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Cellular CD44S as a Determinant of Human Immunodeficiency Virus Type 1 Infection and Cellular Tropism

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CD4 is the predominant cell membrane protein that binds human immunodeficiency virus type 1 (HIV-1) gp120 and facilitates HIV-1 infection, but other membrane-associated molecules may be involved in determining HIV-1 cellular infection. Our prior work had suggested that CD44, the transmembrane receptor for hyaluronan, might play a role in the infection of mononuclear phagocytes with HIV-1. In the present work, we have used cells of the CD4-positive, CD44-negative human T-lymphoblast cell line Jurkat to study the role of CD44 in HIV-1 infection and tropism. Cells were transfected with cDNA for the standard (S, or hematopoietic) CD44 isoform CD44S or the epithelial isoform CD44E. The resultant lines expressed appropriate CD44S or CD44E mRNA and protein. While the parent Jurkat cells, those transfected with vector alone, and those transfected with CD44E could be productively infected with only the lymphocytotropic strain HIV-1-LAI, cells transfected with CD44S were rendered susceptible to productive infection with the monocytotropic strains HIV-1-BaL and HIV-1-ADA. Also, CD44S-transfected cells displayed higher levels of infection with HIV-1-LAI than did the other transfected Jurkat cells. The transfected cell line cells all had comparable growth rates and expressed similar levels of the membrane antigens CD4, CD7, major histocompatibility complex (MHC) class I, MHC class II, and CD11a, while levels of CD3 were slightly higher in cells transfected with vector alone and in one of the clones transfected with CD44S. Hyaluronan binding was increased in cells transfected with either CD44S or CD44E. Mouse NIH 3T3 fibroblasts transfected with human CD4, human CD44S, or both human CD4 and CD44S displayed the appropriate antigens, but they could not be productively infected with lymphocytotropic or monocytotropic strains of HIV-1. The results indicate that in human leukocytes, CD44S is an important determinant of HIV-1 productive infection and may be involved in viral cellular tropism.

Cellular CD4 has been recognized as the predominant membrane protein that interacts with human immunodeficiency virus type 1 (HIV-1). In most cells, CD4 serves as the receptor that binds HIV-1 gp120, initiating events that lead to cell-virus membrane fusion and internalization of HIV into the susceptible cell (12, 13, 47). The ability of HIV to infect some CD4-negative cells (21, 22, 53) and the observation that gp120-CD4 interactions may not be the sole determinants of HIV-1 infectivity and spread (23, 37) have prompted the search for additional cell surface molecules involved in HIV-1 infection. The lymphocyte function-associated type 1 (LFA-1) molecule was thought to be an important determinant of HIV-1 infection of cells; however, it was eventually established that LFA-1 was not required for infection, but that it was important for efficient fusion of HIV-1-infected cells and syncytium formation (20, 28, 42, 43, 55). Investigators have found in CD4-negative neural cell lines and colon epithelial cells that the membrane lipid galactosyl ceramide can serve as a receptor for HIV-1 and bind gp120 (21, 22). Furthermore, although certain virus strains preferentially infect either monocytes or lymphocytes (monocytotropic or lymphocytotropic viral strains), both types of HIV-1 variants interact with CD4 (29, 41). This may indicate that other cellular molecules play a role in determining HIV-1 infection and HIV-1 tropism.

To determine the role of non-CD4 cell membrane molecules in HIV-1 infection of monocytes and lymphocytes, we have been studying various antibodies against cell adhesion molecules in in vitro assays of HIV-1 infection. We recently reported that antibodies against CD44, the transmembrane receptor for hyaluronan, effectively inhibited infection of human mononuclear phagocytes (monocytes and peritoneal macrophages) with the monocytotropic HIV-1 strains HIV-1-BaL and HIV-1-ADA (46). However, these antibodies had no effect on HIV-1 infection of normal blood lymphocytes and T-lymphoid cell line cells. These studies indicated that CD44 may be a determinant of HIV-1 infection of mononuclear phagocytes and of HIV-1 cellular tropism in general.

To study the role of CD44 in HIV-1 infection and tropism, we have used cells of the human T-lymphoblast line Jurkat, a CD4-positive, CD44-negative cell line which can be infected by lymphocytotropic strains of HIV-1 but not by monocytotropic HIV-1 strains. We show here that transfection of Jurkat cells with the standard (S) low-molecular-weight isoform CD44S (also called the CD44H, or hematopoietic, isoform), but not transfection with the high-molecular-weight epithelial isoform CD44E renders the Jurkat T cells capable of being infected by monocytotropic strains of HIV-1.

MATERIALS AND METHODS

Cell lines. Unless otherwise designated, all cell lines were from the American Tissue Culture Collection (Baltimore, Md.). The human T-cell line Jurkat, obtained from Warner C. Greene (University of California at San Francisco) was originally from the American Tissue Culture Collection. The Jurkat cell line expresses membrane CD4 antigen but does not express CD44 antigen or mRNA (35). This parent Jurkat line was maintained by serial passage in low-endotoxin-content RPMI 1640 with 10% fetal bovine serum (FBS; Hyclone, Logan, Utah)

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and used for all transfections. Cells of the CEM cell line, a human T-lymphoblast cell line, were maintained in low-endotoxin-content RPMI 1640 with 10% FBS. The mouse cell line NIH 3T3 was obtained from the American Tissue Culture Collection and cultured in Dulbecco's modified Eagle's medium with 10% FBS, 2 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml.

Virus strains. Cell-free stocks of HIV-1-BaL, a monocytotropic strain, and HIV-1-LAI, a lymphocytotropic strain, of HIV-1 (16) were produced in CEM cells (30, 49). Virus pools were titrated on T cells and mononuclear phagocytes by using p24 antigen release or reverse transcriptase (RT) activity as an endpoint at 10 days after infection for macrophages and 20 days after infection for monocytes. HIV-1-ADA, a second monocytotropic strain, was obtained from the AIDS Research and Reference Reagent Program (National Institutes of Health, Bethesda, Md.); virus stocks of this strain were produced by using normal blood monocytes.

Plasmids. Plasmid CDw44, containing the full-length human CD44S cDNA, and plasmid CD44E, containing the full-length human CD44E cDNA (51), were from Brian Seed (Harvard University, Boston, Mass.). CD44S and CD44E cDNAs were cleaved with *Hin*dIII and *Not*I restriction enzymes and cloned into the pCEP4 vector (InVitrogen, San Diego, Calif.). This vector contains a human cytomegalovirus immediate-early gene enhancer-promoter and a hygromycin resistance gene. Plasmid T4-pMV7, containing the human CD4 receptor cDNA, was obtained from the AIDS Research and Reference Program.

Monoclonal antibodies. Mouse monoclonal antibodies against various epitopes of human CD44 (A3D8, A1G3, and 5F12) have been described before (25, 54). The anti-CD4 antibody SIM.4 recognizes human CD4, binds to the Leu3A epitope, and blocks HIV-induced syncytium formation (38). SIM.4 antibody was from a CD4 hybridoma obtained from the AIDS Research and Reference Reagent Program. P3x63 immunoglobulin G1 (IgG1) paraprotein (P3) was produced by the P3x63 Ag 8.652 myeloma cell line and used as a negative control. Antibodies against CD7 (3A1), CD3 (Leu4), ICAM-1 or CD59 (RR1.1.1), CD11a of LFA-1 (TS-122), major histocompatibility complex (MHC) class I (3F10), and MHC class II (L243) have been described before (2).

Assessment of viral infection. Jurkat cells were cultured in 6-mm-diameter wells of microtiter plates at 10^5 per well in 0.2 ml. NIH 3T3 cells were cultured in the 6-mm-diameter wells at 5×10^3 cells per well in 0.2 ml. Cells were inoculated with HIV-1 at a multiplicity of infection (MOI) of approximately 0.01.

Morphologic cytopathic effects were determined by observation of live cells with an inverted phase-contrast microscope. RT activity was measured from culture supernatants as noted before (57). RT activity was quantitated by use of a phosphoimager. The 50% tissue culture infectious dose ($TCID_{50}$) was determined as noted before (31). For experiments with NIH 3T3 cells, the cells were inoculated and cultured for up to 20 days, with assessment of cytopathology and supernatant medium RT activity. In other work, to improve the ability to detect productive infection in NIH 3T3 cells, cells were inoculated, cultured overnight, washed, treated with trypsin (2.5 mg/ml for 4 min at room temperature) to remove extracellular virus, and then incubated for another 18 h. Supernatant medium was then recovered and cocultivated with CEM cells for 14 days, and supernatant medium RT activity was measured.

Transfections. Jurkat cells were transfected by the calcium phosphate coprecipitation technique as directed by the manufacturer (GIBCO-BRL, Grand Island, N.Y.). Briefly, 10^6 cells were washed with Dulbecco's modified Eagle's medium. Then 10 μg of plasmid DNA (the pCEP4 vector containing CD44S or CD44E or the vector alone) diluted in calcium phosphate buffer was added dropwise to the cells and incubated at 37°C overnight; the medium was then replaced with fresh medium containing 10% FBS for another 48 h. Transfected clones were selected by culturing with 550 U of hygromycin (Calbiochem, La Jolla, Calif.) per ml and screened for CD44 antigen expression by flow cytometry. At least four independent transfectants in each experiment were cloned and continuously cultured with 275 U of hygromycin per ml.

NIH 3T3 cells were transfected by comparable techniques. For CD44 transfection, cells were cotransfected with the CDM8 plasmid, containing CDw44, and the pSVneo plasmid, containing the neomycin resistance gene. For CD4 transfections, the cells were transfected with the T4-mPV7 plasmid. For combined CD4 and CD44 transfections, cells were cotransfected with the CDM8 plasmid, containing CDw44, and the T4-pMV7 plasmids. Individual colonies were selected by culturing in 800 μg of G418 per ml and maintained in medium with 400 μg of G418 per ml.

Flow cytometry. Cell surface antigen analysis was done as described before (35) by indirect immunofluorescence with a FACSTAR analyzer. Hyaluronan-fluorescein isothiocyanate (FITC) binding was quantified as noted before (35).

Immunoblot analysis. Cells (5×10^6) were washed twice with phosphate-buffered saline (PBS), lysed in 1% sodium dodecyl sulfate (SDS) in 10 mM Tris (pH 7.4), and boiled for 5 min. The viscosity of the sample was diminished by several passages through a 26-gauge needle. Samples were then centrifuged for 5 min to remove insoluble material. The protein concentration was adjusted to 1 mg/ml. Ten micrograms of protein was loaded into each lane and separated by electrophoresis through SDS–7.5% polyacrylamide. Proteins were transferred to a polyvinyl difluoride membrane. The membrane was then blocked with 5% dry milk and 2% bovine serum albumin at 0°C for 18 h and subsequently incubated with the primary antibody (A3D8, A1G3, 5F12, or P3). The membrane was processed by the enhanced chemiluminescence (ECL) method (Amersham, Arlington Heights, Ill.). After being washed, the membrane was incubated for 1 h with horseradish peroxidase-conjugated anti-mouse IgG, washed, and then developed according to the manufacturer's instructions.

TABLE 1. Phenotypic characterization of Jurkat cell lines

Antibody[a]	Antigen	% of cells positive				
		Jurkat	JP3	JH3	JH8	JE5
P3	None	0	2	1	1	4
A3D8	CD44	2	9	71	66	67
A1G3	CD44	2	8	70	35	51
5F12	CD44	2	8	59	48	70
SIM4	CD4	81	83	59	89	55
3F10	MHC-I	100	92	89	93	96
DR	MHC-II	0	1	0	1	3
Leu4	CD3	4	36	14	2	2
R.R.1	ICAM-1	83	95	73	95	89
3A1	CD7	100	100	96	98	97
TS-122	CD11a	65	72	73	77	24
Hyaluronan-FITC		2	6	27	13	33

[a] All antibodies were used at saturating concentrations. The hyaluronan-FITC assays were done by direct analysis of binding of hyaluronan-FITC to cells and did not involve antibodies.

Northern (RNA blot) analysis. Twenty micrograms of total cellular RNA was separated in 1.2% formaldehyde–agarose gels and immobilized on a nylon membrane by standard capillary transfer. cDNA probes were prepared by random-primed incorporation of digoxygenin-labeled deoxynucleoside triphosphates (dNTPs; Boehringer-Mannheim) into gel-purified CD44 DNA. Hybridization was done at 42°C in 1× Denhardt's solution and 50% formamide. Chemiluminescent detection was done according to the manufacturer's instructions (Boehringer-Mannheim).

RT-PCR. cDNA from Jurkat cells was synthesized from RNA with Superscript RT from GIBCO-BRL. Thirty-five cycles of PCR were done under the following conditions: 95°C for 80 s, 52°C for 1 min, and 72°C for 3 min. A negative control (no template cDNA in the reaction mix) was done with each reaction. The PCR primers for CD44 sequences were as follows: U1, forward, 5′ ACATC AGTCA CAGAC CTGCC 3′, and D1, reverse, 5′ GCAAA CTGCA AGAAT CAAAG CC 3′.

RESULTS

Cell phenotypes. Several stable, transfected cell lines were obtained. Five lines were characterized in detail: the Jurkat parent (J), two lines transfected with CD44S (JH3 and JH8), one line transfected with CD44E (JE5), and one line transfected with the vector pCEP4 alone (JP3). The doubling times of the five cell lines were similar; for example, J, 1.2 days; JH3, 1.2 days; JH8, 1.0 day; JH3 in hygromycin, 1.6 days; and JH8 in hygromycin, 1.4 days. All five lines were comparable in appearance in culture except for cellular aggregation. Cells of the parent J line and the line transfected with the vector alone (JP3) grew as floating single cells, while those expressing CD44S or CD44E formed floating homoaggregates in liquid culture. By flow cytometry (Table 1), nontransfected J cells and those transfected with the vector alone (JP3) did not express CD44, while JH3, JH8, and JE5 expressed high levels of CD44, as determined by reaction with antibodies A3D8, A1G3, and 5F12. Both CD44S- and CD44E-transfected cell lines bound FITC-conjugated hyaluronan (Table 1). Analysis of expression of other surface membrane molecules that might influence HIV-1 infection of the cells showed no changes in the expression of CD4, MHC class I or II antigens, ICAM-I (CD59), CD7, or CD11a of LFA-1 (CD11a/CD18) (Table 1). JP3 cells (those transfected with the vector alone) and JH3 cells (those from one of the cell lines transfected with CD44S) displayed more CD3 than did the parent J cells and the other transfected cell lines JH8 and JE5.

Immunoblot analysis of the cells (Fig. 1) showed that J cells and JP3 cells contained no CD44 antigen detectable by antibodies A3D8, A1G3, and 5F12. JH3 and JH8 cells contained

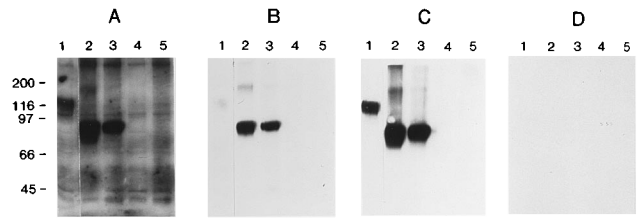

FIG. 1. Immunoblot analysis of Jurkat cell lines for CD44 expression. Lysates from Jurkat parent cells (J, lane 5) and cell lines JP3 (lane 4), JH3 (lane 3), JH8 (lane 2), and JE5 (lane 1) were analyzed with antibody 5F12 (A), A1G3 (B), A3D8 (C), and control antibody P3 (D). Ten micrograms of total cellular protein was used for each lane.

an antigen at about 80 kDa that was recognized by antibodies A3D8, A1G3, and 5F12, while JE5 cells contained an antigen at about 130 kDa that was recognized by antibodies A3D8 and 5F12 (and not strongly by A1G3). This pattern of reactivity of these antibodies for CD44S and CD44E is similar to that described before (25, 27, 54).

A conventional Northern blot analysis of total RNA extracted from the different cell line cells showed that cell lines J and JP3 expressed no detectable CD44S or CD44E message, while cell lines JH3 and JH8 expressed an mRNA of 2.2 kb and JE5 expressed an mRNA of 3.3 kb (data not shown). To analyze the expression of mRNA for the various CD44 isoforms by a more sensitive and specific technique, we did RT-PCR with primers that flanked the potential variant exons (19, 36). CD44S mRNA contains none of the variant exons and was predicted to yield a DNA product of 470 bp. CD44E mRNA contains variant exons 8, 9, and 10 and was predicted to yield a DNA product of 878 bp. Figure 2 demonstrates that all five cell lines contained the predicted mRNAs: J and JP3 had no CD44 mRNA, while JH3 and JH8 had CD44S (470-bp product) and JE5 had CD44E (878-bp product).

Susceptibility of transfected cells to HIV-1 infection. Prior work by us and others has demonstrated that Jurkat cell line cells are susceptible to infection with the lymphocytotropic strain HIV-1-LAI but not by monocytotropic strains (9) (data not shown). As noted in Fig. 3, the parent J cells and those transfected with vector alone (JP3 cells) could be infected with HIV-1-LAI but not with HIV-1-BaL or HIV-1-ADA. Likewise, transfected cells expressing CD44E (JE5 cells) could be

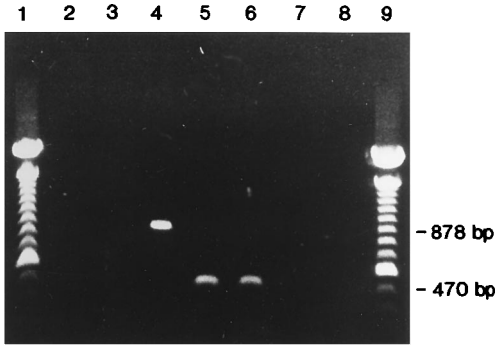

FIG. 2. RT-PCR analysis of Jurkat cell lines for CD44 mRNA expression. mRNA was reverse transcribed into DNA and amplified by PCR (see text). The resultant DNA was electrophoresed and stained with ethidium bromide. Lane 1, standards; lane 2, reagent only, without primers; lane 3, reagent control with primers; lane 4, JE5; lane 5, JH8; lane 6, JH3; lane 7, JP3; lane 8, J; lane 9, size standards. The 470-bp band predicted for the product of CD44S is seen in lanes 5 (JH8) and 6 (JH3), while the 878-bp band predicted for the product of CD44E is seen in lane 4 (JE5).

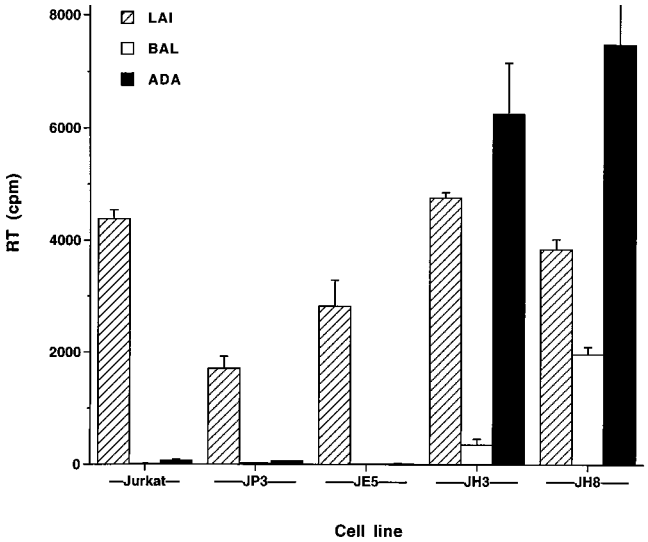

FIG. 3. Level of HIV-1 infection in Jurkat cell lines. Cells from the different Jurkat cell lines were inoculated with HIV-1-LAI, HIV-1-BaL, or HIV-1-ADA. Supernatant RT activity was measured at day 20 after inoculation.

infected with HIV-1-LAI but not with the monocytotropic strains. In contrast, cells from each of the two lines expressing CD44S (JH3 and JH8 cells) could be infected with HIV-1-BaL and HIV-1-ADA in addition to HIV-1-LAI. The data displayed in Fig. 3 are representative of five separate identical experiments, each of which gave comparable results. Also, using two other stable lines transfected with CD44S and one other stable line transfected with CD44E not discussed above, we found comparable patterns of infectivity (data not shown).

Table 2 shows the time course for infection with the different virus strains and $TCID_{50}$ values for the different strains in the parent J cells and the two cell lines expressing CD44S (JH3 and JH8). Cells of the CD44S-transfected JH8 cell line displayed evidence of HIV-1 infection earlier than did those of the other Jurkat cell lines. When assessed at day 16 after inoculation, CD44S-transfected lines JH3 and JH8 had higher $TCID_{50}$s for HIV-1-LAI than did the parent J cells. Also, as noted above, cells from lines JH3 and JH8 could be infected with HIV-1-BaL and HIV-1-ADA, while the parent line J could not be infected with either of these strains.

To determine if anti-CD44 antibodies modified HIV-1 infection of the cells, we cultured cells of lines J, JP3, JH3, JH8, and JE5 with 35 or 70 μg of purified A3D8 antibody or control P3 antibody per ml, with and without inoculation with HIV-1-LAI, HIV-1-BaL, or HIV-1-ADA. The antibodies did not

TABLE 2. Characteristics of HIV-1 infection in Jurkat cell lines[a]

Cell line	Day of appearance of RT activity			$TCID_{50}$/ml		
	HIV-1-LAI	HIV-1-BaL	HIV-1-ADA	HIV-1-LAI	HIV-1-BaL	HIV-1-ADA
Jurkat	12	—[b]	—	1,600	<100	<100
JH3	12	16	16	3,600	<100	<100
JH8	8	12	12	29,100	3,200	900

[a] Cells were inoculated (10 μl of virus stock into 200 μl of total culture volume), and supernatant samples were measured every 2 days for RT activity. The $TCID_{50}$ values were calculated from RT values of day 16 samples. These values, in general, were maximal for the period of observation.

[b] —, no RT activity was detected through 32 days of culture after inoculation.

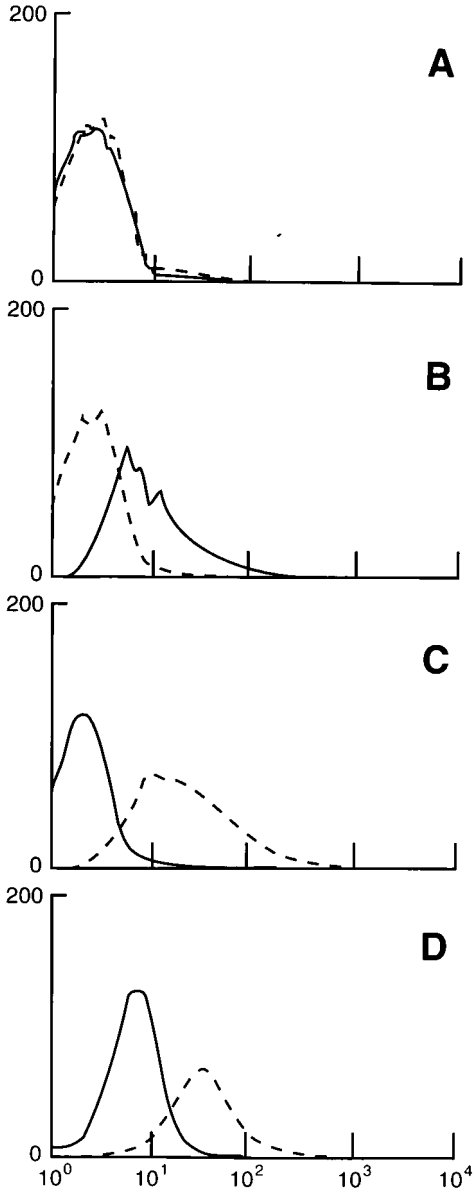

FIG. 4. Flow cytometric analysis of antigen expression by NIH 3T3 cells. (A) Control NIH 3T3 cells, (B) NIH 3T3-T4 cells (transfected with CD4), (C) NIH 3T3-CD44 cells (transfected with CD44), and (D) NIH 3T3-T4/CD44 cells (transfected with CD4 and CD44) were analyzed with antibodies against CD4 (SIM.4, solid lines), CD44 (A3D8, dashed lines), and control antibody P3. The results with P3 are not displayed; they were essentially identical to those of CD4 and CD44 in panel A. *y* axis, cell number; *x* axis, intensity of fluorescence.

modify the ability of the virus to cause productive infection of the cells (data not shown). This is in contrast to our previous studies with normal mononuclear phagocytes, in which anti-CD44 antibodies inhibited productive HIV-1 infection (46).

Investigators have noted that murine cells, including those expressing murine or human CD4, cannot be productively infected with HIV-1 virions. To determine if human CD44S would enable mouse cells expressing human CD4 to become infected with lymphocytotropic or monocytotropic strains of HIV-1, we transfected mouse NIH 3T3 cells with human CD4 alone, human CD44S alone, or a combination of human CD4 and CD44. These cells expressed high levels of human CD4 and human CD44, as determined by studies with anti-CD4 and anti-CD44 antibodies (Fig. 4). When we inoculated the cells with HIV-1-LAI, HIV-1-BaL, or HIV-1-ADA, there was no evidence of supernatant RT activity (for periods of culture of up to 28 days). Likewise, when supernatants from the various transfected NIH 3T3 that had been inoculated with the different HIV-1 strains were cocultured with cells of the human T-cell line CEM, we still could not detect any RT activity. Thus, although human CD44S enhanced the ability of human Jurkat cells to be infected with lymphocytotropic and monocytotropic strains of HIV-1, human CD44S (alone or in combination with human CD4) could not enable mouse NIH 3T3 cells to be infected with HIV-1.

DISCUSSION

Our prior work has shown that anti-CD44 antibodies can inhibit productive HIV-1 infection of mononuclear phagocytes but not lymphocytes (46). In the present work, to study the role of CD44 isoforms in cellular HIV-1 infection and tropism, we have used cells of the CD4-positive, CD44-negative human T-lymphoblast cell line Jurkat. Jurkat cells can be infected with lymphocytotropic strains of HIV-1 but not with monocytotropic strains (9). We show here that by inducing high-level membrane expression of isoform CD44S (but not isoform CD44E) on Jurkat T cells by CD44 gene transfections, we render Jurkat cells infectable by the monocytotropic strains HIV-1-BaL and HIV-1-ADA and more infectable by the lymphocytotropic strain HIV-1-LAI. The mechanisms underlying these changes are not fully known.

The human CD44 molecules (previously known as Pgp-1, HCAM, Hermes antigen, and the lymphocyte homing receptor) are a family of related glycoproteins formed by alternative RNA splicing (17, 26, 34, 36, 48, 51). Different CD44 isoforms may mediate various functions, including (i) serving as the membrane receptor for hyaluronan, (ii) anchoring cells to the extracellular matrix by binding hyaluronan, fibronectin, or collagen, (iii) binding to the cytoskeletal protein ankyrin, (iv) mediating leukocyte binding to endothelial cells and leukocyte aggregation, (v) serving as a leukocyte receptor involved in lymphocyte comitogenesis and monocyte monokine secretion, and (vi) determining the metastatic behavior of certain tumor cells (26). Soluble CD44 has been found in tissue fluids and plasma (24), and soluble CD44 or CD44 in liposomes can interfere with the normal function of CD44 in vitro or in vivo (24, 52, 56). An intact CD44 cytoplasmic domain is critical for the functional activity of CD44 (33); interaction with protein kinase C may be important in the cell signaling pathways (3, 4). Hematopoietic cells such as lymphocytes and monocytes express predominantly the CD44S isoform, while epithelial cells express predominantly the CD44E isoform; expression of CD44 isoforms can be modulated by cytokines and cellular activation (27, 36).

While CD4 functions as the predominant membrane receptor for HIV-1, there is evidence that another membrane component(s) may be required for HIV-1 binding, entry, and productive infection. Potential cofactors include galactosyl ceramide, LFA-1 (CD11a/CD18), and CD26. In CD4-negative neural cell lines and colon epithelial cells, galactosyl ceramide has been noted to bind gp120 and to serve as a "receptor" for HIV-1 (21, 22). LFA-1 appears to be important for fusion of HIV-1-infected cells but not for HIV-1 infection of cells per se (20, 28, 42, 43, 55). Callebaut and coworkers reported that the T-cell activation antigen CD26 (dipeptidyl peptidase IV) appears to be a cofactor for entry of HIV into $CD4^+$ cells (6, 7), but others could not confirm this (1, 5, 8, 40, 44).

Our experiments demonstrate that expression of CD44S by

Jurkat cells enhances their ability to be infected by monocytotropic strains of HIV-1. The mechanism(s) of this alteration is not known. Cells transfected with either CD44S or CD44E transcribed the predicted mRNA, expressed high levels of membrane CD44S or CD44E, and bound hyaluronan. There were no changes in cell behavior or expression of other molecules which might alter HIV-1 infectability—cell growth rates and cell expression of CD4, MHC class I and II, CD3, CD7, LFA-1 (CD11a/CD18), and ICAM-I (CD59) were essentially unchanged. Anti-CD44 antibodies did not modify HIV-1 infection in any of the cell line cells, and we have not been able to demonstrate any direct binding of CD44 with HIV-1 gp120 (56a). Although this suggests that CD44 is probably not functioning at the cell membrane-virus envelope interaction step, more work is required to precisely determine the mechanism(s) involved. It is important to note that our earlier work showed that anti-CD44 antibodies inhibited productive HIV-1 infection of normal mononuclear phagocytes (46); however, our current studies show that the antibodies do not block infection of any of the Jurkat cell line cells with either lymphocytotropic or monocytotropic strains. This suggests that different mechanisms of modulation of HIV-1 infection involving CD44 may be operative in normal mononuclear phagocytes and in the various CD44-transfected Jurkat cell line cells.

Gallatin and coworkers noted earlier that in macaques infected with simian immunodeficiency virus, there was a selective depletion of CD4-positive CD44 (heterotypic adhesion receptor) "high" cells (15). Furthermore, the $CD4^+$ $CD44^{hi}$ cells were much more susceptible to productive simian immunodeficiency virus infection in vitro (15). This suggests that CD44 could be a determinant of lentivirus infection of cells in vivo. Guo and Hildreth noted that HIV-1 infection of the human monocytic cell lines MonoMac and THP-1 caused a decrease in CD44 expression coincident with an induction of CD18-dependent homotypic cell aggregation (18). It is possible that cells expressing CD44S would be more responsive to signals which would cause "activation" of cells to express certain proviral mechanisms (e.g., expression of tumor necrosis factor alpha or beta [45]) or diminish certain antiviral mechanisms (e.g., expression of alpha interferon [14]). Our findings suggest that cellular CD44 (in addition to cellular CD4 and viral gp120) may be an important determinant of viral infection and tropism. Other investigators have noted that cells of the human myeloid cell lines HL-60, U937, and THP-1 (cells usually only infectable by lymphocytotropic strains of HIV-1) can also be rendered susceptible to infection with monocytotropic strains of HIV-1 after differentiation in vitro (32, 39). It is possible that this is related to a differentiation-linked induction of expression of the cellular CD44S isoform.

Recent evidence suggests that CD44 may play a role in the infection of human cells with poliovirus. Shepley and Racaniello showed that the anti-CD44S monoclonal antibody AF3 blocked poliovirus binding to and infection of human HeLa cells, although it did not bind to the poliovirus receptor per se (50). Furthermore, they demonstrated that anti-CD44 antibodies A3D8 and IM7 also blocked poliovirus binding to and infection of HeLa cells. However, mouse L-cell transformants expressing high levels of membrane CD44S could not bind or be infected with poliovirus, indicating that CD44S could not solely act as the poliovirus receptor (50). Similarly, in our experiments, we showed that high-level expression of human CD44S alone or human CD44S with human CD4 did not render mouse fibroblasts susceptible to infection with HIV-1. Human HeLa cells may express both CD44E and CD44S. HeLa cells transfected with CD4 (presumably then expressing both CD44S and CD4) can be infected with lymphocytotropic but not monocytotropic HIV-1 strains (10, 11). However, in the CD4-positive Jurkat T-cell lines examined here (normally infectable by lymphocytotropic but not monocytotropic HIV-1 strains), expression of CD44S increases the cells' infectability by the lymphocytotropic stain HIV-1-LAI and renders the cells infectable by the monocytotropic strains HIV-1-BaL and HIV-1-ADA. These data indicate clearly that in certain cell types (e.g., mouse fibroblasts and human HeLa cells), expression of human CD4 and CD44S is not sufficient to allow infection with monocytotropic strains of HIV-1, while in the human Jurkat T-cell line, expression of CD44S does modify HIV-1 infection and tropism. Further study in these systems may add to our understanding of the role of various CD44 isoforms and other molecules in HIV-1 infection.

Researchers have noted the critical importance of mononuclear phagocytes and monocytotropic strains of HIV-1 in the transmission of HIV-1—despite the existence of several different quasi-species of HIV-1 in infected individuals, monocytotropic (non-syncytium-inducing) strains appear to be preferentially transmitted in vivo to mononuclear phagocytes (58, 59). The full understanding of the mechanisms by which CD44S influences HIV-1 infection and tropism for human leukocytes will aid in our overall understanding of the biology of HIV-1 infection. Furthermore, this work may help in the development of novel strategies for preventing or controlling HIV-1 infection.

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REFERENCES

1. **Alizon, M., and T. Dragic.** 1994. Technical comments: CD26 antigen and HIV fusion? Science **264:**1161–1162.
2. **Barry, T. S., D. M. Jones, C. B. Richter, and B. F. Haynes.** 1991. Successful engraftment of human postnatal thymus in severe combined immune deficient (SCID) mice: differential engraftment of thymic components with irradiation versus anti-asialo GM-1 immunosuppressive regimens. J. Exp. Med. **173:**167–180.
3. **Bourguignon, L. Y., V. B. Lokeshwar, J. He, X. Chen, and G. J. Bourguignon.** 1992. A CD44-like endothelial cell transmembrane glycoprotein (GP116) interacts with extracellular matrix and ankyrin. Mol. Cell. Biol. **12:**4464–4471.
4. **Bourguignon, L. Y. W., E. L. Kalomiris, and V. B. Lokeshwar.** 1991. Acylation of the lymphoma transmembrane glycoprotein GP85 may be required for GP85-ankyrin interaction. J. Biol. Chem. **266:**11761–11765.
5. **Broder, C. C., O. Nussbaum, W. G. Gutheil, W. W. Bachochin, and E. A. Berger.** 1994. Technical comments: CD26 antigen and HIV fusion? Science **264:**1156–1159.
6. **Callebaut, C., E. Jacotot, B. Krust, and A. G. Hovanessian.** 1994. Technical comments: CD26 antigen and HIV fusion?, response. Science **264:**1162–1165.
7. **Callebaut, C., B. Krust, E. Jacotot, and A. G. Hovanessian.** 1993. T cell activation antigen, CD26, as a cofactor for entry of HIV in $CD4^+$ cells. Science **262:**2045–2050.
8. **Camerini, D., V. Planelles, and I. S. Y. Chen.** 1994. Technical comments: CD26 antigen and HIV fusion? Science **264:**1160–1161.
9. **Carrillo, A., D. B. Trowbridge, P. Westervelt, and L. Ratner.** 1993. Identification of HIV1 determinants for T lymphoid cell line infection. Virology **197:**817–824.
10. **Chesebro, B., J. Nishio, S. Perryman, A. Cann, W. O'Brien, I. S. Chen, and K. Wehrly.** 1991. Identification of human immunodeficiency virus envelope gene sequences influencing viral entry into CD4-positive HeLa cells, T-leukemia cells, and macrophages. J. Virol. **65:**5782–5789.
11. **Chesebro, B., K. Wehrly, J. Nishio, and S. Perryman.** 1992. Macrophage-tropic human immunodeficiency virus isolates from different patients exhibit unusual V3 envelope sequence homogeneity in comparison with T-cell-tropic isolates: definition of critical amino acids involved in cell tropism. J. Virol. **66:**6547–6554.
12. **Dalgleish, A. G., P. C. L. 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.
13. **Fisher, R. A., J. M. Bertonis, W. Meier, V. A. Johnson, D. S. Costopoulos, T. Liu, R. Tizard, B. D. Walker, M. S. Hirsch, R. T. Schooley, and R. A. Flavell.** 1988. HIV infection is blocked in vitro by recombinant soluble CD4. Nature (London) **331:**76–78.
14. **Francis, M. L., M. S. Meltzer, and H. E. Gendelman.** 1992. Interferons in the persistence, pathogenesis, and treatment of HIV infection. AIDS Res. Hum. Retroviruses **8:**199–207.
15. **Gallatin, W. M., M. J. Gale, P. A. Hoffman, D. M. Willerford, K. E. Draves, R. E. Beneveniste, W. R. Morton, and E. A. Clark.** 1989. Selective replication of simian immunodeficiency virus in a subset of $CD4^+$ lymphocytes. Proc. Natl. Acad. Sci. USA **86:**3301–3305.
16. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science **233:**215–219.
17. **Goldstein, L. A., D. F. H. Zhou, L. J. Picker, C. N. Minty, R. F. Bargatze, J. F. Ding, and E. C. Butcher.** 1989. A human lymphocyte homing receptor, the Hermes antigen, is related to cartilage proteoglycan core and link proteins. Cell **56:**1063–1072.
18. **Guo, M. M., and J. E. Hildreth.** 1993. HIV-induced loss of CD44 expression in monocytic cell lines. J. Immunol. **151:**2225–2236.
19. **Hale, L. P., B. F. Haynes, and S. S. McCachren.** Expression of CD44 variants in human inflammatory synovitis. Submitted for publication.
20. **Hansen, J.-E. S., C. Nielsen, L. R. Mathiesen, and J. O. Nielsen.** 1991. Involvement of lymphocyte function-associated antigen-1 (LFA-1) in HIV infection: inhibition by monoclonal antibody. Scand. J. Infect. Dis. **23:**31–36.
21. **Harouse, J. M., S. Bhat, S. L. Spitalnik, M. Laughlin, S. Stefano, D. H. Silberberg, and F. Gonzalez-Scarano.** 1991. Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. Science **253:**320–323.
22. **Harouse, J. M., M. A. Laughlin, C. Pletcher, H. M. Friedman, and F. Gonzalez-Scarano.** 1991. Entry of human immunodeficiency virus-1 into glial cells proceeds via an alternate, efficient pathway. J. Leukocyte Biol. **49:**605–609.
23. **Harrington, R. D., and A. P. Geballe.** 1993. Cofactor requirement for human immunodeficiency virus type 1 entry into a CD4-expressing human cell line. J. Virol. **67:**5939–5947.
24. **Haynes, B. F., L. P. Hale, K. L. Patton, M. E. Martin, and R. M. McCallum.** 1991. Measurement of an adhesion molecule as an indicator of inflammatory disease activity: up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. Arthritis Rheum. **34:**1434–1443.
25. **Haynes, B. F., E. A. Harden, M. J. Telen, M. E. Hemler, J. L. Strominger, T. J. Palker, R. M. Scearce, and G. S. Eisenbarth.** 1983. Differentiation of human T lymphocytes. I. Acquisition of a novel human cell surface protein (p80) during normal intrathymic T cell maturation. J. Immunol. **131:**1195–1200.
26. **Haynes, B. F., H. X. Liao, and K. L. Patton.** 1991. The transmembrane hyaluronate receptor (CD44): multiple functions, multiple forms. Cancer Cells **3:**347–350.
27. **Haynes, B. F., M. J. Telen, L. P. Hale, and S. M. Denning.** 1989. CD44—a molecule involved in leukocyte adherence and T-cell activation. Immunol. Today **10:**423–428.
28. **Hildreth, J. E. K., and R. J. Orentas.** 1989. Involvement of a leukocyte adhesion receptor (LFA-1) in HIV-induced syncytium formation. Science **244:**1075–1078.
29. **Hwang, S. S., T. J. Boyle, H. K. Lyerley, and B. R. Cullen.** 1992. Identification of envelope V3 loop as the major determinant of CD4 neutralization sensitivity of HIV-1. Science **257:**535–537.
30. **Hwang, S. S., T. J. Boyle, H. K. Lyerley, and B. R. Cullen.** 1992. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science **253:**71–74.
31. **Johnson, V. A., and R. E. Byington.** 1990. Quantitative assays for virus infectivity, p. 71–76. *In* A. Aldovini and B. D. Walker (ed.), Techniques in HIV research. Stockton Press, New York.
32. **Kitano, K., G. C. Baldwin, M. A. Raines, and D. W. Golde.** 1990. Differentiating agents facilitate infection of myeloid leukemia cell lines by monocytotropic HIV-1 strains. Blood **76:**1980–1988. (Erratum, **77:**1624, 1991.)
33. **Lesley, J., Q. He, K. Miyake, A. Hamann, R. Hyman, and P. W. Kincade.** 1992. Requirements for hyaluronic acid binding by CD44: a role for the cytoplasmic domain and activation by antibody. J. Exp. Med. **175:**257–266.
34. **Lesley, J., and R. Hyman.** 1992. CD44 can be activated to function as a hyaluronic acid receptor in normal murine T cells. Eur. J. Immunol. **22:**2719–2723.
35. **Liao, H. X., M. C. Levesque, K. Patton, B. Bergamo, D. Jones, M. A. Moody, M. J. Telen, and B. F. Haynes.** 1993. Regulation of human CD44H and CD44E isoform binding to hyaluronan by phorbol myristate acetate and anti-CD44 monoclonal and polyclonal antibodies. J. Immunol. **151:**6490–6499.
36. **Mackay, C. R., H. J. Terpe, R. Stauder, W. L. Marston, H. Stark, and U. Gunthert.** 1994. Expression and modulation of CD44 variant isoforms in humans. J. Cell. Biol. **124:**71–82.
37. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, and R. A. Weiss.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47:**333–348.
38. **McCallus, D. E., K. E. Ugen, A. I. Sato, W. V. Williams, and D. B. Weiner.** 1992. Construction of a recombinant bacterial human CD4 expression system producing a bioactive CD4 molecule. Viral Immunol. **5:**163–172.
39. **Meylan, P. R., C. A. Spina, D. D. Richman, and R. S. Kornbluth.** 1993. In vitro differentiation of monocytoid THP-1 cells affects their permissiveness for HIV strains: a model system for studying the cellular basis of HIV differential tropism. Virology **193:**256–267.
40. **Morimoto, C., C. I. Lord, C. Zhang, J. S. Duke-Cohan, N. L. Letvin, and S. F. Schlossman.** 1994. Role of CD26/dipeptidyl peptidase IV in human immunodeficiency virus type infection and apoptosis. Proc. Natl. Acad. Sci. USA **91:**9960–9964.
41. **O'Brien, W. A., I. S. Y. Chen, D. D. Ho, and E. S. Daar.** 1992. Mapping genetic determinants for human immunodeficiency virus type 1 resistance to soluble CD4. J. Virol. **66:**3125–3130.
42. **Pantaleo, G., L. Butini, C. Graziosi, G. Poli, S. M. Schnittman, J. J. Greenhouse, J. I. Gallin, and A. S. Fauci.** 1991. Human immunodeficiency virus (HIV) infection in $CD4^+$ T lymphocytes genetically deficient in LFA-1: LFA-1 is required for HIV-mediated cell fusion but not for viral transmission. J. Exp. Med. **173:**511–514.
43. **Pantaleo, G., G. Poli, L. Butini, C. Fox, A. I. Dayton, and A. S. Fauci.** 1991. Dissociation between syncytia formation and HIV spreading—suppression of syncytia formation does not necessarily reflect inhibition of HIV infection. Eur. J. Immunol. **21:**1771–1774.
44. **Patience, C., A. McKnight, P. R. Clapham, M. T. Boyd, R. A. Weiss, and T. F. Schulz.** 1994. Technical comments: CD26 antigen and HIV fusion? Science **264:**1159–1160.
45. **Poli, G., and A. S. Fauci.** 1993. Cytokine modulation of HIV expression. Semin. Immunol. **5:**165–173.
46. **Rivadeneira, E. D., D. L. Sauls, Y. Yu, B. F. Haynes, and J. B. Weinberg.** 1995. Inhibition of HIV-1 infection of mononuclear phagocytes by anti-CD44 antibodies. AIDS Res. Hum. Retroviruses **11:**541–546.
47. **Sattentau, Q. J., and R. A. Weiss.** 1988. The CD4 antigen: physiological ligand and HIV receptor. Cell **52:**631–633.
48. **Screaton, G. R., V. B. Martyn, D. G. Jackson, F. B. Cornelis, U. Gerth, and J. I. Bell.** 1992. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. Proc. Natl. Acad. Sci. USA **89:**12160–12164.
49. **Shadduck, P. P., J. B. Weinberg, A. F. Haney, J. A. Bartlett, A. J. Langlois, D. P. Bolognesi, and T. J. Matthews.** 1991. Lack of enhancing effect of human anti-human immunodeficiency type 1 (HIV-1) antibody on HIV-1 infection of human blood monocytes and peritoneal macrophages. J. Virol. **65:**4309–4316.
50. **Shepley, M. P., and V. R. Racaniello.** 1994. A monoclonal antibody that blocks poliovirus attachment recognizes the lymphocyte homing receptor CD44. J. Virol. **68:**1301–1308.
51. **Stamenkovic, I., A. Aruffo, M. Amiot, and B. Seed.** 1991. The hematopoietic and epithelial forms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate-bearing cells. EMBO J. **10:**343–348.
52. **Sy, M.-S., Y.-J. Guo, and I. Stamenkovic.** 1992. Inhibition of tumor growth in vivo with a soluble CD44-immunoglobulin fusion protein. J. Exp. Med. **176:**623–627.
53. **Tateno, M., F. Gonzalez-Scarano, and J. A. Levy.** 1989. Human immunodeficiency virus can infect CD4-negative human fibroblastoid cells. Proc. Natl. Acad. Sci. USA **86:**4287–4290.
54. **Telen, M. J., G. S. Eisenbarth, and B. F. Haynes.** 1983. Human erythrocyte antigens: regulation of expression of a novel red cell surface antigen by the inhibitor Lutheran In(Lu) gene. J. Clin. Invest. **71:**1878–1886.
55. **Valentin, A., K. Lundin, M. Patarroyo, and B. Asjo.** 1990. The leukocyte adhesion glycoprotein CD18 participates in HIV-1-induced syncytia formation in monocytoid and T cells. J. Immunol. **144:**934–937.
56. **Watson, S. R., C. Fennie, and L. A. Lasky.** 1991. Neutrophil influx into an inflammatory site inhibited by a soluble homing receptor-IgG chimaera. Nature (London) **349:**164–167.
56a. **Weinberg, J. B., and B. F. Haynes.** Unpublished data.
57. **Weinberg, J. B., T. J. Matthews, B. R. Cullen, and M. H. Malim.** 1991. Productive human immunodeficiency virus type-1 (HIV-1) infection of nonproliferating human monocytes. J. Exp. Med. **174:**1477–1482.
58. **Wolinsky, S. M., C. M. Wike, B. Korber, C. Hutto, W. P. Parks, L. L. Rosenblum, K. J. Kunstman, M. R. Furtado, and J. L. Munoz.** 1992. Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. Science **255:**1134–1137.
59. **Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho.** 1993. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science **261:**1179–1181.