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Transcellular Transactivation by the Human Immunodeficiency Virus Type 1 *tat* Protein

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The human immunodeficiency virus type 1 (HIV-1) transactivator (*tat*) protein produced in one cell activated HIV-1 promoter-directed gene expression in a second cell, provided the cells were in direct contact with one another. This observation suggests that the *tat* protein produced in HIV-1-infected cells has a physiological effect on neighboring cells.

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of AIDS. In addition to encoding the viral *gag*, *pol*, and *env* proteins, the HIV-1 genome also encodes at least seven other proteins which are of importance for regulation of the virus life cycle. Although the phenotype of most of these regulatory proteins has been described, the mode of action is not fully understood. It is also not known to what extent, if any, these regulatory proteins interact with each other.

The most studied of the regulatory viral proteins is that encoded by the *tat* gene (7, 18). Viruses deleted in the *tat* gene are unable to replicate or to induce cytopathic effects (3). The *tat*-encoded protein is 86 amino acids long and is highly conserved among viral isolates (1, 18). The *tat* protein acts through the *cis*-acting *trans*-activation response element sequence located within the first 56 nucleotides of the viral mRNA (6, 11, 13, 16). Purified *tat* protein transactivates the HIV-1 long terminal repeat (LTR) when added to cells carrying LTR-reporter gene constructs (9, 12). A peptide corresponding to the 58 N-terminal amino acids of the *tat* protein is specifically taken up by cells and transactivates HIV-1 LTR gene expression. Such transactivation is specifically competed for by an inactive *tat* peptide (8). It has been reported that *tat* protein can be found free in medium from *tat*-producing cell lines or cells infected with HIV-1 (4). The free *tat* could be from either cell death or secretion. To determine whether *tat* protein produced by one cell can activate genes in uninfected neighboring cells, the ability of a cell constitutively producing the *tat* protein to activate the HIV-1 LTR stably integrated into a second cell line was determined.

The *tat*-producing cell lines used were Jurkat-*tat* cells obtained by transfection of Jurkat human T cells with a BK virus plasmid expression vector containing the HIV-1 *tat* cDNA (2). The HeLa 3T1 cell line containing the HIV-1 LTR located 5′ to the bacterial chloramphenicol acetyltransferase (CAT) gene were used as recipients (5). Cells were grown in RPMI 1640 (GIBCO) supplemented with 10% fetal calf serum and antibiotics (penicillin and streptomycin). For cocultivation experiments, 7×10^5 HeLa 3T1 cells were seeded in 25-mm-diameter petri dishes. After overnight incubation at 37°C, the medium was removed and replaced with 5 ml of medium containing different amounts of Jurkat-*tat* or Jurkat cells. For some cocultivation experiments, HeLa 3T1 cells were seeded in Transwell plates (Costar) to separate the reporter cells physically from the Jurkat-*tat* or Jurkat cells. In other experiments, HeLa 3T1 cells were cultivated for 24 h in the presence of cell-free conditioned medium from Jurkat-*tat* cells. In some experiments, chloroquine was added at a concentration of 100 μM to reduce lysosomal degradation of *tat* protein (9). We observed no increase in transactivation following addition of chloroquine. In all of the experiments, incubation was performed for 24 h. After this time, supernatants containing T cells were removed and adherent HeLa 3T1 cells were washed three times with phosphate-buffered saline. CAT activity was determined by standard methods (10), using approximately 1.5 μg of protein cell extract (Bio-Rad assay) per reaction. Immunoprecipitation was performed as described previously (2, 14). Immunoprecipitation using monospecific anti-*tat* serum showed that the *tat* protein is constitutively produced by the Jurkat-*tat* cell line. As shown in Fig. 1 (lane 2), a protein of approximately 16 kDa is expressed in extracts of Jurkat-*tat* cells. Precipitation of this protein is specifically competed for by preincubation of the serum with the synthetic peptide used to raise the antibodies (Fig. 1, lane 3) but not by the keyhole limpet hemocyanin (Fig. 1, lane 4) used to conjugate the peptide, indicating that an authentic *tat* protein is produced by Jurkat-*tat* cells. The *tat* protein was not detected in the supernatant of these cultures by immunoprecipitation, Western blot (immunoblot) analysis, or enzyme-linked immunosorbent assay. It is possible that *tat* protein is not secreted by Jurkat-*tat* cells. It is also possible that secreted *tat* protein is unstable or degraded by cellular proteases present at the cell surface or in the medium. Alternatively, secreted *tat* protein may bind strongly to the surface of the cell.

When HeLa 3T1 reporter cells were cocultivated for 24 h with Jurkat-*tat* cells, a 5- to 10-fold increase in CAT activity was observed (Fig. 2A, lanes 2 to 4, and Table 1) suggesting that the *tat* protein produced by Jurkat-*tat* cells was able to transactivate the HIV-1 LTR in the reporter HeLa cells. Cocultivation of HeLa 3T1 cells with control Jurkat cells which do not express *tat* protein had no effect on HIV-1 LTR-CAT expression (Fig. 2A, lane 6). The transcellular transactivation of HIV-1 LTR-CAT in HeLa 3T1 cells was slightly reduced by including Jurkat cells with the Jurkat-*tat* cells in the culture medium. This observation indicates that the Jurkat cells competed with the HeLa cells for *tat* uptake (Fig. 2B). Analogous results were obtained with several Jurkat-*tat* cell lines, as shown in Table 1.

To determine whether the stimulatory effect is abolished by anti-*tat* antibody binding, rabbit *tat* antiserum raised

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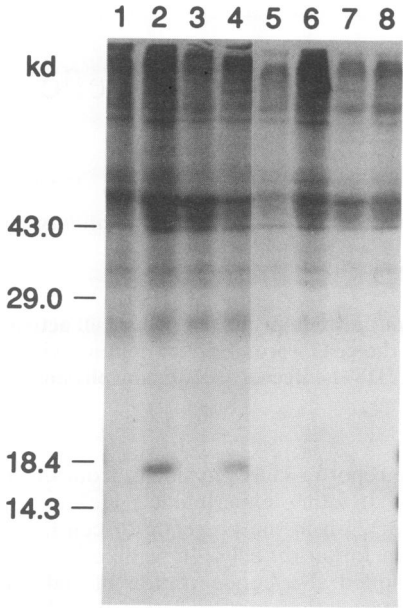

FIG. 1. Immunoprecipitation of metabolically [^{35}S]cysteine-labelled Jurkat-*tat* (lanes 1 to 4) and Jurkat (lanes 5 to 8) cells. Lanes: 1 and 5, rabbit preimmune serum; 2 and 6, anti-*tat* serum; 3 and 7, anti-*tat* serum plus *tat* peptide; 4 and 8, anti-*tat* serum plus keyhole limpet hemocyanin. Molecular size markers: 14.3 kDa, lysozyme; 18.4 kDa, β-lactoglobulin; 29 kDa, carbonic anhydrase; 43 kDa, ovalbumin.

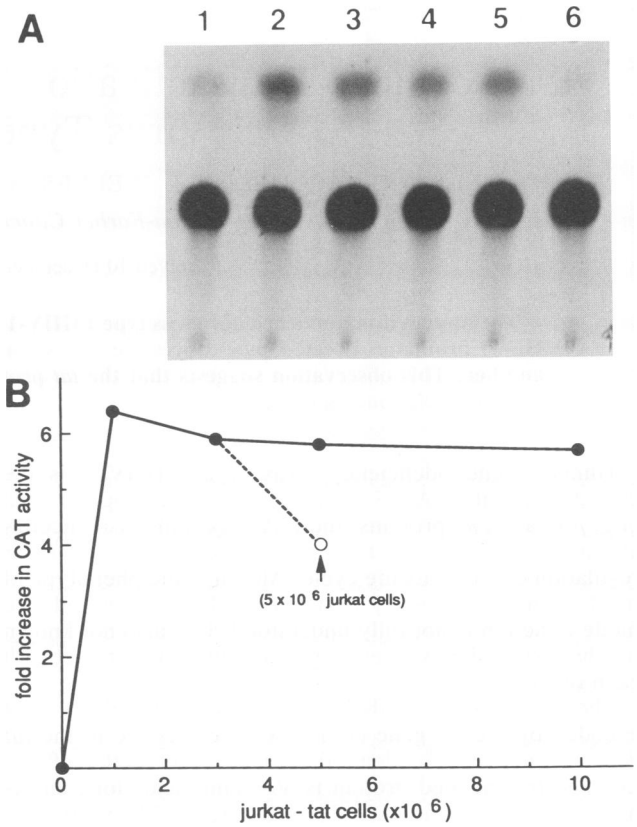

FIG. 2. Transactivation of HIV-1 LTR-CAT after cocultivation of HeLa 3T1 cells with increasing concentrations of Jurkat-*tat* cells. (A) Lanes: 1, only HeLa 3T1 cells; 2, 5×10^5 Jurkat-*tat* cells; 3, 2.5×10^6 Jurkat-*tat* cells; 4, 10×10^6 Jurkat-*tat* cells; 5, 5×10^6 Jurkat-*tat* cells and 5×10^6 Jurkat cells; 6, 10×10^6 Jurkat cells. (B) Graphic presentation of the data from panel A based on the amount of radioactivity transferred to acetyl chloramphenicol in the CAT assay.

against (i) a synthetic peptide corresponding to the first 20 amino acids of the *tat* protein or (ii) a highly purified fraction of *tat* expressed in *Escherichia coli* was added to the culture medium. No inhibition of transcellular transactivation was observed when Jurkat-*tat* cells were cocultivated with HeLa 3T1 reporter cells in the presence of antisera (Fig. 3). This result supports the notion that *tat* is transferred by close contact between donor and acceptor cells.

In a second series of experiments, HeLa 3T1 cells were physically separated by a membrane from Jurkat-*tat* cells by seeding them into Costar Transwell plates. The membrane prevents cell-to-cell contact and allows only passage of the medium. As shown in Fig. 4A (lanes 1 to 4), the HIV-1 LTR in HeLa 3T1 cells was not transactivated when the cells were physically separated from *tat*-producing cells. The membrane did not prevent transport of purified *tat* through the membrane.

Figure 4B shows the data from an experiment in which HeLa 3T1 cells were incubated with conditioned medium from either Jurkat (Fig. 4B, lane 2) or Jurkat-*tat* (Fig. 4B, lane 3) cells, indicating that *tat* is not present in the culture in sufficient concentrations to cause transactivation of HeLa 3T1 cells. These results demonstrate that *tat* protein produced in one cell can transactivate an HIV-1 LTR in another cell. Moreover, direct cell-to-cell contact is required for transcellular transactivation. Transcellular transactivation may result from transfer of *tat* protein through special junctions between HeLa 3T1 cells and Jurkat-*tat* cells or by fusion of the two different cell types. Alternatively, the *tat* protein may bind to the surface of Jurkat-*tat* cells following secretion and therefore be transferred to HeLa 3T1 cells only by direct cell-to-cell contact.

It was recently reported that *tat* protein released from HIV-1-infected H9 or COS-1 cells transfected with a *tat*-encoding plasmid stimulated growth of Kaposi's sarcoma cells derived from patients with AIDS (4). It was also reported that free *tat* protein was detected in the supernatant of *tat*-producing cells in the culture medium. However, in the experiments reported here, no *tat* protein was found in the medium. The difference in the results may be due to the use of Jurkat rather than COS cells.

The mechanism by which the *tat* protein is transferred from one cell to another is still unclear. The *tat* protein has no N-terminal sequence similar to a signal sequence found on proteins secreted through the Golgi apparatus directly to the plasma membrane or secreting granules (15). A possible

TABLE 1. Transactivation of HIV-1 LTR-CAT in HeLa 3T1 cells following cocultivation with Jurkat-*tat* cells

Cell pairs[a]	CAT activity (acetyl chloramphenicol cpm [per 1.5 μg of protein]/total cpm)	% Conversion	Fold activation
HeLa 3T1-Jurkat	4,464/66,701	6.7	
HeLa 3T1-clone 34	39,230/84,729	46.3	7
HeLa 3T1-clone 42	41,637/119,287	34.9	5
HeLa 3T1/clone 44	24,931/63,611	39.2	6

[a] The Jurkat-*tat* cell lines used were the same as those described by Caputo et al. (2).

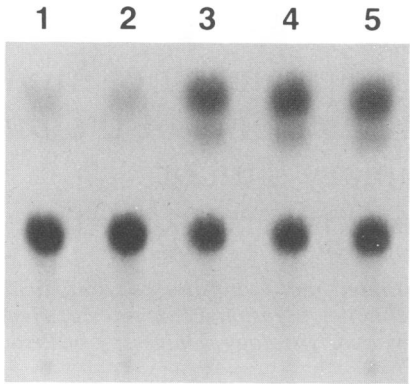

FIG. 3. Transactivation in the presence of antibodies toward the *tat* protein. HeLa 3T1 cells were seeded at a concentration of 7×10^5 per well 24 h before addition of Jurkat or Jurkat-*tat* cells. Lanes: 1, HeLa 3T1; 2, HeLa 3T1 plus Jurkat cells (10^6); 3, HeLa 3T1 plus Jurkat-*tat* cells (10^6); 4 and 5, HeLa 3T1 plus Jurkat-*tat* cells plus *tat* peptide antiserum (50 μl).

mechanism for secretion of the *tat* protein is translocation of intracellular membranes, as has recently been proposed for export of interleukin-1β (17). The results presented here demonstrate that *tat* protein produced in one cell can be active in adjacent uninfected cells. These results raise the possibility that transcellular activation by *tat* protein contributes to the complex pathogenicity of HIV infection.

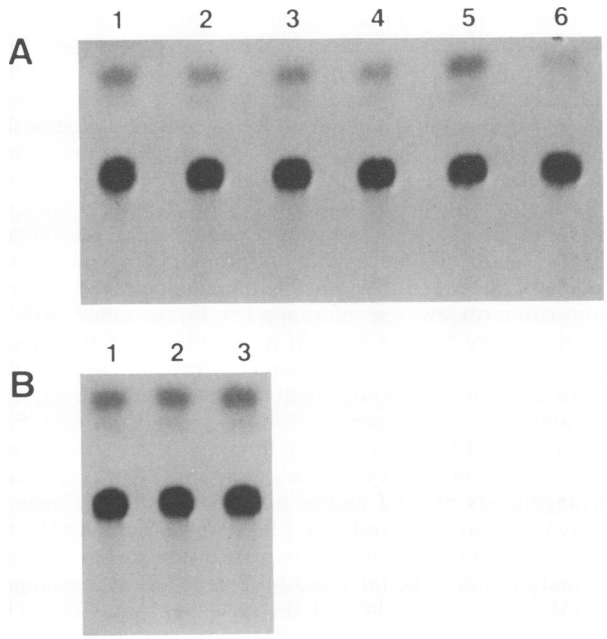

FIG. 4. (A) Absence of transactivation of HIV-1 LTR-CAT after cocultivation of HeLa 3T1 cells with increasing concentrations of Jurkat-*tat* cells physically separated by Costar Transwell membranes. Lanes: 1, 5×10^5 Jurkat-*tat* cells; 2, 2.5×10^6 Jurkat-*tat* cells; 3, 10×10^6 Jurkat-*tat* cells; 4, 5×10^6 Jurkat-*tat* cells and 5×10^6 Jurkat cells; 5, 10×10^6 Jurkat cells; 6, HeLa 3T1 cells only. (B) Absence of transactivation of HIV-1 LTR-CAT following cultivation of HeLa 3T1 cells with conditioned medium from Jurkat-*tat* cells. HeLa 3T1 cells were incubated with 5 ml of cell-free conditioned medium from Jurkat cells (lane 1) or 5 (lane 2) and 15 (lane 3) ml of cell-free conditioned medium from Jurkat-*tat* cells.

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