Brief Definitive Report

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

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Summary

In the present study, we demonstrated that expression of the LFA-1 molecule is necessary for cell fusion and syncytia formation in human immunodeficiency virus (HIV)-infected CD4+ T lymphocytes. In contrast, the lack of expression of LFA-1 does not influence significantly cell-to-cell transmission of HIV. In fact, LFA-1- T lymphocytes obtained from a leukocyte adhesion deficiency patient were unable to fuse and form syncytia when infected with HIV-1 or HIV-2, despite the fact that efficiency of HIV infection (i.e., virus entry, HIV spreading, and levels of virus replication) was comparable with that observed in LFA-1+ T lymphocytes. In addition, we provide evidence that LFA-1 by mediating cell fusion contributes to the depletion of HIV-infected CD4+ T lymphocytes in vitro.

Cell fusion and syncytia formation of HIV-infected cells are dependent on a direct interaction of HIV envelope glycoprotein with the CD4 molecule (1–4). However, molecules that regulate cell-to-cell adhesion, such as LFA-1 (5), may play a potentially important role in the spreading of HIV and in the process of cell fusion of HIV-infected CD4+ lymphocytes (6, 7). In fact, it has been previously shown that mAbs directed against the LFA-1 molecule block direct cell-to-cell transmission of HIV (7), as indicated by inhibition of syncytia formation (6) and virus replication (7) in HIV-infected cells treated with anti-LFA-1 mAb. However, the relative importance of the interaction between HIV envelope and the CD4 molecule vs. LFA-1-related cell-to-cell adhesion in the process of HIV transmission and cell fusion remains to be elucidated.

To address this question, we have used leukocytes genetically deficient in LFA-1 expression as target cells for HIV infection. Leukocytes from patients with leukocyte adhesion deficiency (LAD) are deficient in the expression of three transmembrane glycoproteins, termed LFA-1, Mac-1, and p150,95, which are heterodimers formed between one of three CD11 subunits and a common CD18 subunit (8). Leukocytes from LAD patients, therefore, represent an ideal and natural model system to assess the role of LFA-1 in the spreading of HIV and in HIV-mediated cell fusion.

We demonstrate that cell fusion and syncytia formation do not occur in LFA-1- mononuclear cells despite efficient HIV infection, thus indicating that LFA-1 is required for HIV-mediated cell fusion but does not seem to be involved in cell-to-cell transmission of HIV. In addition, HIV-mediated cytopathicity was more pronounced in LFA-1+ vs. LFA-1- mononuclear cells.

Materials and Methods

mAb and Flow Cytofluorometric Analysis. FITC-conjugated Leu-3 a+b mAb (Becton Dickinson Immunocytometry Systems, San Jose, CA), purified anti-LFA-1 (MHM23) mAb, which recognizes the β chain of LFA-1 molecule (9) (a generous gift of Dr. S. Shaw, Experimental Immunology Branch, NCI, NIH, Bethesda, MD), anti-ICAM-1 (RR11) and anti-ICAM-2 mAb (kindly provided by Dr. T. Springer, Department of Pathology, Harvard Medical School, and the Center for Blood Research, Boston, MA), and human anti-gp41 mAb (kindly provided by Dr. S. Zolla-Pazner, Department of Pathology, New York University Medical Center, New York) were used for phenotypic analyses, which were performed as previously described (10). OKT4a mAb (Ortho Pharmaceutical, Raritan, NJ) was used for inhibition of syncytia formation.

Isolation of Lymphoid Cells. Lymphocytes were isolated from peripheral blood over a Hypaque-Ficoll density gradient.

Cell Cultures. LFA-1- and LFA-1+ PBMC were stimulated

for 72 h with PHA (2 μg/ml) (Sigma Chemical Co., St. Louis, MO) and inoculated either with supernatants (SNs) containing HIV-1_{LAV} (10^{-4} dilution of the viral stock) or HIV-2_{ROD} (10^{-3} dilution of the viral stock). 10^7 HIV-1- or HIV-2-infected PHA-activated cells (6×10^5/well) resuspended in culture medium (RPMI 1640 plus 10% FCS and rIL-2 [50 U/ml]) were plated in 48-well flat-bottomed plates (Costar, Cambridge, MA). Beginning on day 2 after infection, 50 μl of culture SN was removed daily from each well, pooled, stored at −70°C, and subsequently assayed for reverse transcriptase (RT) activity, which was measured as previously described (11).

PCR Analysis. PHA-activated (3 d) LFA-1$^-$ and LFA-1$^+$ cells (2×10^6/ml) were inoculated with SN (10^{-2} dilutions of the viral stock) containing HIV-1 (strain LAV) for 6 h, washed twice, replaced in culture for an additional 18 h and prepared for PCR as previously described (12). Primer pairs used in these experiments included SK 145/101 (gag), SK 29/30 (LTR), and QH 26/27 (HLA-DQa control) (Synthetic Genetics, San Diego, CA). Amplified products were hybridized to γ-[^{32}P]ATP end-labeled probes (SK 102 and SK 31 for the gag and LTR primers, respectively) and analyzed on 10% polyacrylamide gels. Autoradiograms (Kodak, Rochester, NY) of the gels were obtained after 6 h of exposure.

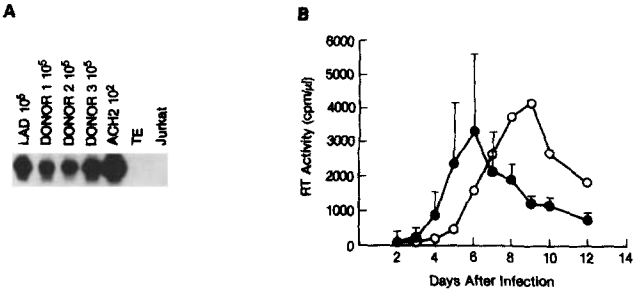

Figure 1. (*A*) Oligomer hybridization of amplified HIV-1 (LAV) DNA from in vitro infected PHA-activated LFA-1$^-$ and LFA-1$^+$ cells. Intensity of the PCR signals in 10^5 LFA-1$^-$ cells and 10^5 LFA-1$^+$ cells from three different donors are compared (TE, buffer control; DNA lysates from 10^5 Jurkat, negative control; DNA lysates from 10^2 ACH2, a cell line containing a single proviral copy of HIV per cell [12], positive control). The results shown were obtained using gag primer pairs and probe. Similar results were obtained with LTR primer pairs and probe. (*B*) Comparative analysis of the levels of virus replication in LFA-1$^-$ and LFA-1$^+$ cell cultures. Levels of virus replication in LFA-1$^-$ (○) and LFA-1$^+$ (●) cells infected with HIV-1. LFA-1$^-$ and LFA-1$^+$ PBMC were stimulated for 72 h with PHA and inoculated with SNs containing HIV-1_{LAV}. The levels of RT activity observed in HIV-infected LFA-1$^-$ cell cultures are representative of three independent experiments and are compared with levels of RT activity (mean ± SD) in HIV-infected LFA-1$^+$ cell cultures from six normal donors.

Results and Discussion

LFA-1$^-$ PBMC obtained from one LAD patient (13) and LFA-1$^+$ PBMC from three normal donors were activated for 72 h with PHA, exposed to SNs containing an HIV-1 isolate, and then analyzed for the presence of HIV DNA by PCR amplification, followed by hybridization with probes for HIV gag and LTR gene segments. Comparable proviral DNA signals were found in both LFA-1$^-$ and LFA-1$^+$ cells (Fig. 1 *A*), indicating that lack of expression of the LFA-1 molecule does not prevent HIV entry into cells. Moreover, similar peak levels of virus replication, as measured by RT activity, were observed in SNs from LFA-1$^-$ and LFA-1$^+$ cell cultures infected with HIV-1; however, a delay in the peak of virus replication was observed in the LFA-1$^-$ cell cultures (Fig. 1 *B*). Similar results were obtained in the LFA-1$^-$ cell cultures infected with HIV-2 (data not shown). Therefore, lack of expression of the LFA-1 molecule may moderately slow the spreading of HIV in T lymphocytes, but transmission of HIV will nonetheless occur as a consequence of the well-recognized mechanism of CD4-gp120 interaction (1–4).

We then assessed the role of the LFA-1 molecule in the process of HIV-mediated syncytia formation. LFA-1$^+$ cell cultures infected either with HIV-1 or HIV-2 showed formation of syncytia beginning at day 3 after infection (data not shown) and reached a peak at day 5 (Fig. 2, *C* and *D*). In contrast, formation of syncytia was never observed in the LFA-1$^-$ cell cultures productively infected either with HIV-1 (Fig. 2 *B*) or HIV-2 (data not shown). Of note is the fact that expression of the gp41 fusogenic component of HIV envelope (14, 15) was similar (~20%) in both HIV-1- and HIV-2-infected LFA-1$^+$ and LFA-1$^-$ cell cultures, as determined by positive staining with anti-gp41 mAb (16) (data not shown). Further evidence that LFA-1 is the key molecule in the process of HIV-mediated cell fusion was obtained by restoring LFA-1-mediated cell-to-cell interactions by coculture experiments. Maximal syncytia formation was observed 12 h after coculturing HIV-1 or HIV-2-infected LFA-1$^-$ cells (3.5×10^5 cells), which expressed normal levels of ICAM-1 and ICAM-2 antigens (data not shown), with 3.5×10^5 uninfected LFA-1$^+$ PHA-activated lymphocytes (data not shown). In addition, pretreatment (45 min at 37°C) of uninfected LFA-1$^+$ cells with either anti-LFA-1 (10 μg/ml) or OKT4a (1 μg/ml) mAb before coculture with HIV-2-infected LFA-1$^-$ cells resulted in complete suppression of syncytia formation (data not shown). These results demonstrate that: (*a*) surface expression of the LFA-1 molecule is necessary for cell fusion and syncytia formation in HIV-infected CD4$^+$ T lymphocytes; (*b*) CD4-gp120 interaction and significant surface expression of the gp41 fusion domain of HIV envelope are events not sufficient to cause cell fusion; (*c*) CD4-gp120 interaction, however, is required for LFA-1-mediated cell fusion, as indicated by the inhibition of syncytia formation with OKT4a mAb in the coculture experiments; and (*d*) syncytia formation is not necessary for extensive spreading of HIV and virus replication.

We next investigated the importance of LFA-1-mediated cell fusion of HIV-infected T lymphocytes as a potential mechanism for HIV-mediated depletion of CD4$^+$ T lymphocytes. In LFA-1$^+$ cell cultures infected either with HIV-1 or HIV-2, a major depletion in CD4$^+$ T lymphocytes (50–70% reduction) was observed between days 4 and 6 (Fig. 3, *A–C* and *E–G*), the period of time corresponding to the presence of syncytia and to the peak of virus replication. 10–12 d after infection, depletion of CD4$^+$ cells in HIV-infected LFA-1$^+$ cultures was >90% compared with uninfected cultures (Fig. 3, *A–C* and *E–G*). In contrast, in LFA-1$^-$ cell cultures infected either with HIV-1 or HIV-2, syncytia formation was never observed, and the rate and the extent of depletion of CD4$^+$ T lymphocytes were significantly less than those ob-

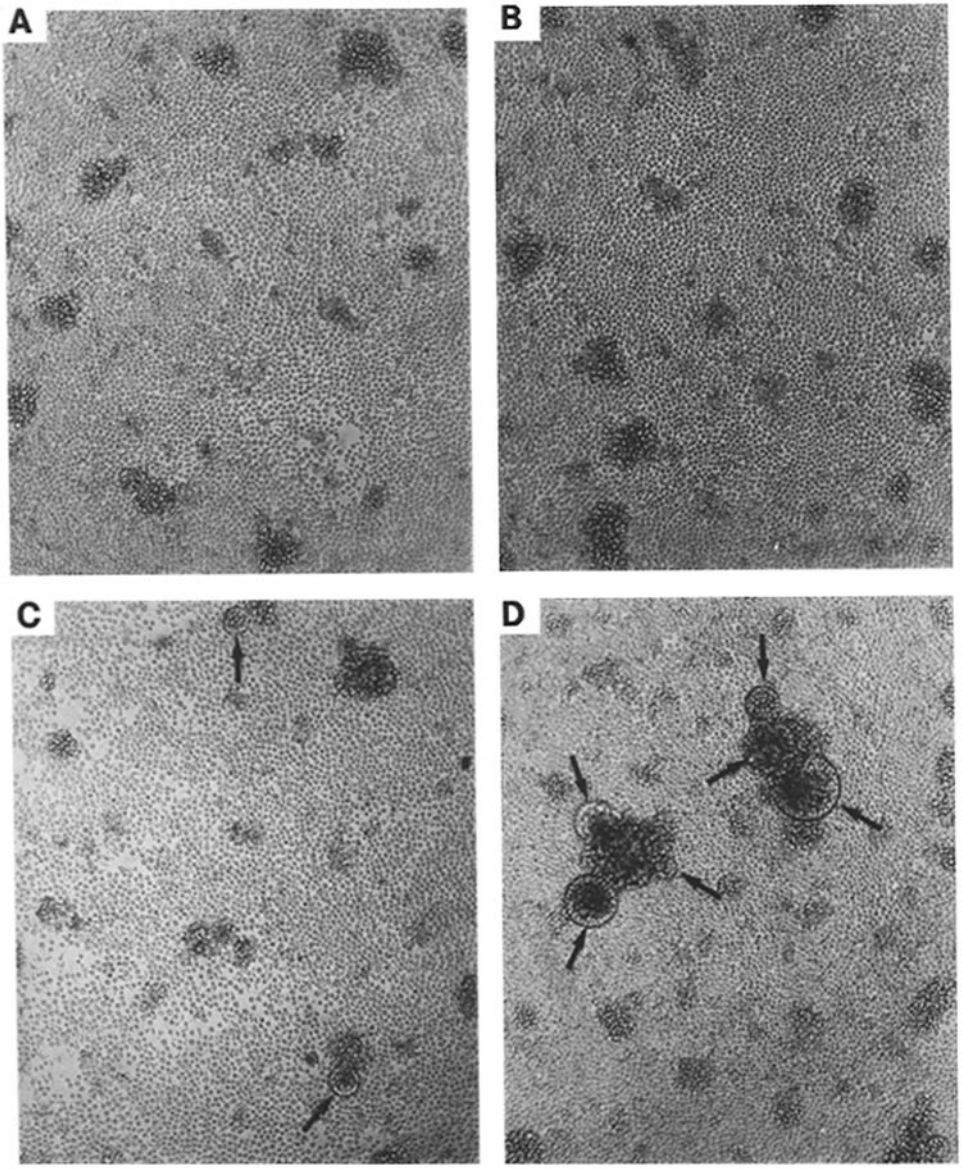

Figure 2. Role of the LFA-1 molecule in cell fusion and syncytia formation. Small clumps, but not syncytia, were observed in uninfected (*A*) and in HIV-1 (*B*) or HIV-2 (data not shown) -infected LFA-1$^-$ cell cultures. In contrast, syncytia formation (*arrows*) occurred in LFA-1$^+$ cell cultures infected with HIV-1 (*C*), and to a greater extent in those infected with HIV-2 (*D*). Photomicrographs (×75) were taken at day 5 after infection in the experiments in *B–D*. Cultures were scored daily for syncytia formation. Infection of PHA-activated LFA-1$^-$ and LFA-1$^+$ cells with HIV-1 and HIV-2 were performed as described in Materials and Methods.

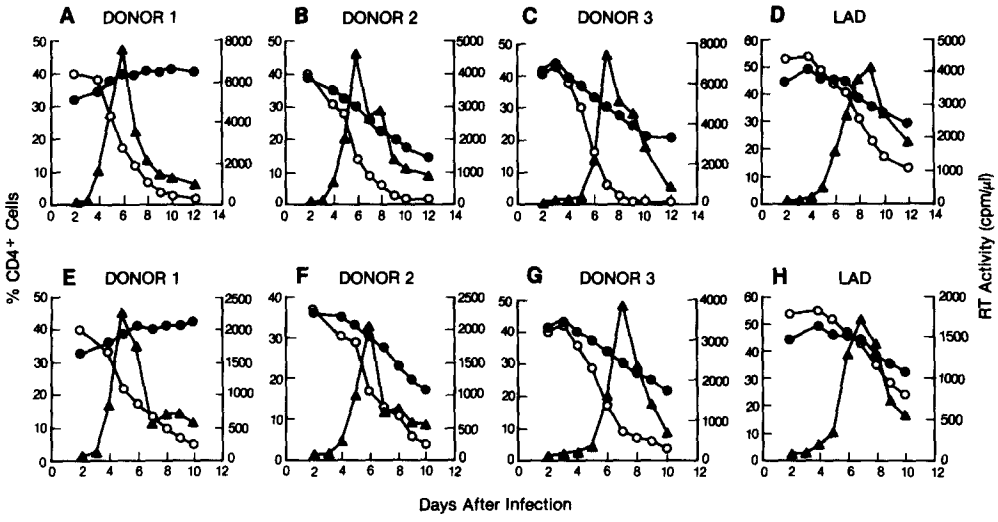

Figure 3. Involvement of the LFA-1 molecule in HIV-mediated depletion of CD4$^+$ T lymphocytes. In *A–C* and *E–G*, PHA-activated LFA-1$^+$ cells infected with HIV-1 and HIV-2, respectively, were obtained from three normal donors. In *D* and *H*, PHA-activated LFA-1$^-$ cells were infected with HIV-1 and HIV-2, respectively. (▲) Levels of RT activity; (●) percentage of CD4$^+$ cells in uninfected cultures; (○) percentage of CD4$^+$ cells in cell cultures infected either with HIV-1 or HIV-2. Infection of PHA-activated LFA-1$^-$ and LFA-1$^+$ cells with HIV-1 and HIV-2 and the staining of CD4$^+$ cells were performed as described in Materials and Methods.

served in HIV-infected LFA-1$^+$ cell cultures (Fig. 3, *D* and *H*). For example, 10–12 d after HIV-1 or HIV-2 infection of LFA-1$^-$ cell cultures, the depletion of CD4$^+$ T lymphocytes was 30–50% compared with uninfected cell cultures (Fig. 3, *D* and *H*). However, an almost complete depletion of LFA-1$^-$ CD4$^+$ T lymphocytes was observed by adding uninfected PHA-activated LFA-1$^+$ cells and thus restoring LFA-1 mediated cell fusion (90% reduction in CD4$^+$ cells 5 d after initiating the coculture; data not shown). Taken together, these results indicate that LFA-1 by mediating cell fusion in HIV-infected CD4$^+$ T lymphocytes contributes significantly to the depletion of CD4$^+$ T lymphocytes by HIV infection in vitro.

In conclusion, expression of the LFA-1 molecule in HIV-infected CD4$^+$ T lymphocytes is associated with optimal spreading, cell fusion, and high cytopathicity. In contrast, the lack of surface expression of LFA-1 abolishes the process of cell fusion in HIV-infected CD4$^+$ T lymphocytes, despite the fact that the gp41 fusion domain is expressed.

The observation that LFA-1 is critically involved in syncytia formation, together with the previously described role of viral gp41 in this process (14, 15), leads us to hypothesize that a direct interaction between gp41 and LFA-1 is required for HIV-mediated cell fusion. If this hypothesis is correct, disrupting this interaction could provide an attractive new therapeutic strategy for HIV infection.

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