavy chains.

CD4 expression on mammalian cell lines

Ψ-AM/CD4 and PA317/CD4 cells were provided by P. Maddon (Columbia University, New York) and B. Chesebro (National Institutes of Health, Hamilton, MT), respectively. Both cell lines produce amphotropic retrovirus particles encoding the cDNA of the CD4 message downstream from the MoMLV LTR promoter as well as a neomycin resistance-selectable marker (Maddon *et al.*, 1986). Target cell lines, subconfluent in exponential growth phase, were infected with virus-containing supernatant from either Ψ-AM/CD4 or PA317/CD4 cell cultures. Cells were incubated for 2 days at 37°, before dividing into 10 new cultures and adding selection medium containing geneticin G418 (GIBCO), usually at 1 mg/ml. Cultures were fed twice weekly with fresh G418-containing medium until resistant colonies of cells emerged. Resistant cells were tested for CD4 expression by immunofluorescent staining and either used as a pool or cloned by limiting dilution to select cells expressing high levels of CD4.

Production and assay of HIV and SIV stocks

Production and assay of HIV-1 and HIV-2 stocks were as described previously (Clapham *et al.*, 1987; Schulz *et al.*, 1990). The optimum conditions for the preparation of SIV stocks are less well defined. Chronically infected H9 or C8166 cells were mixed with four times as many MOLT 4 cells and resuspended in medium at 5×10^5 cells/ml. Supernatant was harvested when extensive cytopathology (syncytia) was apparent. SIV infection was titrated on MOLT 4 cells using the end-point dilution protocol described previously for HIV (Clapham *et al.*, 1987).

Syncytium induction assays

Cell fusion was carried out by mixing equal numbers of virus-producing cells and appropriate indicator cells (5×10^5 cells of each type in 1 ml per 1-cm diameter well). After overnight incubation, syncytia were scored. Cells were washed once in serum-free phosphate-buffered saline and fixed and stained in methanol containing 1% methylene blue and 0.25% basic fuchsin for 10 min. Cell layers were then washed in tap water and examined for syncytia by low-power microscopy.

Cell fusion was also used as an indication of HIV or SIV production. C8166 cells were used as indicators

for HIV-1 and HIV-2 syncytia and MOLT 4 cells were preferred for SIV fusion. In these assays, the presence of giant syncytia was determined in suspension without staining.

An estimate of the fraction of nuclei incorporated into syncytia in adherent cells was made and recorded as follows; −, <2%; +, 2–5%; ++, 5–20%; +++, 20–50%; ++++, >50%.

Vesicular stomatitis virus (VSV) pseudotypes

Production and assay of VSV pseudotypes bearing envelope glycoproteins of HIV were similar to those previously described for human retroviruses (Clapham *et al.*, 1984, 1987). Briefly, excess neutralizing sheep anti-VSV serum (provided by J. Zavada) was added to VSV harvested from cells chronically infected with HIV-1 or HIV-2. Pseudotypes were plated on cell layers seeded at 2.5×10^5 cells per 3-cm-diameter well 1 day before infection. Pseudotype was adsorbed to polybrene-treated (20 μg/ml) cells for 1 hr before washing and adding excess mink lung cells (Mv-1-lu, not susceptible to HIV). Mink cells acted as a plaque indicator layer for progeny VSV released from test cells infected with pseudotype. After attachment of the mink cells (1.5 hr), the cells were overlaid with agar medium. Plaques were counted 1–2 days after plating.

Immunofluorescence assays

Adherent cells were detached by Versene and stained as for suspension cells. Appropriate concentrations of anti-CD4 monoclonal antibody (Leu3a) were added to 5×10^5 cells containing 0.02% sodium azide and incubated at 37° for 30 min. After two washes in phosphate-buffered saline containing azide and 1% FCS, the cells were incubated for a further 30 min in fluorescein isothiocyanate (FITC) conjugated with rabbit anti-mouse IgG. A sample of cells were then examined by fluorescence microscopy and the percentage of fluorescing cells recorded. Samples for flow cytometry analysis were prepared in the same way.

Radiolabeling assays for estimating surface CD4 and gp120 binding

CD4 assay. The anti-CD4 monoclonal antibody, mAb 318/4120 (Evans and Co.), was used for most cell lines. Adherent cells expressing surface CD4 and $CD4^-$ control cells were plated in 1-cm wells (10^5 cells in 1 ml) 2 days before assay. All reagents were diluted in complete phosphate-buffered saline containing 1% FCS and 0.02% sodium azide (cPBS/FCS/NaN_3). Initially mAb 318 was titrated on the highest-expressing CD4 cell line to ensure that saturating levels were used in each assay.

One hundred microliters of mAb 318 (10 μg/ml) was added to cell layers and incubated at 4° for 60 min and washed twice in cPBS and 100 μl of anti-mouse IgG/^{125}I conjugate (10^5 cpm/ml, Amersham) was added for a further 60 min at 4°. The cell layer was washed three times and solubilized in 100 μl 4 *M* sodium hydroxide and 100 μl 1% empigen before counting in a gamma counter (Beckman 5500B). For suspension cells, assays were carried out in V-bottom 96-well plates. One hundred thousand cells were tested in each sample and washes were performed by pelleting cells at low speed. CD4 expression on CHO, XC, 2H3, and Daudi cells was measured in the same way but using mAb Leu 3a.

gp120 binding assay. Purified recombinant gp120 (derived from HIV-1 IIIB) produced from Chinese hamster ovary (CHO) cells was provided by P. Stephens (Celltech). One hundred microliters gp120 (2 μg/ml) was added to cells as described in the CD4 assay and incubated at 4° for 60 min before washing twice in cPBS/FCS/NaN_3. One hundred microliters of anti-gp120 mAb 110.5 (Kinney Thomas *et al.*, 1988) (Genetic Systems) ascites diluted 1/400 was added and the cells again were incubated at 4° for 60 min and washed as before. One hundred microliters of IgG/^{125}I conjugate (10^5 cpm/ml) (Amersham) was added and incubated at 4° for 60 min as before. Cell layers were then solubilized and counted as described for the CD4 assay. Suspension cells were treated as in the CD4 assay. gp120 binding on $CD4^+$ TE671, U87, and Daudi cells was also estimated by direct labeling with an ^{125}I-gp120 conjugate (provided by A. Pelchen-Matthews).

Activation of 2H3 cells

$CD4^+$ and $CD4^-$ 2H3 rat leukemic basophil cells (10^6 cells/ml) were primed by incubating with rat IgE (10 μg/ml) (provided by C. Dean) in growth medium at 37° on a rotary mixer. The cells were washed once and goat anti-mouse immunoglobulin (Serolabs, 1/1000) was added with 100 μl of IIIB virus or with 100 μl of medium (mock-infected), for 30 min at 37°. The mock-infected cells were assessed for activation of basophil granules by testing for the release of proteases as follows. Clarified cell lysate 2×50 μl was added to 50 μl of substrate, 1 m*M* *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Sigma) in 0.2 *M* sodium citrate, pH 4.5. Samples were placed in wells in a 96-well plate and incubated for 45 min at room temperature. Unstimulated 2H3 cells were used as a negative control and the reaction was quenched by addition of 100 μl of bicarbonate

buffer (0.5 *M*, pH 9). Protease activity was assessed by reading color development in wells at 400 n*M*. Infected cells were incubated at 37° and assessed each day for cpe as well as harvesting supernatant every 4 days to test for p24 antigen presence.

ELISA for detection of HIV-1 p24 gag antigen

One hundred microliters of p24 capture antibody D7320 (Aalto Bioreagents, Dublin) (10 μg/ml in 100 m*M* sodium bicarbonate, pH 8.5) was added to each well of a 96-well plate and incubated overnight at room temperature. D7320 was raised against three conserved HIV-1 *gag* peptides. Wells were then washed twice with 200 μl TBS buffer (25 m*M* Tris–HCl, 144 m*M* NaCl, pH 7.5) and treated with 2% milk powder (Cadbury's Marvel) in TBS for 30 min at room temperature. Supernatants for testing (from virus-producing cultures) were inactivated by adding empigen zwitterionic detergent (Surfachem Ltd.) to 1% and further diluted to give a final concentration of empigen of 0.05%. Further dilutions were made in TBS + 0.05% empigen where appropriate. One hundred-microliter samples in duplicate were added to wells and incubated for 3–4 hr at room temperature. The wells were washed twice in 200 μl TBS and an anti-p24/alkaline phosphatase conjugate (EH12E1-AP) (Novo Biolabs, Cambridge) added. The EH12E1-AP was used at a predetermined concentration of 1/1000 in TMT-SS (TMT-SS is TBS containing 4% milk powder, 20% sheep serum, and 0.5% tween 20), for 1 hr at room temperature. This time wells were washed 6 times in 200 μl of AMPAK-WASH, before amplifying using an AMPAK kit (Novo Biolabs, Cambridge). The optical density was then measured at 490 nm on a Dynatech MR710 microplate reader. The assay was calibrated with recombinant p24 (HTLV-IIIB) purified from Ty yeast particles (British Biotechnology Ltd.).

Reverse transcriptase assay

Reverse transcriptase assays followed the method of Hoffman *et al.* (1985) precisely. Supernatant culture fluid was harvested from HIV- or SIV-producing cultures. The RNA-directed DNA polymerase activity was assayed without concentrating the virus, using an exogenous, synthetic poly(rA)·oligo(dT) template-primer with Mg^{2+} as the divalent cation.

Polymerase chain reaction

Polymerase chain reactions (PCRs) for DNA amplification were carried out essentially as described by Saiki *et al.* (1986). Between 0.1 and 1 μg of DNA extracted from test cells was mixed with 250 ng of each primer (see below) and added to the four dNTPs (0.2 μ*M*) in 10 m*M* Tris–HCl, pH 8, 50 m*M* KCl, 2 m*M* $MgCl_2$. *Taq* polymerase enzyme (2.5 units) was added to each reaction mix in a total volume of 100 μl. 30 sequential cycles of amplification were carried out; 95° for 1.5 min, 55° for 2 min and 72° for 3 min.

Primers used were derived from the HTLV-IIIB *gag* sequence:

DP 818 5′-GAGGAAGCTGCAGAATGG-3′

1407–1425

ADP 819 5′-GGTCCTTGTCTTATGTC

CAGAATGCTG-3′ 1620–1646

These primers generated a 240-bp band fragment in positive samples. Fifty microliters of each amplification product was run on a 1.5% agarose gel and photographed after ethidium bromide staining and uv transillumination.

Polyethylene glycol-induced infection of CD4⁺ mink cells

The fusigen polyethylene glycol (PEG) was added to HIV-1-adsorbed mink-CD4⁺ cells to induce fusion of cell and virus membranes. One hundred thousand mink-CD4⁺ cells were seeded in 1-cm-diameter wells of a 24-well cluster dish, 2 days before infection. One hundred microliters of HIV-1/RF virus (10^6 TCIDs for C8166 cells) was added on ice and allowed to adsorb for 1 hr. One-half milliliter of 50% PEG (1500) in growth medium prewarmed to 37° was added and the cell tray kept at 37°. After 1 min an equal volume of medium was added dropwise over a 2-min period. The well was then filled up with growth medium dropwise, before washing, adding fresh medium, and incubating at 37°. After 2 days incubation and subsequently twice weekly, the cell layers were trypsinized and passaged. After 1 and 2 weeks, cell samples were cocultivated with C8166 cells to detect progeny virus. Supernatant was also collected for p24 and reverse transcriptase assays.

RESULTS

CD4 expression and gp120 binding of CD4⁺ cell lines

CD4⁺ cell lines of various mammalian species were prepared as described under Materials and Methods. Figure 1 shows the flow cytometry analysis of Leu 3a-stained mink cells before and after CD4 introduction, and also the fluorescence microscopy of the same cells. A wide range of CD4 expression is apparent on uncloned CD4⁺ mink cells. CD4 expression by each

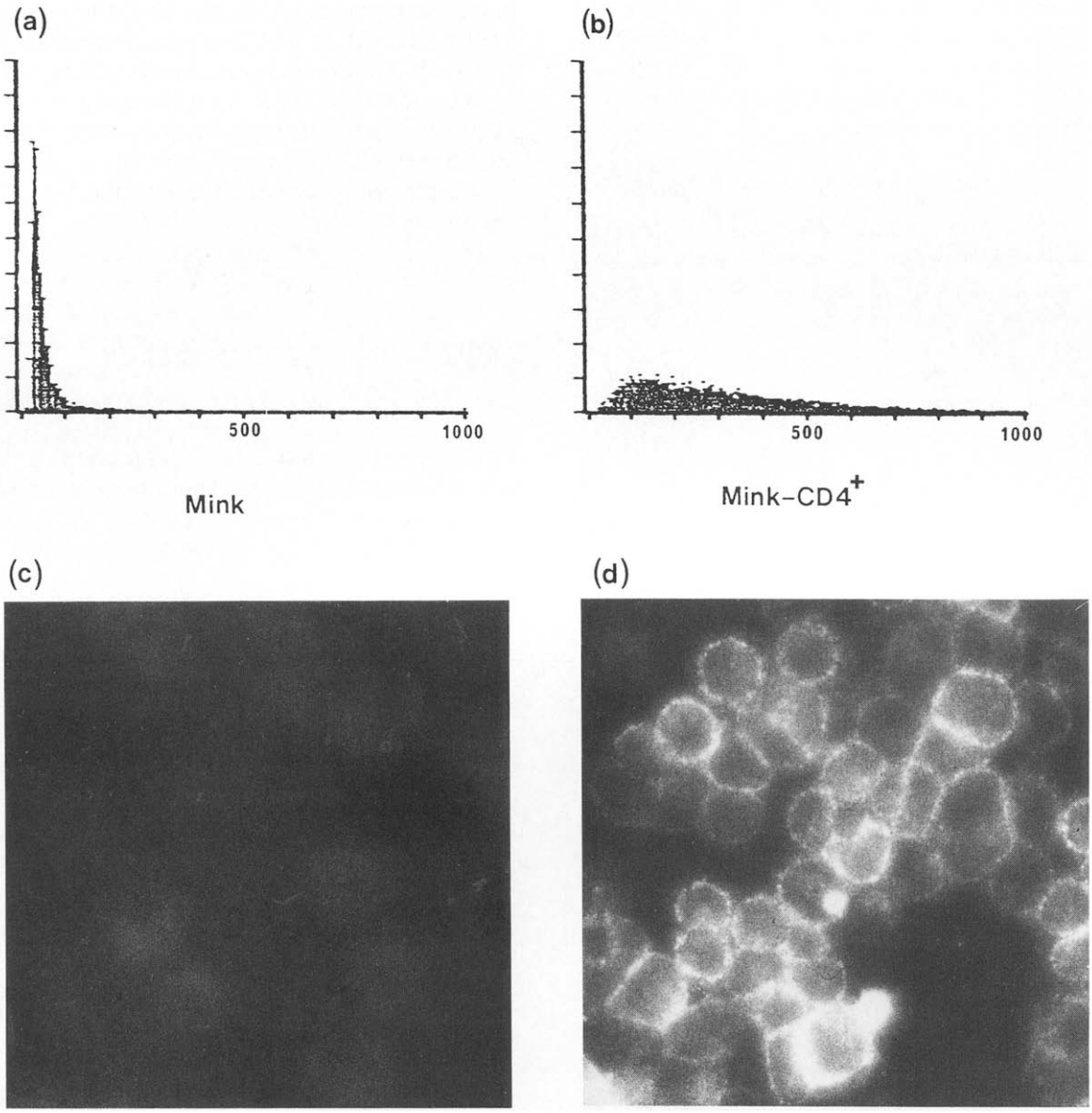

FIG. 1. CD4 expression on mink lung cells, before and after introduction of human CD4 cDNA. FACS analysis of anti-CD4 immunofluorescence on (a) $CD4^-$, and (b) $CD4^+$ cells; fluorescence microscopy of stained $CD4^-$ (c) and $CD4^+$ (d) cells.

cell type was then measured quantitatively by radiolabeling each cell line. Figure 2a shows the level of anti-CD4 mAb 318 binding detected on selected cell lines compared to $CD4^-$ parental cells. CD4 expression varied from cell type to cell type and between individual clones (not shown). In general, mink cells expressed the most, and human TE671 and Daudi cells, the least. The transfected cell lines, XC, CHO, and 2H3, also expressed significant levels of surface CD4 (data not shown). The ability of surface CD4 to bind recombinant gp120 derived from HIV-1/IIIB was also measured. Figure 2b shows that each $CD4^+$ cell line bound gp120 at levels roughly proportional to the amount of CD4 expression measured by anti-CD4 mAb binding.

It may be noted that surface CD4 expression was detectable on TE671 cells both by anti-CD4 mAb binding and by gp120 binding, yet it was barely above the background, nonspecific level of $CD4^-$ parental TE671 cells. $CD4^-$ U87 glioma cells bound more gp120 than did $CD4^-$ TE671 cells (Fig. 2b). Whereas $CD4^-$ TE671 cells are weakly permissive to HIV-1 infection by a CD4-independent entry route, U87 cells are resistant (Clapham *et al.*, 1989). There was thus no apparent relationship between the amount of gp120 bound to

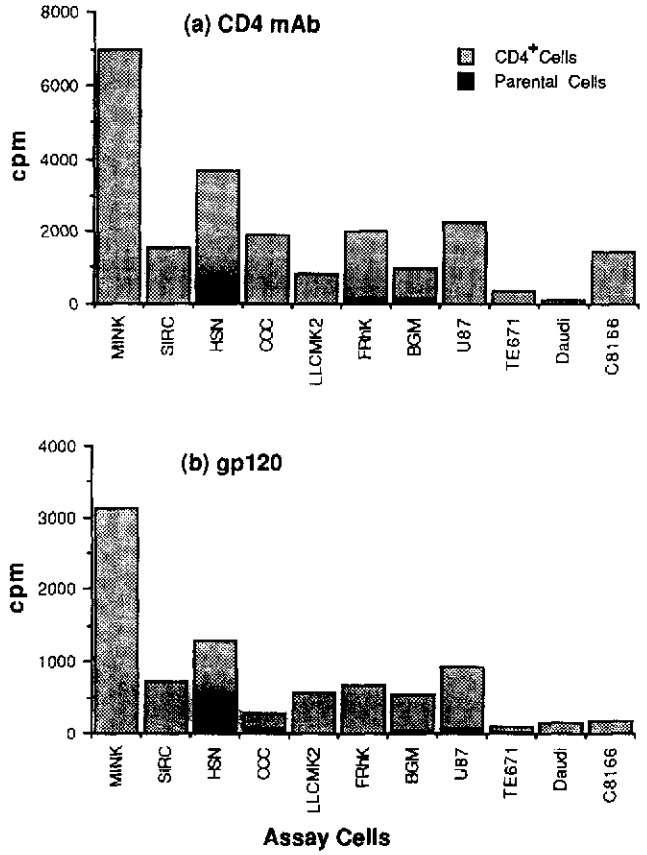

FIG. 2. CD4 expression (a) and gp120 binding (b) on mammalian cell lines after introduction of human CD4 cDNA.

$CD4^-$ cells and susceptibility to HIV infection by routes independent of CD4.

Susceptibility of $CD4^+$ cell lines to HIV-1 infection and cell fusion

$CD4^+$ non-human cell lines (from four taxonomic orders of mammal) were exposed to 10^5 or more TCID of HIV-1/RF or IIIB, and the cells were split twice weekly and monitored for virus replication. Reverse transcriptase activity was not detected in the supernatant on any occasion, nor could infectivity be rescued by cocultivation with the highly permissive human T-cell line, C8166. Supernatant samples from XC-$CD4^+$, CHO-$CD4^+$, and 2H3-$CD4^+$ were negative for p24 antigen, 1 and 2 weeks postinfection. In contrast, each of the $CD4^+$ human cell lines, constructed in parallel using the same CD4 expression vector, were readily infected with HIV-1, with the exception of U87 glioma cells (Table 1).

Each $CD4^+$ cell line was also tested for susceptibility to HIV-1-induced fusion by cocultivation with a "high" HIV-1 producer cell line (H9/RF or H9/IIIB). No syncytia were observed in $CD4^+$ non-human cell lines after overnight or more prolonged cocultivation with either H9/RF or H9/IIIB (Table 1). These producer cells both caused massive syncytium induction after cocultivation with the $CD4^+$ human cell lines C8166, Daudi-$CD4^+$, HeLa-$CD4^+$, and TE671-$CD4^+$. No syncytia were apparent in U87-$CD4^+$ cells and only moderate cell fusion occurred in HOS-$CD4^+$ cells.

VSV(HIV-1) pseudotypes are phenotypically mixed virus particles that bear the envelope glycoproteins of HIV and require a functional HIV surface receptor for attachment and membrane penetration (Dalgleish *et al.*, 1984; Clapham *et al.*, 1987). After cell entry and uncoating, the VSV genome replicates to release viral progeny and cause cell death within a few hours. VSV(HIV) pseudotypes can thus be used as a plaque-forming assay specific for HIV penetration and envelope uncoating (Dalgleish *et al.*, 1984; McClure *et al.*, 1988). Table 1 shows that VSV(HIV-1) pseudotypes

TABLE 1

SUSCEPTIBILITY OF $CD4^+$ CELL LINES TO HIV-1

Species of cell	$CD4^+$ cells	HIV-1 infection[a]	Cell fusion[b]	VSV pseudotype infection[c]
Rat	HSN	–	–	–
	XC	–	–	NT[d]
	2H3	–	–	NT
Cat	CCC	–	–	–
Mink	Mv-1-lu	–	–	–
Hamster	CHO	–	(–)	–
Rabbit	SIRC	–	–	–
Rhesus	LLCMK2	–	–	–
	FRhK/4T	–	–	–
AGM[e]	BGM	–	–	–
Human[f]	U87	–	–	–
	HOS	3.0	+	3.4
	TE671	4.0	++++	4.5
	HeLa	4.0	+++	4.0
	Daudi	3.0	+++	NT
	C8166	5.0	++++	4.5

[a] HIV-1 infection is shown as the $\log_{10}$ TCID end-point titration of a stock of HIV/RF. Infection was detected by cell fusion of appropriate $CD4^+$ indicator cells as well as reverse transcriptase or p24 detection in cell culture supernatants.

[b] Cell fusion was tested by cocultivating virus producer cells with $CD4^+$ test cells and examining for the presence of syncytia after overnight incubation.

[c] Titers of VSV(HIV-1) pseudotype prepared with the RF strain of HIV-1 are shown as $\log_{10}$ PFU/ml. Nonpseudotype VSV plated at $>10^8$ PFU/ml on each cell type.

[d] NT, not tested.

[e] AGM, African green monkey.

[f] HIV-1 cell fusion, infection, and VSV pseudotypes were completely blocked by CD4 mAb (Leu 3a) and by sCD4.

failed to produce plaques (above background) on the CD4+ non-human cell lines. Titers ranging from 10^3 to $>10^4$ PFU/ml were recorded on CD4+ human cells, except U87-CD4+.

Proteolytic cleavage of gp120 in the V3 loop has been implicated in the entry of HIV-1 (Hattori *et al.*, 1989; Stephens *et al.*, 1990). Cleavage of gp70 of ecotropic murine leukemia virus (MLV-E) occurs after cell uptake, probably in the endosomes (Andersen, 1985, 1987). MLV-E infection of mouse fibroblasts is pH dependent, whereas infection of fusion-sensitive rat XC cells is pH-independent, suggesting that a pH-independent protease might be active on the surface of XC cells (McClure *et al.*, 1990). CD4+ rat XC cells were therefore constructed and included in tests for HIV infection as well as the rat leukemic basophil line, 2H3, which degranulates after activation to release histamine and many proteases. However, no evidence of infection of XC-CD4+ or 2H3-CD4+ cells by HIV-1 was obtained (Table 1), even after activation.

Recombinant gp120 derived from aging hamster CHO cells was shown to be cleaved in the V3 loop (Stephens *et al.*, 1990), so CHO-CD4+ cells were also constructed and tested for HIV infection. As described above, these cells, like the other CD4+ non-primate cells, were not infectible by HIV-1 (Table 1). Since our CHO cells generated a background level of spontaneous syncytia during culture, it was difficult to assess precisely whether further fusion occurred after HIV infection. For this reason, proviral evidence of HIV infection was sought. DNA was extracted from CHO cells exposed to HIV-1 and subjected to PCR analysis using *gag*-specific primers. While PCR amplification of DNA *gag* sequences yielded bands of the predicted size on an agarose gel (Fig. 3) using DNA from infected human CD4+ cells (confirmed by Southern blot), none was detected in CD4+ CHO or mink cells. The lack of proviral DNA in these cells provides further evidence that the restriction to infection occurs early in the HIV replication cycle.

Taken together, these results show that CD4 expression on the non-human cell lines studied was not sufficient to render these cells permissive to HIV-1 infection or cell fusion. Syncytium induction in CD4+ cells by chronically infected, HIV-1 producer cells occurs within a few hours and does not require HIV replication. The failure of the CD4+ non-primate cells to form syncytia indicates that the block to infection occurs at or before membrane fusion. The lack of VSV(HIV-1) pseudotype infection provides further evidence that the site of the restriction occurs before viral penetration and uncoating, as all cell types were fully permissive for VSV replication.

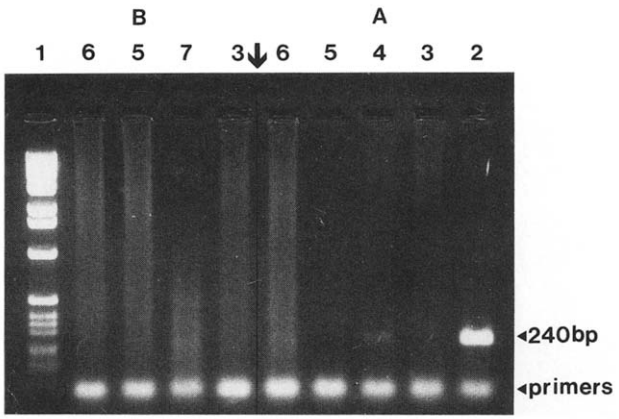

FIG. 3. Analysis of PCR-amplified DNA from human C8166, HeLa, and hamster CHO cells. DNA was extracted from HIV-1/IIIB-exposed cells after 1 (A) or 2 (B) weeks. 0.1 μg of DNA was amplified from the permissive C8166 T-cell line whereas 1 μg of DNA was amplified from the other HIV-1/IIIB-exposed cells. Lane 1, 1-kb ladder; lane 2, C8166 cells; lane 3, mink-CD4+ cells; lane 4, HeLa-CD4+ cells; lane 5, CHO cells; lane 6, CHO-CD4+ cells; lane 7, HeLa cells.

Since gp120 bound to CD4 on transfected non-human cells, but the cells were not permissive to cell fusion or VSV(HIV-1) infection, we assumed that an essential step in infection was lacking after virion binding but before uncoating. We therefore investigated whether bound HIV-1 virions would functionally infect cells after treatment with the membrane fusigen, PEG, as the infection of *env*⁻ Rous sarcoma virus has previously been reported by a similar technique (Weiss *et al.*, 1977). HIV-1 (RF strain, 10^6 TCID) was adsorbed to mink-CD4+ cells, which were subsequently exposed to PEG. HIV-1 infection was detected by p24 antigen and by rescue of progeny virus upon cocultivation with the permissive cell line, C8166. The results (Table 2) demonstrate that mink-CD4+ cells are permissive to low-titer replication of HIV-1, provided that entry is mediated through PEG fusion. Thus cell penetration appears to be the block to infection in CD4+ nonpermissive cells.

Susceptibility of CD4+ cell lines to HIV-2 and SIV infection

We investigated whether selected CD4+ cell lines were susceptible to infection by HIV-2 and SIV. Titration of these viruses in parallel to HIV-1 revealed significant differences in patterns of susceptibility (Fig. 4a), and these were reflected in the plating of VSV(HIV-1) and VSV(HIV-2) pseudotypes (Fig. 4b). For example, human TE671-CD4+ cells were susceptible to both HIV-1 and HIV-2 but not to SIV_{MAC}, whereas human

TABLE 2

POLYETHYLENE GLYCOL-INDUCED INFECTION OF CD4+ MINK CELLS BY HIV-1

Cell Type	Cocultivation with C8166 cells	Treatment: None p24[b]	None RT[b]	PEG[a] p24	PEG[a] RT
Mink	–	0.2	–	0.9	–
	+[c]	0.3	-	>2.0	14,800
H9	–	>2.0	27,900	NT[d]	NT

[a] 10^6 TCID HIV-1/RF were adsorbed to mink-CD4+ cells for 1 hr before PEG treatment (Materials and Methods).

[b] Infection of mink-CD4+ cells was assessed by p24 detection (measured in OD units) or reverse transcriptase activity (RT) in the cell supernatant 10 days postinfection.

[c] C8166 cells were added to mink-CD4- cells 10 days after infection and rescued virus was measured 5 days later.

[d] NT, not tested.

U87 cells were selectively sensitive only to HIV-2 and SIV.

The rhesus macaque kidney line LLCMK2 was sensitive only to SIV_{MAC}, whereas another rhesus kidney line, FRhK, also showed a low susceptibility to HIV-2 (Fig. 4). The two rhesus cell lines were also sensitive to infection by SIV_{SM} (data not shown). The rhesus monkey cell lines supported chronic production of SIV_{MAC}. CD4- parental cells were not permissive, and infection of the CD4+ lines was blocked by Leu 3a antibody. The Buffalo green monkey (BGM) cell line of AGM did not support HIV-1, HIV-2, SIV_{MAC}, SIV_{SM}, or SIV_{AGM} replication; however, a low though significant plating of VSV(HIV-2) pseudotype was observed.

The susceptibility of CD4+ primate cell lines was next tested in syncytium induction assays. Figure 5 shows the induction of syncytia by HIV-1, HIV-2, and SIV_{MAC} in CD4+ TE671 and U87 cells. To investigate whether the selective tropisms of HIV-1, HIV-2, and SIV for the different cell lines was a general property of these virus types, 19 distinct virus strains were examined (Table 3). Ten strains of HIV-1, including 6 African strains with widely divergent *env* sequences, induced cell fusion in TE671-CD4+ cells but not in U87-CD4+ cells. Conversely, 6 distinct isolates of HIV-2 induced syncytia in both types of human cell, though LAV-2_{ROD} and CBL-22 did so more efficiently than the other strains in U87 cells. Table 3 also shows differential sensitivity to cell fusion of the simian cell lines expressing human CD4. The FRhK rhesus line was sensitive to all strains of HIV-2 and SIV whereas the LLCMK2 line was sensitive only to SIV_{MAC} and SIV_{SM}. The BGM line of African green monkey cells showed differential sensitivity to HIV-2 isolates. It is interesting to note that while the BGM line was sensitive to cell fusion by HIV-2 LAV-2_{ROD} and by all SIV strains (Table 3), and to a lesser extent to VSV(HIV-2) pseudotypes (Fig. 4b), it did not support replication of LAV-2_{ROD} or SIV_{MAC} (Fig. 4a). The BGM-CD4+ cells thus appear to be permissive for HIV-2 and SIV entry but exert a postpenetration restriction of viral replication.

DISCUSSION

We have shown that HIV-1, HIV-2, and SIV strains exhibit distinct tropisms for human and simian cell lines expressing recombinant human CD4. Among the human cell lines, CD4+ TE671 cells are permissive for 10 HIV-1 and 6 HIV-2 strains but not for 3 SIV strains, and CD4+ U87 cells are susceptible to the HIV-2 and SIV strains but not to HIV-1. VSV(HIV) pseudotype assays gave results parallel to HIV infection, indicating that the differences were determined at the cell surface; this

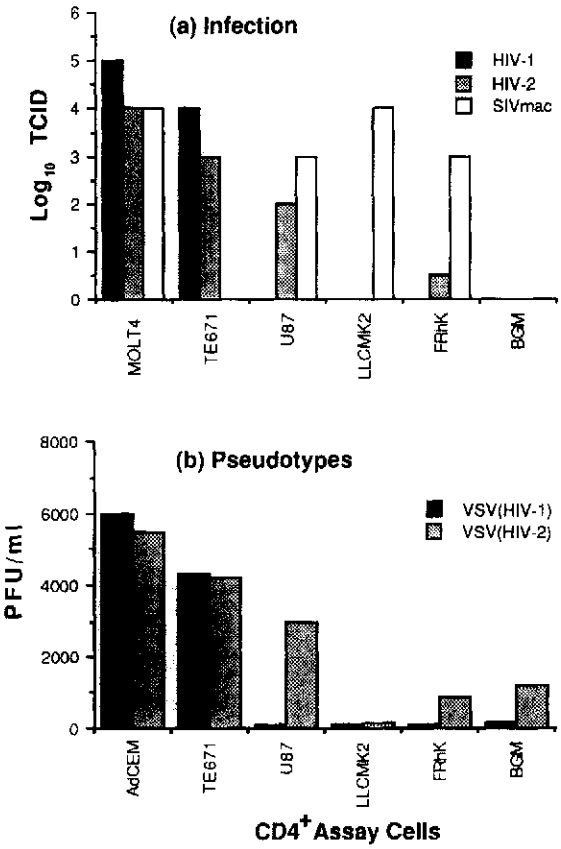

FIG. 4. Infection of CD4+ primate cell lines by (a) titration of HIV-1, HIV-2, and SIV; TCID end-point estimation of HIV-1/RF, HIV-2/LAV-2_{ROD}, and SIV_{MAC32H}. (b) VSV(HIV-1) and VSV(HIV-2) pseudotype titration.

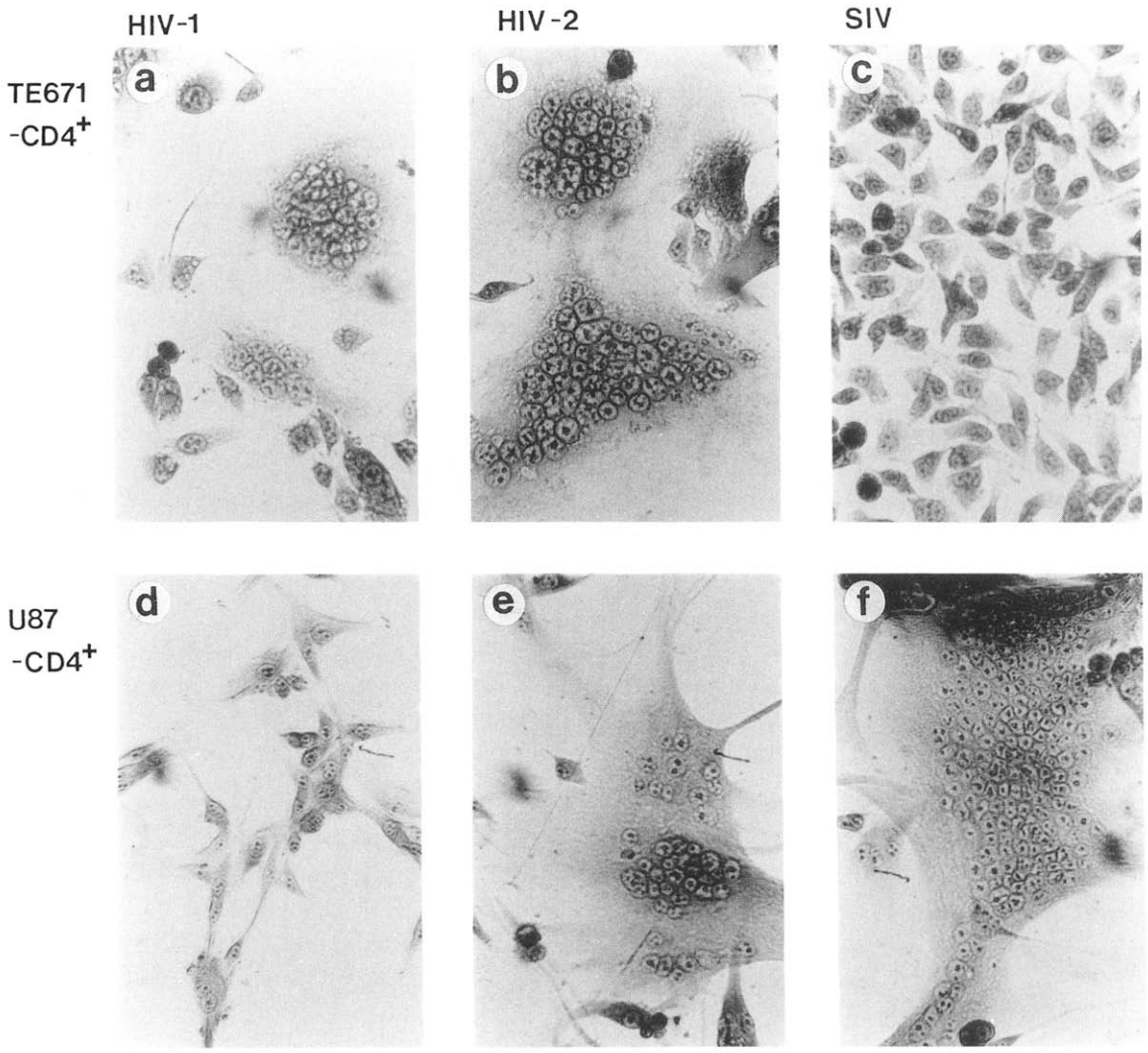

FIG. 5. Cell fusion induced by HIV-1/RF (a and d), HIV-2/LAV-2_{ROD} (b and e), or SIV_{MAC} (c and f) producer cell lines cocultivated overnight with either TE671-CD4+ (a–c) or U87-CD4+ (d–f) cells.

was further supported by cell fusion assays presumed to be independent of virus replication. The lack of HIV-1 infection of U87-CD4+ has also been reported by Chesebro *et al.* (1990) using independently isolated CD4+ clones. These authors also showed that transfected HIV-1 DNA resulted in productive replication, indicating that the block to virion infection occurred before proviral integration.

Efficient SIV_{MAC} replication in human T-cells involves specific selection of mutant viruses with a truncated transmembrane glycoprotein (gp32 instead of a gp41) (Hirsch *et al.*, 1989; Cranage *et al.*, 1989). The status and role of the transmembrane glycoproteins of the SIVs used in this study of nonlymphoid human CD4+ cells were not determined, though they were preadapted to growth in human T-cell lines.

Like the CD4+ U87 and simian cell lines studied, CD4+ non-primate cell lines expressing human CD4 bound gp120 but were nonpermissive for HIV-1 replication. CD4+ cell lines of rat, cat, mink, hamster, and rabbit origin were examined, and their resistance to VSV(HIV-1) and to syncytium formation again indicated that a cell surface restriction was the cause.

Successful infection of mink-CD4+ cells by polyethylene glycol treatment following HIV adsorption showed that the block to infection could be overcome by inducing membrane fusion. CD4− mink cells have recently been reported to be permissive to infection by HIV-1 phenotypically mixed with amphotropic murine leukemia virus (Canivet *et al.*, 1990). The failure of HIV-1 to infect rabbit cells was interesting in view of the reported infection of two T-cell lines and a macrophage

TABLE 3

HIV- AND SIV-INDUCED FUSION OF CD4+ PRIMATE CELL LINES[a]

		Fusion of CD4+ cell line					
		Human			Rhesus		AGM
Virus	Strain	MOLT4#8	TE671	U87	LLCMK2	FRhK	BGM
HIV-1	RF	++++	++++	−	−	−	−
	IIIB	++++	++++	−	−	−	−
	NY5	++++	++++	−	−	−	−
	SF-2	++++	++++	−	−	−	−
	CBL-4	++++	++++	−	−	−	−
	Z39	++++	++++	−	−	−	−
	Z84	++++	++++	−	−	−	−
	Z129	++++	++++	−	−	−	−
	NDK	++++	++++	−	−	−	−
	U455	++++	++++	−	−	−	−
HIV-2	$LAV\text{-}2_{ROD}$	++++	++++	++++	−	+++	+++
	SBL6669	++++	++++	+	−	+++	+++
	CBL-20	++++	++++	+	−	+	−
	CBL-21	++++	++++	+	−	+	−
	CBL-22	++++	++++	++++	−	++−	−
	CBL-23	++++	++++	+	−	+	−
SIV	MAC	++++	−	++++	+	++++	++++
	SM	++++	−	++++	+	++++	+++
	AGM	++++	−	+	−	++++	+++

[a] Cell fusion assays were carried out by mixing HIV or SIV producer cells with CD4+ cells as described under Materials and Methods. Each CD4− parent line was negative for cell fusion after mixing with cells producing HIV-1 RF, HIV-2 CBL20, and SIV_{MAC}.

line of rabbits (Kulaga *et al.*, 1988), and infection of rabbits *in vivo* (Filice *et al.*, 1988).

Our results are consistent with those we previously reported for CD4+ mouse cell lines (Maddon *et al.*, 1986, 1988) and for most of the CD4+ non-primate cell lines studied recently with vaccinia virus vectors by Ashorn *et al.* (1990). However, Ashorn *et al.* identified a minority of non-primate cell lines expressing very high levels of CD4 from a vaccinia vector that supported HIV-1-directed cell fusion after cocultivation with human cells expressing HIV-1 envelope glycoproteins. Interestingly, while these cells supported fusion mediated by HIV-1 envelope glycoproteins expressed on human cells, they were resistant to fusion by cocultivation with mouse cells expressing the HIV-1 glycoproteins, although the latter cells were competent for fusion of CD4+ human cells. These findings suggest that events between virion attachment to CD4 and gp41-mediated membrane fusion may be more complex than previously thought. Two surface components additional to CD4 may be needed, one associated with the target cell membrane and the other provided by either the target cell or virus-providing cell.

Little is known about the steps between the gp120/CD4 interaction and the gp41-induced fusion of viral and cell membranes that allows the introduction of the virion nucleocapsid into the cell cytoplasm. The major histocompatability (MHC) class I antigen has been implicated as an additional requirement to CD4 for HIV-1 infection (Corbeau *et al.*, 1990; Devaux *et al.*, 1990). However, we found that CD4+, class I-deficient Daudi cells became fully sensitive to HIV-1 infection and syncytium induction after CD4 transfection, although they remained negative for surface expression of MHC class I antigen.

Hattori *et al.* (1989) and Stephens *et al.* (1990) proposed that one step may involve the cleavage of the gp120 V3 loop by a cellular protease. Trypstatin, an inhibitor of the proteases trypsin and tryptase, inhibited HIV-1 infection and has homology to the GPGR tip of the V3 loop (Hattori *et al.*, 1989). The V3 loop on gp120 is highly variable but conserves either trypsin-like and chymotrypsin-like cleavage sites (Clements *et al.*, 1991). It is possible that cell surface proteases specifically cleaving the V3 loop may determine this step in infection as a prerequisite for fusion. The importance of the V3 loop in HIV tropism has been demonstrated by Takeuchi *et al.* (1991), who analyzed a variant of $HIV\text{-}1_{GUN}$ that infects CD4+ human brain-derived cells, whereas the parental virus replicates in T-cells but not

brain cells; a single amino acid substitution in the V3 loop determines this change in cell tropism.

Cell surface proteases have also been implicated as important in the entry of other enveloped viruses including retroviruses (Andersen, 1983; Appleyard and Tisdale, 1985). Several different proteases including trypsin, α-chymotrypsin, and thermolysin were shown to enhance MLV-induced cell fusion of mouse fibroblasts (Andersen and Skov, 1989). Cleavage of MLV gp70 normally occurs in the endosomes or lysosomes (Andersen, 1987), but the XC cell line which is susceptible to MLV-induced cell fusion and is pH-independent for MLV entry may express a cell surface protease that cleaves gp120 as well as MLV gp70 (McClure *et al.*, 1990). CD4+ XC cells were therefore included in this study but were not permissive for HIV-1 entry. CD4+ rat 2H3 cells secrete an array of proteases when activated by IgE treatment, but these cells were also resistant to HIV infection even after IgE activation. Stephens *et al.* (1990) showed that recombinant gp120 produced from aging CHO cells was partially cleaved at the V3 loop; CD4 expression on the cell surface of CHO cells did not render them permissive to HIV infection.

Analysis of diverse strains of HIV-1, HIV-2, and SIV has revealed a surprising degree of group specificity for a postbinding, prepenetration event early in the infection of a set of CD4+ human and non-human primate cell lines. The viral tropisms we have examined did not correlate with MHC class I expression or with known cell surface protease activity. Selection and analysis of HIV or SIV variants with an altered cell tropism may reveal which region of the virus genome (presumably in the envelope gene) determines this early step during infection.

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REFERENCES

ALBERT, J., BREDBERG, U., CHIODI, F., BOTTIGER, B., FENYO, E. M., NORRBY, E., and BIBERFELD, G. (1987). A new human retrovirus isolate of West African origin (SBL-6669) and its relationship to HTLV-IV, LAV-II, and HTLV-IIIB. *AIDS Res. Hum. Retroviruses* **3**, 3–10.

ANDERSEN, K. B. (1983). Leupeptin inhibits retrovirus infection in mouse fibroblasts. *J. Virol.* **48**, 765–769.

ANDERSEN, K. B. (1985). The fate of surface protein gp70 during entry of retrovirus into mouse fibroblasts. *J. Gen. Virol.* **142**, 112–120.

ANDERSEN, K. B. (1987). Cleavage fragments of the retrovirus surface protein gp70 during virus entry. *J. Gen. Virol.* **68**, 2193–2202.

ANDERSEN, K. B., and SKOV, H. (1989). Retrovirus-induced cell fusion is enhanced by protease treatment. *J. Gen. Virol.* **70**, 1921–1927.

APPLEYARD, G., and TISDALE, M. (1985). Inhibition of the growth of human coronavirus 229E by leupeptin. *J. Gen. Virol.* **66**, 363–366.

ASHORN, P. A., BERGER, E. A., and MOSS, B. (1990). Human immunodeficiency virus envelope glycoprotein/CD4-mediated fusion of nonprimate cells with human cells. *J. Virol.* **64**, 2149–2156.

BARRON, A. L., OLSHEVSKY, C., and COHEN, M. M. (1970). Characteristics of the BGM line of cells from african green monkey kidney. *Arch. Virusforsch.* **32**, 389–392.

BEDINGER, P., MORIARTY, A., VON BORSTEL, R. C., DONOVAN, N. J., STEIMER, K. S., and LITTMAN, D. R. (1988). Internalization of the human immunodeficiency virus does not require the cytoplasmic domain of CD4. *Nature* **334**, 162–165.

BENN, S., RUTLEDGE, R., FOLKS, T., GOLD, J., BAKER, L., MCCORMICK, J., FEORINO, P., PIOT, P., QUINN, T., and MARTIN, M. (1985). Genomic heterogeneity of AIDS retroviral isolates from North America and Zaire. *Science* **230**, 949–951.

CAMERINI, D., and SEED, B. (1990). A CD4 domain important for HIV-mediated syncytium formation lies outside the virus binding site. *Cell* **60**, 747–754.

CANIVET, M., HOFFMAN, A. D., HARDY, D., SERNATINGER, and LEVY, J. A. (1990). Replication of HIV-1 in a wide variety of animal cells following phenotypic mixing with murine retroviruses. *Virology* **178**, 543–551.

CHESEBRO, B., BULLER, R., PORTIS, J., and WEHRLY, K. (1990). Failure of human immunodeficiency virus entry and infection in CD4-positive human brain and skin cells. *J. Virol.* **64**, 215–221.

CLAPHAM, P. R., NAGY, K., and WEISS, R. A. (1984). Pseudotypes of human T-cell leukemia virus type 1 and 2: Neutralisation by patient's sera. *Proc. Natl. Acad. Sci. USA* **81**, 2886–2889.

CLAPHAM, P. R., WEISS, R. A., DALGLEISH, A. G., EXLEY, M., WHITBY, D., and HOGG, N. (1987). Human immunodeficiency virus infection of monocytic and T-lymphocytic cells: Receptor modulation and differentiation induced by phorbol ester. *Virology* **158**, 44–51.

CLAPHAM, P. R., WEBER, J. N., WHITBY, D., MCINTOSH, K., DALGLEISH, A. G., MADDON, P. J., DEEN, K. C., SWEET, R. W., and WEISS, R. A. (1989). Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells. *Nature* **337**, 368–370.

CLAVEL, F., GUETARD, D., BRUN-VEZINET, F., CHAMARET, S., REY, M. A., SANTOS-FERREIRA, M. O., LAURENT, A. G., DAUGUET, C., KATLAMA, C., ROUZIOUX, C., KLATZMANN, D., CHAMPALIMAUD, J. L., and MONTAGNIER, L. (1986). Isolation of a new human retrovirus from West African patients with AIDS. *Science* **233**, 343–346.

CLEMENTS, G. J., PRICE-JONES, M. J., STEPHENS, P. E., SUTTON, C., SCHULZ, T. F., CLAPHAM, P. R., MCKEATING, J. A., MCCLURE, M. O., THOMSON, S., MARSH, M., KAY, J., WEISS, R. A., and MOORE, J. P. (1991). The V3 loop of the HIV-1 surface glycoprotein contains proteolytic cleavage sites: A possible function in viral fusion. *AIDS Res. Hum. Retroviruses*, **7**, 3–16.

CORBEAU, P., DEVAUX, C., KOIRILSKY, F., and CHERMANN, J. C. (1990). An early post infection signal mediated by monoclonal antibody anti beta 2-microglobulin is responsible for delayed production of HIV-1 in PBL cells. *J. Virol.* **64**, 1459–1464.

CRANAGE, M. P., ALMOND, N., JENKINS, A., and KITCHEN, P. A. (1989). Transmembrane protein of SIV. *Nature* **342**, 349.

CRANDELL, R. A., FABRICANT, C. G., and NELSON-REES, W. A. (1973). Development, characterisation and virus susceptibility of a feline (*Felis catus*) renal cell line (CRFK). *In Vitro* **9**, 176–185.

CURRIE, G. A., and GAGE, J. O. (1973). Influence of tumour growth on the evolution of cytotoxic lymphoid cells in rats bearing a spontaneously metastasizing syngeneic fibrosarcoma. *Br. J. Cancer* **28**, 136–146.

DALGLEISH, A. G., BEVERLEY, P. C. L., CLAPHAM, P. R., CRAWFORD, D. H., GREAVES, M. F., and WEISS, R. A. (1984). The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312,** 763–766.

DANIEL, M. D., LETVIN, N. L., KING, N. W., KANNAGI, M., SEHGAL, P. K., HUNT, R. D., KANKI, P. J., ESSEX, M., and DESROSIERS, R. C. (1985). Isolation of a T-cell tropic HTLV-III-like retrovirus from macaques. *Science* **228,** 1201–1204.

DEVAUX, C., BOUCRAUT, J., POIRIR, G., CORBEAU, P., REY, F., BERKINARE, M., PERAIREAU, B., KOIRILSKY, F., and CHERMANN, J. C. (1991). Anti beta 2-microglobulin monoclonal antibodies mediate a delay of HIV-1 cpe in MT4 cells. *Scand. J. Immunol.*, in press.

DIAMOND, D. C., FINBERG, R., CHAUDHURI, S., SLECKMAN, B. P., and BURAKOFF, S. J. (1990). Human immunodeficiency virus infection is efficiently mediated by a glycolipid-anchored form of CD4. *Proc. Natl. Acad. Sci. USA* **87,** 5001–5005.

FILICE, G., CEREDA, P. M., and VARNIER, O. E. (1988). Infection of rabbits with human immunodeficiency virus. *Nature* **335,** 366–369.

GALLAGHER, W. R. (1987). Detection of fusion peptide sequence in the transmembrane protein of human immunodeficiency virus. *Cell* **50,** 327–328.

HATTORI, T., KOITO, A., TAKATSUKI, K., KIDO, H., and KATUNUMA, N. (1989). Involvement of tryptase-related cellular proteinase(s) in human immunodeficiency virus type 1 infection. *FEBS Lett.* **248,** 48–52.

HEALEY, D., DIANDA, L., MOORE, J. P., MCDOUGAL, J. S., MOORE, M. J., ESTESS, P., BUCK, D., KWONG, P. D., BEVERLEY, P. C. L., and SATTENTAU, Q. J. (1990). Novel anti-CD4 monoclonal antibodies separate HIV infection and fusion of $CD4^+$ cells from virus binding. *J. Exp. Med.* **172,** 1233–1242.

HENDERSON, I. C., LIEBER, M. M., and TODARO, G. J. (1974). Mink cell line Mv1Lu (CCL64) focus formation and generation of non-producer transformed cell lines with murine and feline sarcoma viruses. *Virology* **60,** 282–287.

HIRSCH, V. M., EDMONDSON, P., MURPHEY-CORB, M., ARBEILLE, B., JOHNSON, P. R., and MULLINS, J. I. (1989). SIV adaption to human cells. *Nature* **341,** 573–574.

HO, D. D., KAPLAN, J. C., RACKAUSKAS, I. E., and GURNEY, M. E. (1988). Second conserved domain of gp120 is important for HIV infectivity and antibody neutralisation. *Science* **239,** 1021–1023.

HOFFMAN, A. D., BANAPOUR, B., and LEVY, J. A. (1985). Characterisation of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. *Virology* **147,** 326–335.

HULL, R. N., CHERRY, W. R., and TRITCH, O. J. (1962). Growth characteristics of monkey kidney cell strains LLC-MK1, LLC-MK2, and LLC-MK2 (NCTC-3196) and their utility in virus research. *J. Exp. Med.* **115,** 903–918.

KINNEY THOMAS, E., WEBER, J. N., MCCLURE, J., CLAPHAM, P. R., SINGHAL, M. C., SHRIVER, M. K., and WEISS, R. A. (1988). Neutralising monoclonal antibodies to the AIDS virus. *AIDS* **2,** 25–29.

KLATZMANN, D., CHAMPAGNE, E., CHAMARET, S., GRUEST, J., GUETARD, D., HERCEND, T., GLUCKMAN, J-C., and MONTAGNIER, L. (1984). T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature* **312,** 767–768.

KOWALSKY, M., POTZ, J., BASIRIPOUR, L., DORFMAN, T., GOH, W. C., TERWILLIGER, E., DAYTON, A., ROSEN, C., HASELTINE, W., and SODROSKI, J. (1987). Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. *Science* **237,** 1351–1355.

KULAGA, H., FOLKS, T. M., RUTLEDGE, R., and KINDT, T. J. (1988). Infection of rabbit T-cell and macrophage lines with human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **85,** 4455–4459.

LEERHOY, J. (1965). Cytopathic effect of rubella virus in a rabbit-cornea cell line. *Science* **149,** 633–634.

LEVY, J. A., HOFFMAN, A. D., KRAMER, S. M., LANDIS, J. A., SHIMABUKURO, J. M., and OSHIRO, L. S. (1984). Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* **225,** 840–842.

LINSLEY, P. S., LEDBETTER, J. A., KINNEY-THOMAS, E., and HU, S.-L. (1988). Effects of anti-gp120 monoclonal antibodies on CD4 receptor binding by the env protein of human immunodeficiency virus type 1. *J. Virol.* **62,** 3695–3702.

MADDON, P. J., DALGLEISH, A. G., MCDOUGAL, J. S., CLAPHAM, P. R., WEISS, R. A., and AXEL, R. (1986). The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47,** 333–348.

MADDON, P. J., MCDOUGAL, J. S., CLAPHAM, P. R., DALGLEISH, A. G., JAMAL, S., WEISS, R. A., and AXEL, R. (1988). HIV infection does not require endocytosis of its receptor CD4. *Cell* **54,** 865–874.

MARSH, M., and HELENIUS, A. (1989). Virus entry into animal cells. *Adv. Virus Res.* **36,** 107–151.

MCALLISTER, R. M., ISAACS, H., RONGEY, R., PEER, M., AU, W., SOUKUP, S. W., and GARDNER, M. B. (1977). Establishment of a human medulloblastoma cell line. *Int. J. Cancer* **20,** 206–212.

MCCLURE, M. O., MARSH, M., and WEISS, R. A. (1988). Human immunodeficiency virus infection of CD4-bearing cells occurs by a pH-independent mechanism. *EMBO J.* **7,** 513–518.

MCCLURE, M. O., SOMMERFELT, M. A., MARSH, M., and WEISS, R. A. (1990). The pH independence of mammalian retrovirus infection. *J. Gen. Virol.* **71,** 767–773.

MURPHEY-CORB, M., MARTIN, L. N., RANGAN, S. R. S., BASKIN, G. B., GORMUS, B. J., WOLF, R. H., ANDES, W. A., WEST, M., and MONTELARO, R. C. (1986). Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and possible origin in asymptomatic monkeys. *Nature* **321,** 435–437.

ORAM, J. D., DOWNING, R. G., ROFF, M., CLEGG, J. C. S., SERWADDA, D., and CARSWELL, J. W. (1990). Nucleotide sequence of a Ugandan HIV-1 provirus reveals genetic diversity from other HIV-1 isolates. *AIDS Res. Hum. Retroviruses* **6,** 1073–1078.

OHTA, Y., MASUDA, T., TSUJIMOTO, H., ISHIKAWA, K-I., KODAMA, T., MORIKAWA, S., NAKAI, M., HONJO, S., and HAYAMI, M. (1988). Isolation of simian immunodeficiency virus from African green monkeys and seroepidemiologic survey of the virus in various nonhuman primates. *Int. J. Cancer* **41,** 115–122.

PLOEGH, H. L., CANNON, L. E., and STROMINGER, J. L. (1979). Cell free translation of the mRNAs for the heavy and light chains of HLA-A and HLA-B antigens. *Proc. Natl. Acad. Sci. USA* **76,** 2273–2276.

POPOVIC, M., SARNGADHARAN, M. G., READ, E., and GALLO, R. C. (1984). Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224,** 497–500.

SAIKI, R. K., BUGAWAN, T. L., HORN, G. T., MULLIS, K. B., and ERLICH, H. A. (1986). Analysis of enzymatically amplified beta-globin and HLA DQa DNA with allele specific oligonucleotide probes. *Nature* **324,** 206–212.

SALAHUDDIN, S. Z., MARKHAM, P. D., WONG-STAAL, F., FRANCHINI, G., KALYANARAMAN, V. S., and GALLO, R. C. (1983). Restricted expression of human T-cell leukemia lymphoma virus (HTLV) in transformed human umbilical cord blood lymphocytes. *Virology* **129,** 51–54.

SATTENTAU, Q. J., CLAPHAM, P. R., WEISS, R. A., BEVERLEY, P. C. L., MONTAGNIER, L., ALHALABI, M. F., GLUCKMAN, J. C., and KLATZMANN, D. (1988). The human and simian immunodeficiency viruses HIV

1, HIV-2 and SIV interact with similar epitopes on their cellular receptor, the CD4 molecule. *AIDS* **2,** 101–105.

SCHULZ, T. F., WHITBY, D., HOAD, J. G., CORRAH, T., WHITTLE, H., and WEISS, R. A. (1990). Biological and molecular variability of HIV-2 isolates from the Gambia. *J. Virol.* **64,** 5177–5182.

SIMKOVIC, D., SVOBODA, J., and VALENTOVA, N. (1963). Clonal analysis of line XC rat tumor cells grown in vitro. *Folia Biol.* **9,** 82–88.

SIRAGARIAN, R. P., MCGRINEY, A., DASCURIAN, E. L., LEWS, F. T., HIRATA, F., and AXELROD, J. (1982). Variants of the rat basophil leukemia cell line for the study of histamine release. *Fed. Proc.* **41,** 30–34.

SKINNER, M. A., LANGLOIS, A. J., MCDANAL, C. B., MCDOUGAL, J. S., BOLOGNESI, D. P., and MATTHEWS, T. J. (1988). Neutralising antibodies to an immunodominant envelope sequence do not prevent gp120 binding to CD4. *J. Virol.* **62,** 4195–4200.

SPIRE, B., SIRE, J., ZACHAR, V., REY, F., BARRE-SINOUSSI, F., GALIBERT, F., HAMPE, A., and CHERMANN, J-C. (1989). Nucleotide sequence of HIV1-NDK: A highly cytopathic strain of the human immunodeficiency virus. *Gene* **81,** 275–284.

SRINIVASAN, A., ANAND, R., YORK, D., RANGANATHAN, P., FEORINO, P., SCHOCHETMAN, G., CURRAN, J., KALYANARAMAN, V. S., LUCIW, P. A., and SANCHEZ-PESCADOR, R. (1987). Molecular characterization of human immunodeficiency virus from Zaire: Nucleotide sequence analysis identifies conserved and variable domains in the envelope gene. *Gene* **52,** 71–82.

STEIN, B. S., GOWDA, S., LIFSON, J., PENHALLOW, R., BENSCH, K., and ENGLEMAN, E. (1987). pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. *Cell* **49,** 659–668.

STEPHENS, P. E., CLEMENTS, G., YARRANTON, G. T., and MOORE, J. (1990). A chink in HIV's armour. *Nature* **343,** 219.

STRATTON, M. R., REEVES, B. R., and COOPER, C. S. (1989). Misidentified cell. *Nature* **337,** 311–312.

TAKEUCHI, Y., AKUTSU, M., MURAYAMA, K., SHIMUZU, N., and HOSHINO, H. (1991). Host range mutant of human immunodeficiency virus type 1: Modification of cell tropism by a single point mutation at the neutralisation epitope in the env gene. *J. Virol.,* in press.

WALLACE, R. E., VASINGTON, P. J., PETRICCIANI, J. C., HOPPS, H. E., LORENZ, D. E., and KADANKA, Z. (1973). Development and characterization of cell lines from subhuman primates. *In Vitro* **8,** 333–341.

WEISS, R. A., BOETTIGER, D. and MURPHY, H. M. (1977). Pseudotypes of avian sarcoma viruses with the envelope properties of vesicular stomatitis virus. *Virology* **76,** 808–825.

WEISS, R. A., CLAPHAM, P. R., WEBER, J. N., DALGLEISH, A. G., LASKY, L. A., and BERMAN, P. W. (1986). Variable and conserved neutralisation antigens of human immunodeficiency virus. *Nature* **324,** 572–575.

WESTERMARK, B., PONTEN, J., and HUGOSSON, R. (1973). Determinants for the establishment of permanent tissue culture lines from human gliomas. *Acta Pathol. Microbiol. Scand.* **81,** 791–805.

WHITE, J., KIELIAN, M., and HELENIUS, A. (1983). Membrane fusion proteins of enveloped animal viruses. *Q. Rev. Biophys.* **16,** 151–195.

WILLEY, R. L., SMITH, D. H., LASKY, L. A., THEODORE, T. S., EARL, P. L., MOSS, B., CAPON, D. J., and MARTIN, M. A. (1988). In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. *J. Virol.* **62,** 139–147.