in three types of CD4+ cell populations: HeLa cells expressing vaccinia-encoded CD4, the SupT1 CD4+ human T cell line, and phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs) from healthy human donors. Controls experiments (not shown) indicated that in all cases, significant β-galactosidase was produced with wild-type env, but not with the fusion-defective uncleavable mutant env.

To examine the effects of DPP IV inhibitors, we preincubated the indicated CD4+ cell types without or with the designated inhibitor before mixing with the env-expressing fusion partner. In the reporter gene activation assay, IPI had no effect on cell fusion at 10 mM, a concentration greater by >10³ than the reported Ki for DPP IV inhibition (2.2 μM) (14). Similarly, we observed no effects with the extremely potent inhibitor L-Pro-L-boroPro (P-boroP), even at concentrations (0.1 to 1 mM) greater by 10⁶ to 10⁷ than the corresponding Ki value for inhibition of DPP IV, 16 pM (15). In contrast, strong inhibition was observed with 100 μg of dextran sulfate (DS) per milliliter, indicating that fusion monitored by the reporter gene activation assay was sensitive to a well-known inhibitor of HIV env/CD4-mediated fusion (16).

We also tested the effects of the DPP IV inhibitors on cell fusion in vaccinia-free systems, using mixtures of one continuous cell line expressing endogenous CD4 and another expressing endogenous env (Fig. 3, bottom). The results with mixtures of CD4+ SupT1 cells and HIV-1 persistently infected 8E5 cells (Fig. 3, bottom) indicate no significant inhibition by the DPP IV inhibitors IPI (10 mM) or P-boroP (1 mM); under parallel conditions (100 μg/ml) abrogated syncytia formation. Additional experiments (not shown) using mixtures of various combinations of HIV-1 persistently infected cell lines (8E5 or H9/HTLV-IIIIB), and CD4+ continuous human cell lines (A3.01 T cell line, HeLa-CD4 transfectant cell line) similarly showed no inhibition of syncytia formation by the DPP IV inhibitors.

Taken together, these findings suggest that CD26 does not play a critical role in HIV-1 env/CD4-mediated cell fusion. It is possible that the discrepancies between our results and those of Callebaut et al. (8) reflect differences in the biological processes studied and the assays used. Arguments have been raised that env/CD4-mediated virus-cell fusion may be mechanistically different from cell-cell fusion (17). However this notion has been directly challenged by experimental findings (11, 18). A novel criterion for a model has been raised that the specificity of env/CD4-mediated cell fusion in the vaccinia-based system closely parallels that observed for HIV infection and syncytia formation (13). Most critically, both processes require CD4 to be expressed on a human cell type, and the defects with CD4-expressing nonhuman cells can be overcome by the formation of hybrids with human cells. We therefore believe that the discrepancies between our findings with cell fusion and those of Callebaut et al. with virus entry do not reflect a differential involvement of CD26 in these two processes. Definitive resolution of this problem will come when and if one or more factors are identified that render CD4-expressing nonhuman cells permissive for both virus-cell and cell-cell fusion mediated by HIV-1 env.

Christopher C. Broder
Ofer Nussbaum
Laboratory of Viral Diseases,
National Institute of Allergy and Infectious Diseases,
National Institutes of Health,
Bethesda, MD 20892, USA

William G. Gethel
William B. Bachovchin
Department of Biochemistry,
Tufts University School of Medicine,
Boston, MA 22111, USA

Edward A. Berger
Laboratory of Viral Diseases,
National Institute of Allergy and Infectious Diseases,
National Institutes of Health

REFERENCES AND NOTES

22. R. A. Morgan, personal communication.
23. S. Chakrabarti and B. Moss, personal communication.
24. C. C. Broder, unpublished data.

25. Supported in part by an award to E.A.B. from the NIH Intramural AIDS Targeted Antiviral Program, and by NIH grant AI-31866 to W.W.B. C.C.B is a recipient of an NIH Intramural Research Training Award. P. Kennedy provided expert technical assistance. We thank B. Fleischer for the donation of plasmid pK5S-CD26, R. A. Morgan for plasmid pG1NT7β-gal, and B. Moss and S. Chakrabarti for plasmid pS699 and vaccinia recombinant vS699.

30 December 1993; accepted 8 February 1994

Callebaut et al. (1) report that CD26 may act as an accessory receptor for CD4 for HIV infection. We have previously investigated the possible role of cell surface proteases cleaving the V3 loop of HIV gp120 as a pathway to entry into the cell (2). We have therefore tested whether human CD26 co-expressed with human CD4 on the surface of mink (Mv-1-lu) and cat (CCC) cell lines would provide the missing factor required for HIV-1 entry. We chose these cell lines in preference to mouse NIH 3T3 cells because

Table 1. Titration of HIV on cells expressing human CD4 and CD26 antigens. Data are shown in log₁₀ infectious units per milliliter. HeLa, Mv-1-lu (mink lung), and CCC (cat kidney) cells were transduced with human CD4 with the use of a retroviral vector and selected in neomycin as described by Clapham et al. (5). To express human CD26, cells were co-transfected (Lipo-fectamine; Gibco BRL, Paisley, Scotland) with CD26 cDNA in pCDM8 and pS2v-puro and selected in puromycin (3 μg/ml). All (100%) of CD4-transduced cells expressed CD4 as assessed by fluorescence-activated cell sorting analysis with monoclonal antibody (mAb ADP 318). All (100%) of Mv-1-lu-CD4/CD26 cells co-expressed human CD26 (detected with mAb TAS9); Eurogenetics, Teddington, United Kingdom) in amounts 20- to 100-fold higher than those present on H9 human T cells. Approximately 75% of CCC-CD4/CD26 cells expressed human CD26 in amounts similar to those expressed by Mv-1-lu. Cells were seeded and HIV infected by focal antigen assay as described previously (5). Undetectable infection (−) indicates that no infectious units were found in 250 μl of undiluted virus stock. Infection of cell mixtures indicates that a mixture of HeLa-CD4 cells and Mv-1-lu-CD4 cells in a ratio of 1:10,000 yielded detectable infection with the same RF HIV-1 stock.

<table>
<thead>
<tr>
<th>Cells</th>
<th>HIV-1</th>
<th>HIV-2</th>
<th>HIV-1 (HTLV-I) pseudo-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa-CD4</td>
<td>4.3</td>
<td>5.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Mv-1-lu</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Mv-1-lu-CD4</td>
<td>2.5</td>
<td>2.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Mv-1-lu-CD4/CD26</td>
<td>2.6</td>
<td>2.6</td>
<td>2.5</td>
</tr>
<tr>
<td>CCC</td>
<td>2.5</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>CCC-CD4</td>
<td>3.5</td>
<td>3.5</td>
<td>2.6</td>
</tr>
<tr>
<td>CCC-CD4/CD26</td>
<td>3.6</td>
<td>3.6</td>
<td>2.6</td>
</tr>
</tbody>
</table>

*ROD virus stock was rescued from the pA2CR3 molecular clone. **The HIV-1(HTLV-I) pseudo-type was prepared by propagating HIV-1 RF in C91PL cells. Because Mv-1-lu and CCC cells express receptors for HTLV (7), the pseudo-type was used as a positive control for HIV-1 replication in these cells, provided that entry occurred.
murine cells only weakly support HIV replication even if the entry blocks are bypassed (3, 4). In contrast, mink cells readily replicate HIV-1 if entry is facilitated as an amphotropic MLV pseudotype (3) or if nonprimate HIV-1 is adsorbed to mink cells expressing human CD4 and then treated with the nontoxic fusogen, polyethylene glycol (5). Moreover, we have shown that mink Mv-1-Lu cells and feline CCC cells can be infected by several strains of HIV-2 and simian immunodeficiency without the presence of human factors other than CD4 (6).

We used the same CD26 cDNA clone as did Callebaut et al. (1). Stable, dual expression of human CD4 and human CD26 did not render these cells susceptible to infection by the LAI and RF strains of HIV-1 (Table 1). Flow cytometry revealed that more than 99% of mink CD4-CD26 cells expressed substantial amounts of both human antigens at the cell surface, whereas approximately 75% of cat cells expressed both antigens. The CD26 was enzymatically active. In conclusion, our results, obtained with the use of an assay system for CD4-dependent HIV infection in promiscuous carnivore cell lines, indicate that human CD26 is not required for HIV-2 entry and is not sufficient for HIV-1 entry.

Callebaut et al. conclude that the human T cell activation antigen CD26 is a cofactor for HIV infection of CD4+ cells (1). Their data indicate that murine NIH 3T3 cells are permissive to infection by HIV only when human CD4 and CD26 are present. If correct, this observation has important implications for HIV tropism in human cells, as it has previously been reported by ourselves and others that CD4 is necessary but not sufficient for HIV infection of human lymphoblastoid T cell lines and mononuclear phagocytes. Furthermore, it has been shown that human CD4 is not sufficient for HIV infection of mouse (NIH 3T3) cells (2) or of African green monkey cells (COS) (3). Because one of us (D.C.) was involved in the isolation of a CDNA encoding the human CD26 antigen (4), we tested the role of CD26 in HIV-1 infection of murine and nonhuman primate cell lines.

Table 1. CD26 function in HIV infection. Light units are the result of luciferase assay of lysates of the indicated cell lines and transfectants after infection with HIV-1—luciferase transducing particles coated with the indicated envelope. Syncytia data indicate the number of blue syncytia formed when the same cells were transfected with a T7-promoter—lacZ construct and mixed with HeLa cells expressing HIV-1 envelope and T7 RNA polymerase. The percentages of cells expressing CD4, CD26, and both antigens are shown. Values are the average of four luciferase assays with LAI HIV-1 envelope, two luciferase assays with amphotropic envelope, and three syncytia assays; they represent at least 2500 cells analyzed by flow cytometry. For the luciferase assay the background was 113 ± 7 light units for eight measurements of buffer only, and the background values were all within one standard deviation of that value. For the syncytium assay the background values were all less than four. Bckg, background; ND, not determined. COS cells were co-transfected with an envelope-deleted JR-CSF HIV-1 genome bearing the firefly luciferase gene and an HIV1 envelope or murine amphotropic envelope expression vector (7), by electroporation or with lipofectamine (Gibco/BRL, Bethesda, Maryland). Pseudotype HIV-1—luciferase transducing particles were harvested 2 days later and incubated with the cells listed. The cells were lysed 3 days later and assayed for luciferase activity with a Monolight 2010 luminometer and Promega luciferase assay kit. The syncytium assay was done essentially by the protocol of Berger et al. (6). Briefly, 2 × 106 of the cells per well of each indicated cell type were infected with wild-type vaccinia and transfected with the lacZ gene under the control of the T7 promoter (8) with the use of lipofectamine. They were then mixed with HeLa cells infected with recombinant vaccinia encoding LAI HIV-1 envelope and T7 RNA polymerase (9). The cells were fixed 1 day later and incubated with X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; Sigma, St. Louis, Missouri), then syncytia were counted in one-eighth of each well of a 24-well plate. Surface expression of CD4 and CD26 was determined by incubation with Leu-3A-FTC (Becton Dickinson, Mountain View, California) and TS145 ascites, and then with phycoerythrin conjugated goat antibody to mouse IgG (Caltag, South San Francisco, California), respectively, with the use of a FACScan flow cytometer (Becton Dickinson). The percentages of mixed HeLa and HeLa-CD4 cells positive for CD4 were calculated.

<table>
<thead>
<tr>
<th>Cell line (transfected plasmid)</th>
<th>%CD4+</th>
<th>%CD26+</th>
<th>%CD4* \ CD26*</th>
<th>Syncytia</th>
<th>HIV-luciferase + LAI HIV-1 envelope (light units)</th>
<th>HIV-luciferase + amphotropic envelope (light units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa-CD4</td>
<td>&gt;98</td>
<td>&gt;98</td>
<td>&gt;98</td>
<td>Confluent</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HeLa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HeLa-CD4/HeLa = 0.1</td>
<td>10</td>
<td>&gt;98</td>
<td>98</td>
<td>98 ± 13</td>
<td>1719 ± 47</td>
<td>149 ± 10</td>
</tr>
<tr>
<td>HeLa-CD4/HeLa = 0.02</td>
<td>2</td>
<td>&gt;98</td>
<td>2</td>
<td>21 ± 1</td>
<td>1525 ± 1215</td>
<td>206 ± 9</td>
</tr>
<tr>
<td>HeLa-CD4/HeLa = 0.004</td>
<td>0.4</td>
<td>&gt;98</td>
<td>0.4</td>
<td>Bckg</td>
<td>631 ± 51</td>
<td>163 ± 58</td>
</tr>
<tr>
<td>HeLa-CD4/HeLa = 0.0008</td>
<td>0.08</td>
<td>&gt;98</td>
<td>0.08</td>
<td>Bckg</td>
<td>766 ± 29</td>
<td>Bckg</td>
</tr>
<tr>
<td>COS-CD4 (CDM7—CD26)</td>
<td>&gt;98</td>
<td>81.3</td>
<td>81.3</td>
<td>Bckg</td>
<td>1898 ± 531</td>
<td>Bckg</td>
</tr>
<tr>
<td>COS-CD4 (CDM8)</td>
<td>&gt;98</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>Bckg</td>
<td>10235 ± 2207</td>
<td>Bckg*</td>
</tr>
<tr>
<td>A9 (CDM8—CD4)</td>
<td>21.6</td>
<td>39.8</td>
<td>19.6</td>
<td>Bckg</td>
<td>ND</td>
<td>Bckg*</td>
</tr>
<tr>
<td>NIH 3T3—CD4 (CDM7—CD26)</td>
<td>&gt;98</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>ND</td>
<td>4634 ± 95</td>
<td>Bckg</td>
</tr>
<tr>
<td>NIH 3T3—CD4 (CDM8—CD4)</td>
<td>&lt;98</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>ND</td>
<td>4280 ± 276</td>
<td>Bckg</td>
</tr>
</tbody>
</table>

*These values were determined in a separate assay in which 1 HeLa-CD4 cell in 10 HeLa cells could be detected, but 1 in 100 could not.
CD26 antigen and HIV fusion?
C Patience, A McKnight, PR Clapham, MT Boyd, RA Weiss and TF Schulz

Science 264 (5162), 1159-1160.
DOI: 10.1126/science.7909960