The ability of the HIV-1 *envelope* gene products to form syncytia is correlated with infectivity of HIV-1 strains with respect to species and cell type (*5*). Simian and murine cells bearing human CD4 and CD26 were tested in a syncytium assay with the use of vaccinia-expressed *envelope* from the LAI isolate of HIV-1, essentially as described by Berger *et al.* (*6*). In this assay, HeLa-CD4 cells form abundant blue syncytia, detectable in a 50-fold excess of syncytium-resistant HeLa cells (Table 1). In contrast, we found that COS cells or A9 cells expressing human CD4 and CD26, or CD4 alone, did not form detectable HIV-1 envelope–mediated syncytia.

To more directly test the role of CD26 in HIV-1 infection, we constructed an *envelope*-defective molecular clone of the JR-CSF isolate of HIV-1 that bears the firefly *luciferase* gene in place of the *nef* gene. With this clone, luciferase activity could be used as a sensitive assay for HIV-1 infection. We prepared pseudotype defective HIV-1–*luciferase* virions by cotransfecting this modified HIV-1 genome with an LAI HIV-1 envelope expression vector, or with a murine amphotropic retroviral envelope expression vector, and then used them to infect human, simian, and murine cell lines that express both human CD4 and CD26. This assay is not only sensitive, but because it measures intracellular expression of luciferase, potential problems with residual input of HIV-1 [which Callebaut *et al.* address with trypsin treatment (*1*)] are obviated. We incubated HIV-1–*luciferase* virions bearing LAI HIV envelope glycoproteins with simian (COS) and mouse (NIH 3T3 and A9) cells expressing CD4 alone or CD4 and CD26. We assayed cells for luciferase activity 3 days after exposure to the HIV-1 pseudotype. As expected, COS, NIH 3T3, and A9 cells expressing CD4 alone were not infectable. Co-expression of CD26 with CD4 in these cells did not render them infectable by HIV-1–*luciferase* virions bearing LAI HIV-1 envelope glycoproteins as determined by luciferase activity (Table 1). In a reconstruction experiment, infection of HeLa-CD4 cells mixed with a 250-fold excess of HeLa cells resulted in detectable luciferase activity. Therefore, the sensitivity of the assay was sufficient to detect infection of the murine and simian cells expressing human CD4 and CD26 had they been susceptible to HIV-1. Furthermore, all these cells expressed luciferase after infection by HIV-1–*luciferase* virions bearing murine amphotropic retrovirus envelope glycoproteins, which indicates that the transfected cells were competent to support HIV-1 infection and luciferase expression, given a functional envelope-receptor pair.

One potential explanation for the positive NIH 3T3 infection observed by Callebaut *et al.* (*1*) is that CD26 with CD4 may indeed confer HIV susceptibility very inefficiently, detectable only after incubation with CEM cells. In such an assay, even a few infectious virions may be amplified, by viral spread in CEM cells, to detectable amounts. However, if this is the case, one must question the biological relevance of a cofactor that does not confer susceptibility to the majority of target cells harboring it.

In summary, we used sensitive, quantitative assays of HIV-1–mediated syncytium formation and HIV-1 infection to test the role of CD26 in these processes. We are unable to confirm the report of Callebaut *et al.* and conclude that human CD26 does not confer susceptibility to HIV-1 syncytium formation or infection in murine or simian cell lines that express human CD4.

Note added in proof: We recently made an NIH 3T3 cell line that stably expresses human CD4 and CD26. Although 45% of the cells expressed both receptors, we were unable to detect luciferase activity after infecting the cells with HIV-1–*luciferase* coated with LAI HIV-1 envelope.

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Callebaut *et al.* identify the cell surface protease CD26 as the long-sought human cofactor that allows HIV entry into CD4$^+$ cells (*1*). Their principal experimental evidence is the complementation of murine NIH 3T3 cells for productive HIV infection by transient transfection with CD4 and CD26 cDNAs. However, an undetermined and probably minor fraction of the murine cells coexpressed CD4 and CD26, and virus entry could not be measured directly. Hence, virus production was detected after amplification on human CD4$^+$ cells.

We sought to perform more direct assays to evaluate the possible role of CD26 in HIV entry. We stably transfected a CD26 expression vector (*2*) into CD4$^+$ NIH 3T3 cells [SC6 cell line (*3*)]. We performed experiments on a clone (SC6-CD26) that expresses large amounts of surface CD26 (similar to activated human lymphocytes), and on an uncloned population that we had sorted for CD26 expression (SC6-CD26P). Surface CD4 expression was similar in parental and CD26$^+$ cells; DPP IV activity was only detected in CD26$^+$ cells (*4*).

Because HIV entry in murine CD4 cells is blocked at the stage of membrane fusion with the viral envelope (*5*), we tested the ability of CD4$^+$CD26$^+$ murine cells to form syncytia with cells that express HIV envelope proteins. The SC6 cells contain a LTR-*lacZ* transgene induced after fusion with cells that express the viral protein Tat, such as HIV-infected cells (*3*). With the use of this highly sensitive assay, we could not detect fusion between CD26$^+$ SC6 cells and different HIV-1 infected cell lines. We also did not detect virus production after contact of parental or CD26$^+$ SC6 cells with HIV-1 (one infectious unit, IU, per cell), either directly or after coculture with human CD4$^+$ cells (*4*). Because the small amount of HIV-1 expression in murine cells might have limited the sensitivity of assays that are based on virus production, we sought proviral DNA as a marker of virus entry (Fig. 1). HIV-1 DNA was not amplified by polymerase chain reaction (PCR) in lysates from parental or CD26$^+$ SC6 cells after contact with 5,000 IU of HIV-1 (*6*). Proviral DNA was readily detected in parallel CD4-independent infections with 500 IU of HIV-1 coated with the envelope of human T cell leukemia virus type 1 (HTLV-1) (*6*).

In these experiments, we did not confirm the role of CD26 as a cofactor that allows HIV-1 entry into murine CD4$^+$ cells. Callebaut *et al.* did not observe HIV production from murine cells in the presence of zidovudine (AZT), which led them to suggest that HIV replication was necessary and, hence, that virus entry occurred. It can be argued that AZT also has effects at the cell surface, as it reduces the ability of uninfected CD4$^+$ cells to form syncytia (*7*). However, a simpler explanation could be that calcium phosphate transfection of murine cells allowed artifactual virus entry. Indeed, HIV was retrieved by Callebaut *et al.* (*1*) from murine cells transfected with CD4 alone, or with CD26 alone. A simple additive effect could explain the apparently higher quantity of virus rescued from cotransfected cells.

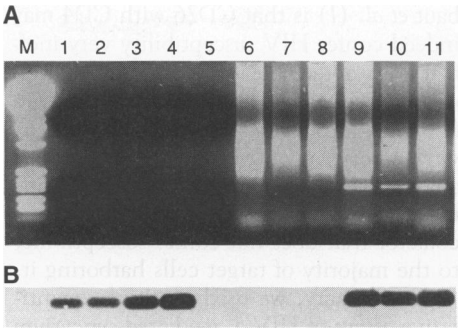

Fig. 1. Detection of HIV-1 entry into murine cells by PCR amplification of an HIV-1 *pol* gene fragment. Ethidium bromide staining of a 1.5% agarose gel (**A**) and hybridization to an HIV-1 *pol* probe after membrane transfer (**B**). DNA size markers, M (BRL 1 kb, ladder). Uninfected human T cells lysates containing 12, 25, 50, and 100 copies, respectively, of HIV-1 proviral DNA (lanes 1–4). Uninfected human T cells cells (lane 5). Lysates of murine cells SC6 (*6, 9*), SC6-CD26 (*7, 10*), or SC6-CD26P (*8, 11*), infected with wild-type HIV-1 (*6–8*) or HIV-1 with mixed HIV-1–HTLV-1 envelope phenotype (*9–11*) (lanes 6–11). After overnight contact with DNase-treated viral supernatants, the cells were washed and trypsinized to remove the inoculum and grown for 48 hours before lysis for PCR (*8*).

Finally, Callebaut *et al.* detected (*1*) CD26 expression in the MOLT-4, U937, and in Jurkat human cell lines, which were previously characterized as CD26-negative (*2*). Although variability among subclones cannot be ruled out a priori, it should be noted that CD26 expression was sought by Callebaut *et al.* with the use of monoclonal antibody BA5 (mAb, Immunotech, Marseille, France). Some background signal can be observed with BA5 in human cell lines, including CEM, that are not stained with other mAbs against CD26 and do not express DPP IV activity (*4*). The effect of agents aimed at blocking an eventual interaction between HIV and CD26 should therefore be confirmed by standard virus neutralization techniques, in authentic $CD26^+$ cells. Meanwhile, the cofactors of HIV entry have yet to be identified.

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Response: The HIV-1 *envelope* gene codes for a precursor polyprotein gp160 which, through proteolytic cleavage, yields extracellular (gp120) and transmembrane (gp41) envelope glycoproteins; gp120 binds to the CD4 receptor, whereas gp41 is involved in the fusion process (*1*). Both gp120 and gp41 are associated in a noncovalent manner to generate a complex that has two major functions in HIV infection. For the HIV particles, this complex is essential to the virus/cell membrane fusion that allows viral entry; whereas in HIV-infected cells, the gp120-gp41 complex expressed on the membrane of one cell interacts with CD4 molecules on another cell to initiate cell/cell membrane fusion, which results in the formation of syncytia. By investigating the virus/cell fusion process, we found that another cell surface antigen, CD26, serves as a cofactor for CD4, probably by interacting with highly conserved motifs in the V3 loop of gp120 (*2*).

Broder *et al.* use an assay for cell/cell fusion, to argue against the involvement of CD26 in the HIV envelope-mediated fusion process. Although virus/cell and cell/cell fusion processes require the interaction of gp120-gp41 complex with the CD4 receptor, there are subtle differences between these two events.

1) Cell surface adhesions molecules (such as LFA-1) have been demonstrated to be essential for cell/cell, but not for virus/cell, fusion: HIV-1 infection can occur in $LFA\text{-}1^-$ lymphocytes or in $LFA\text{-}1^+$ lymphocytes treated with antibody to LFA-1, whereas in a syncytium assay the antibody inhibits cell/cell fusion (*3, 4*).

2) HIV isolates from seropositive individuals manifest a significant variability in their capacity to induce syncytium formation. Nonsyncytium-inducing (NSI) isolates have been detected throughout the course of HIV-1 infection, whereas syncytium-inducing (SI) isolates tend to predominate in AIDS patients (*5*). Both NSI and SI isolates of HIV-1 must be able to induce virus/cell fusion because they can infect cells; however, they differ significantly in their capacity to initiate cell/cell fusion. ROD HIV-2 and EHO HIV-2 are two virulent isolates of HIV-2 (6); however, they manifest different phenotypes in the cell/cell fusion process: ROD is SI whereas EHO is NSI (*7*).

3) HIV-1 isolates of SI phenotye do not systematically initiate cell/cell fusion in all human cells permissive to virus/cell fusion. For example, it has been reported that infection of CEM cells (a $CD4^+$ T cell line) with a SI HIV-1 isolate results in single cell killing in the absence of cell/cell fusion (*8*). We have routinely used a clone of CEM cells characterized by the expression of a high density of CD4 (CEM clone 13, prepared in the laboratory of L. Montagnier). Infection of these cells with SI HIV isolates resulted systematically in the formation of syncytia (*9, 10*). In contrast, others have reported that this specific clone is resistant to cell/cell fusion. However, when conjugate formation is increased by the lectin wheat germ agglutinin, then cell/cell fusion has occurred (*11*). Slight differences in cell culturing conditions, therefore, could generate modifications in the surface structure of cells and may account for such discrepancies.

4) Cell death by apoptosis occurs during infection of CEM cells by SI and NSI HIV isolates; apoptosis is initiated by the interaction of cell membrane–expressed gp120-gp41 complex with the CD4 receptor (9). HIV entry (that is, virus/cell fusion) does not initiate apoptosis, so there might be some structural and conformational differences in the gp120-gp41 complex when it is presented by virus particles or by infected cells.

5) Cell/cell fusion has been reported to require Mg^{2+}. Accordingly, the use of EDTA as a chelating agent results in the suppression of cell/cell fusion without affecting HIV infection (*11*).

6) HIV-1–infected H9 cells (because of their membrane presentation of gp120-gp41 complex) are used routinely to induce cell/cell fusion when they are cocultured with uninfected $CD4^+$ cells (*11*). By comparing this cell/cell fusion assay with virus/cell fusion (that is, HIV infection), we have observed that (i) the monoclonal antibody mAb OKT4A against the gp120 binding site in the CD4 molecule (*12*) blocks both virus/cell and cell/cell fusion;

CD26 antigen and HIV fusion?

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