Influence of the human immunodeficiency virus type 1 Tat protein on the proliferation and differentiation of PC12 rat pheochromocytoma cells

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Rat pheochromocytoma PC12 cells were permanently transfected with a plasmid vector, containing the tat gene of human immunodeficiency virus type 1 (HIV-1). Various clones were obtained showing the production of different levels of bioactive Tat protein (Tat) after transient cotransfection with an HIV-1 long terminal repeat–chloramphenicol acetyltransferase reporter plasmid. Under conditions of serum starvation, tat-positive PC12 clones expressing high levels of Tat showed a significantly (P < 0.05) higher proliferation rate with respect to both mock-transfected PC12 cells and tat-positive PC12 cells expressing lower levels of Tat. Moreover, all tat-positive PC12 cell clones showed a partial morphological differentiation into sympathetic-like neurons, when seeded in low density (5 × 103 cells/cm2) cultures. On the other hand, mock-transfected PC12 cells showed the round shaped morphology typical of untreated PC12 cells and displayed signs of neuronal differentiation only after treatment with 100 ng/ml of nerve growth factor. The addition of 5 μg/ml of anti-Tat monoclonal antibody to the culture medium of tat-positive PC12 cell clones almost completely blocked their increased proliferation rate (P < 0.05), but did not affect neuronal differentiation. A significant (P < 0.05) increase in cell proliferation was consistently observed in PC12 cells supplemented with low concentrations of Tat (5 to 25 ng/ml), whereas neuronal differentiation was hardly affected by exogenous Tat. Our data strongly suggest that Tat exerts a complex influence on the proliferation and differentiation of PC12 cells, and this might help in increasing understanding of the pathogenesis of the frequent neurological disorders observed in AIDS patients.

Introduction

The protein encoded by the tat gene of human immunodeficiency virus type 1 (HIV-1) plays an essential role in viral replication (Rosen et al., 1986; Cullen, 1986; Wright et al., 1986), acting on the cis-acting sequence-responsive TAR element, located at the 5' end of all viral mRNAs (Rosen et al., 1985). In addition to this action on the viral genome, Tat protein, by analogy with the tax gene of human T cell leukaemia virus type 1 (Kim et al., 1991), is able to trans-activate strategic cellular genes, such as those encoding tumour necrosis factor-β (Sastry et al., 1990, Buonaguro et al., 1992), interleukin-6 (IL-6; Rezai et al., 1991) and transforming growth factor-β1 (Zauli et al., 1992). Moreover, recent findings demonstrated that Tat protein can act as an exogenous growth factor, since it can be rapidly taken up by different cell types (Frankel & Pabo, 1988), and displays either stimulatory (Ensoli et al., 1990) or inhibitory (Viscidi et al., 1989) effects on cell proliferation, depending on the cell type considered and the concentrations employed.

Neurological disorders are frequently observed in HIV-1-infected individuals (Prince et al., 1988). It has been suggested that infected peripheral blood monocytes, which are the progenitors of microglial cells, are responsible for introduction of HIV-1 to the central nervous system. Microglial cells appear to carry the greatest viral load (Merrill & Chen, 1991), whereas a direct infection of neuronal cells has not yet been clearly demonstrated in vivo. However, increasing evidence suggests that uninfected neuronal cells can be influenced by both inhibitory cytokines (Wahl et al., 1991) and viral products, such as the envelope glycoprotein gp120 (Brenneman et al., 1988), released by neighbouring infected cells.

In this paper, our aim was to evaluate the effect of Tat protein on the proliferation and differentiation of neuronal cells. For this purpose, we chose to investigate the effect of Tat on PC12 rat pheochromocytoma cells, which have the unique capability to differentiate into sympathetic-like neurons upon treatment with nerve...
growth factor (NGF) or IL-6 (Greene, 1978; Rudkin et al., 1989; Satoh et al., 1988).

Methods

Transfection of PC12 cells with HIV-1 tat gene. Pheochromocytoma PC12 cells (Greene & Tischler, 1976) were kindly provided by Dr Lloyd Greene (Columbia University, N.Y., U.S.A.). Cells were usually grown in tissue culture flasks in Dulbecco’s MEM (D-MEM) with 10% heat-inactivated horse serum (HS, Gibco) and 5% heat-inactivated fetal bovine serum (FBS, Gibco) at 37 °C in a fully humidified atmosphere of 7.5% CO₂ in air. PC12 cells were transfected with the expression vector pRPneo-c-Tat/S (Grossi et al., 1988), kindly provided by Professor Barbanti-Brodano (Institute of Microbiology, University of Ferrara, Italy), using the liposome kit Transfectam (Promega). The pRP neo-c-Tat/S vector contains the bacterial gene neo, which confers resistance to the amino-glycoside antibiotic G418 and the cDNA of HIV-1 tat gene in the sense orientation, both under the control of simian virus 40 (SV40) early region promoter and polyadenylation sequences. Unlike in human cells where the vector remains episomal, in rat cells it integrates into genomic DNA. PC12 cells were also mock-transfected with pRPneo-c vector alone which does not contain the HIV-1 tat gene.

Assay of chloramphenicol acetyltransferase (CAT) activity in Tat-positive and mock-transfected PC12 cell clones. To evaluate the production of biologically active Tat protein, different tat-transfected and mock-transfected PC12 cell clones were transiently cotransfected with the plasmid pTZIIICAT, containing the long terminal repeat (LTR) promoter of HIV-1 in front of the bacterial CAT gene (Rosen et al., 1985), using Transfectam. The CAT assay was performed as described by Gorman et al. (1982). Briefly, cell extracts were made 48 to 72 h after transfection by sonicating washed, centrifuged cells in 100 μl of 0.25 M-Tris-HCl pH 7.8. After this the cells were centrifuged for 15 min in an Eppendorf microfuge at 4 °C, the supernatants were then removed and assayed for enzyme activity. The assay mixture had a final volume of 100 μl of distilled water and contained 100 μg of cell extract, 0.25 μg of [¹⁴C]chloramphenicol (50 μCi/mmole, DuPont) and 2 μl of 40 mm-acetyl coenzyme A. Controls were used that contained CAT instead of cell extract. After a 60 min reaction, an estimate of the levels of protein was performed by scraping the spots from each plate and counting the radioactivity in a β-counter. The percentage of [¹⁴C]chloramphenicol conversion was represented by the ratio of the monocystylate plus bi-acetyl forms of [¹⁴C]chloramphenicol/total [¹⁴C]chloramphenicol.

Tat protein detection by in situ immunocytochemistry. PC12 cells, plated on coverslips, were processed for immunocytochemistry after 3 days of culture, using fixation with 4% freshly made paraformaldehyde in 1 x PBS pH 7.3 followed by permeabilization with cold (~20 °C) methanol. Nonspecific binding of antibodies was blocked by pre-incubating samples with 1 x PBS, 2% BSA, 5% NGS (PBB buffer) for 1 h at 37 °C. Primary monoclonal or rabbit polyclonal anti-Tat antibody (American Biotechnologies) were used at 1:20 and 1:25 dilution, respectively, in PBB buffer. After 3 h at 37 °C, coverslips were washed three times with 1 x PBS and incubated for 1 h at 37 °C with a goat anti-rabbit or an anti-mouse IgG antibody, conjugated with alkaline phosphatase. In negative controls, primary antibodies were omitted. Alkaline phosphatase was then detected as previously described (Martelli et al., 1991).

Treatment of PC12 cells with recombinant Tat protein or NGF. Full-length recombinant Tat protein, produced in Escherichia coli (American Biotechnologies), was stored at −70 °C with 0.1 mm-DL-dithiothreitol to prevent oxidation and resuspended in PBS containing 0.1% BSA (Sigma) before use. The Tat protein preparations used in this study were shown to be free of endotoxin contamination by using the limulus amoebocyte lysate test (Whittakers M.A. Bioproducts). PC12 cells (50 x 10⁵) were pelleted in an Eppendorf tube and supplemented with serial concentrations (from 1 ng to 1 μg/ml) of Tat in 50 μl of D-MEM plus 1% HS for 2 h at 37 °C. Cells were then seeded in plastic Petri dishes at the concentration of 5 x 10⁴/cm² in a final volume of 3 ml of D-MEM containing 10% HS plus 5% FCS. In parallel, the different Tat protein concentrations were pre-incubated with 5 μg of a monoclonal anti-Tat antibody, 1:100 dilution of a polyclonal anti-Tat antibody, or 1:100 dilution of control anti-mouse polyclonal antibody for 1 h at 37 °C before being added to PC12 cells. 5-bromo-2-deoxyuridine (BrDU) incorporation was evaluated after 24 to 72 h of culture.

In some experiments, PC12 cells were seeded in culture at low density (5 x 10²/cm²) in the absence or presence of 100 ng/ml of purified murine NGF (U.B.I.). Every 3 days, fresh medium containing NGF was added to the cultures. After 1, 5 and 10 days of culture, cell proliferation and differentiation were evaluated by measuring BrDU incorporation and visualizing neurite outgrowth using a light microscope, respectively.

Evaluation of BrDU uptake. Tat-positive and mock-transfected PC12 cells were seeded at low density (5 x 10²/cm²) on poly-L-lysine- and collagen-coated glass coverslips in six multiwell plates. In some experiments, 5 μg/ml of a monoclonal anti-Tat antibody (American Biotechnologies) or 1:100 dilution of a control anti-mouse polyclonal antibody were added to the culture medium of tat-transfected or mock-transfected PC12 cell clones. After 24 h of culture, 100 μl BrDU was added to each plate for 30 min, and then samples were treated as previously described (Neri et al., 1992). Briefly, cells were fixed in 4% paraformaldehyde/Dulbecco’s PBS pH 7.3 for 30 min at room temperature, washed in PBS and incubated with a 1:10 dilution of a fluorescein isothiocyanate (FITC)-conjugated anti-BrDU antibody (Becton-Dickinson). Coverslips were mounted in glycerol-PBS-1,4-diazabicyclo-2,2,2-octane and slides were examined under a Zeiss Axioskop microscope.

Criteria of morphological differentiation into sympathetic-like neurons. The proportion of neurite-bearing cells was determined by the method described by Rydel & Greene (1987). A cell was considered differentiated when neurite length reached at least twice that of the cell body (i.e. 20 μm). Neurite length was determined using printed micrographs on at least 100 randomly selected cells.

Statistical analysis. The results were expressed as mean ± s.d., obtained from three or more experiments performed in duplicate. Statistical analysis was performed using the two-tailed Student’s t-test.

Results

Establishment of tat-positive PC12 cell clones producing different amounts of Tat

To examine the role of the HIV-1 Tat protein in proliferation and differentiation of neuronal cells, we first developed several PC12 clones that were constitutively expressing Tat protein and PC12 clones mock-transfected with the parental pRPneo-c vector. The function of the stably expressed Tat protein was examined by transiently transfecting the newly established cell clones with the HIV-1 LTR–CAT reporter plasmid pTZIIICAT. Various tat-positive clones dis-
Effect of Tat protein on PC12 cells

Fig. 1. Production of bioactive Tat in five tat-positive PC12 cell clones, evaluated as percentage of 
$[^{14}C]$chloramphenicol conversion (CAT enzyme activity) in mono- and bi-acetylate forms after transient
transfection with plasmid pTZIIIICAT. Three different PC12 cell clones mock-transfected with pRPneo-c vector (negative controls) showed a
$[^{14}C]$chloramphenicol conversion of 0.1 to 0.2% (data not shown). 
Data are expressed as mean ± S.D. of three separate experiments.

Table 1. Increased proliferation rate of tat-positive 
PC12 cell clones no. 1 and no. 2 compared to
mock-transfected PC12 cell clone no. 1 after 24 h in
serum-containing* culture

<table>
<thead>
<tr>
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<th>Cells incorporating BrdU (%)†</th>
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<tr>
<td>Untreated PC12 cells</td>
<td>32.8 ± 3.7</td>
</tr>
<tr>
<td>Mock-transfected PC12 cell clone no. 1</td>
<td>31.6 ± 3.0</td>
</tr>
<tr>
<td>Tat-positive PC12 cell clone no. 1</td>
<td>48.6 ± 3.5</td>
</tr>
<tr>
<td>Tat-positive PC12 cell clone no. 2</td>
<td>49.5 ± 2.5</td>
</tr>
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</table>

* 10% HS with 5% FCS.
† The data represent the percentage of cells in S phase as detected by 
BrdU incorporation, and are expressed as mean ± S.D. of four separate 
experiments performed in duplicate. A statistically significant difference 
($P < 0.05$) was observed between tat-positive PC12 cell clones and 
untreated PC12 cells or mock-transfected PC12 cell clone no. 1.

playing an increase in HIV-1 LTR transcriptional
activity were selected for further studies (Fig. 1).
Expression of the HIV-1 tat gene was also confirmed in
tat-positive PC12 cells by direct visualization of Tat
protein by immunohistochemistry with cells being
probed with a monoclonal anti-Tat antibody (Fig. 2). An
intense labelling was observed in both nuclear and
cytoplasmic compartments.

Increased proliferation rate of tat-positive PC12 cell
clones

The extent of cell proliferation was determined by means
of a BrdU incorporation assay (Neri et al., 1992). After

24 h of culture, tat-positive PC12 cell clones no. 1 and 2
showed a significant ($P < 0.05$) increase in BrdU uptake
with respect to mock-transfected PC12 cells (Table 1).
Moreover, under conditions of serum starvation (Table
2) tat-positive PC12 cell clones with a high expression
performed in duplicate. Tat (5 to 50 ng/ml) induced a statistically significant (P < 0.05) increase in PC12 cell proliferation. Table 2. Differential proliferation rate of tat-positive PC12 cell clones expressing various levels of Tat after 24 h of serum starvation, in absence or presence of 5 µg/ml anti-Tat monoclonal antibody

<table>
<thead>
<tr>
<th>Tat protein concentration (ng/ml)</th>
<th>Cells incorporating BrdU (%)*</th>
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<tbody>
<tr>
<td></td>
<td>D-MEM + 1% HS</td>
</tr>
<tr>
<td>Untreated PC12 cells</td>
<td></td>
</tr>
<tr>
<td>tat-positive PC12 cell clones</td>
<td></td>
</tr>
<tr>
<td>no. 1</td>
<td>17.2±5.3</td>
</tr>
<tr>
<td>no. 2</td>
<td>39.3±5.5</td>
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<tr>
<td>no. 3</td>
<td>38.6±4.2</td>
</tr>
<tr>
<td>no. 4</td>
<td>42.4±5.8</td>
</tr>
<tr>
<td>no. 5</td>
<td>28.4±5.3</td>
</tr>
<tr>
<td>Mock-transfected PC12 cell clones</td>
<td></td>
</tr>
<tr>
<td>no. 1</td>
<td>18.3±6.0</td>
</tr>
<tr>
<td>no. 2</td>
<td>16.4±5.2</td>
</tr>
<tr>
<td>no. 3</td>
<td>19.4±4.7</td>
</tr>
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</table>

* The data represent the percentage of cells in S-phase as detected by BrdU incorporation, and are expressed as mean ± s.d. of four to six separate experiments performed in duplicate. Tat-positive PC12 cell clones no. 1, 2 and 3 showed a significantly (P < 0.05) higher BrdU uptake with respect to tat-positive PC12 cell clones no. 4 and 5, mock-transfected PC12 cell clones no. 1 to 3, and untreated PC12 cells. The addition to the culture medium of 5 µg/ml of an anti-Tat antibody induced a significant (P < 0.05) decrease of BrdU uptake in tat-positive PC12 cell clones.

Table 3. Effect of recombinant Tat on the proliferation rate of PC12 cells in absence or presence of 5 µg monoclonal anti-Tat antibody

<table>
<thead>
<tr>
<th>Tat protein concentration (ng/ml)</th>
<th>Cells incorporating BrdU (%)*</th>
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<tbody>
<tr>
<td>0 (control)</td>
<td>33.6±2.8</td>
</tr>
<tr>
<td>0 plus anti-Tat</td>
<td>33.1±2.5</td>
</tr>
<tr>
<td>1</td>
<td>34.5±3.3</td>
</tr>
<tr>
<td>1 plus anti-Tat</td>
<td>33.3±3.2</td>
</tr>
<tr>
<td>5</td>
<td>39.5±2.2</td>
</tr>
<tr>
<td>5 plus anti-Tat</td>
<td>33.8±3.5</td>
</tr>
<tr>
<td>10</td>
<td>48.5±3.2</td>
</tr>
<tr>
<td>10 plus anti-Tat</td>
<td>33.2±2.2</td>
</tr>
<tr>
<td>25</td>
<td>44.2±3.4</td>
</tr>
<tr>
<td>25 plus anti-Tat</td>
<td>33.5±4.1</td>
</tr>
<tr>
<td>50</td>
<td>41.3±3.4</td>
</tr>
<tr>
<td>50 plus anti-Tat</td>
<td>32.7±3.3</td>
</tr>
<tr>
<td>100</td>
<td>34.5±4.5</td>
</tr>
<tr>
<td>100 plus anti-Tat</td>
<td>32.2±3.2</td>
</tr>
<tr>
<td>500</td>
<td>34.2±3.7</td>
</tr>
<tr>
<td>500 plus anti-Tat</td>
<td>33.0±3.8</td>
</tr>
<tr>
<td>1000</td>
<td>28.5±2.9</td>
</tr>
<tr>
<td>1000 plus anti-Tat</td>
<td>32.1±3.5</td>
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* Data are expressed as mean ± s.d. of four separate experiments performed in duplicate. Tat (5 to 50 ng/ml) induced a statistically significant (P < 0.05) increase in PC12 cell proliferation.

The differential growth rate observed in tat-positive PC12 cell clones expressing various levels of Tat strongly suggested that Tat was directly involved in the increased proliferation observed in tat-positive PC12 cells. This hypothesis was further supported by the demonstration that addition of 5 µg/ml of anti-Tat antibody to the culture medium of PC12 cell clones was able to reduce significantly (P < 0.05) the increased proliferation rate of tat-positive PC12 cells to levels comparable to those of mock-transfected PC12 cells (Table 2).

We then tried to elucidate whether recombinant Tat protein was able to increase proliferation of PC12 cells also. PC12 cells were treated with serial concentrations (from 1 ng/ml to 1 µg/ml) of recombinant Tat (Table 3). After 24 h of culture with serum a significant (P < 0.05) increase in cell proliferation was observed in cultures supplemented with 10 to 50 ng/ml of Tat. On the other hand, higher concentrations (0.1 to 1 µg/ml) of Tat were unable to promote PC12 cell growth and in the presence of 1 µg/ml of Tat an increase in cell death was observed using trypan blue dye exclusion (data not shown). The influence of the Tat protein on PC12 cell proliferation was specific, since pre-treatment of Tat protein with 5 µg of a monoclonal anti-Tat antibody (Table 3) completely blocked the Tat-mediated increase of cell proliferation.

Partial morphological differentiation of tat-positive PC12 cell clones into sympathetic-like neurons

Untreated PC12 cells typically showed a round shape (Fig. 3a), which resembled adrenal medullary chromaffin cells (Greene, 1978) and did not change with the culture conditions (cell density, days of culture, etc.). On the other hand, a long-term exposure to 100 ng/ml of NGF was able to induce an initial neurite outgrowth after only 1 day of culture (Fig. 3b), which became more evident after 3 to 5 days of culture (Fig. 3c). After 10 to 12 days of culture, NGF-treated PC12 cells were converted into fully differentiated sympathetic-like neurons (Fig. 3d).

Mock-transfected PC12 cell clones showed a morphology which was indistinguishable from that of untreated PC12 cells. On the other hand, tat-positive cell lines showed cell flattening and production of cytoplasmic extensions (spiking) within 24 h after seeding in culture (Fig. 3e) and after 3 to 5 days of low-density culture the development of neurite-like processes was clearly evident (Fig. 3f). Nevertheless, at variance with...
Fig. 3. Induction of a partial morphological differentiation of PC12 cells into sympathetic-like neurons by Tat. (a) PC12 cells seeded at low density (5 x 10^5/cm^2) in complete medium after 10 days of culture (control cultures). PC12 cells supplemented with 100 ng/ml of NGF after 1 (b), 5 (c) and 10 (d) days of culture. Tat-positive PC12 cell clone no. 1 after 1 (e), 5 (f) and 10 (g) days of culture. Note that in (e) and (f) cells already show branches and flattened neurite-like structures, longer than two cell body diameters. After 10 days of culture, a complex pattern of neurites was observed in NGF-treated cells (d), whereas tat-positive PC12 cells showed a confluent monolayer (g), with neurite structures being hardly detectable.
NGF-treated PC12 cells, the different tat-positive PC12 cell clones continued to grow in spite of their partial morphological differentiation into sympathetic-like neurons. After 10 to 12 days of culture, a confluent monolayer of cells was usually observed, and the neurite-like processes were no longer clearly distinguishable (Fig. 3g). The percentage of cells incorporating BrdU only slightly decreased with time in tat-positive PC12 cell lines and a significant fraction of cells was still proliferating after 10 days of culture (Table 4). On the contrary, the proportion of NGF-treated PC12 cells incorporating BrdU decreased progressively and dropped near to 0% after 10 days of culture when full morphological differentiation was achieved.

The addition of 100 ng/ml of recombinant Tat to PC12 cells induced some very early morphological changes, such as cell flattening and production of cytoplasmic extensions, but was unable to induce a clear-cut morphological differentiation with emission of neurite-like processes, like those observed in tat-positive PC12 cells (Fig. 3f). Moreover, whereas the addition of 5 µg/ml of a monoclonal anti-Tat antibody to the culture medium of tat-positive PC12 cell clones significantly reduced their proliferation rate (Table 2), it did not affect the differentiated morphology at all (data not shown).

**Discussion**

The clinical course of AIDS is frequently complicated by multiple neurological disorders (Merrill & Chen, 1991). The evidence of a significant correlation between the levels of HIV-1 expression in the brain and clinical severity of the neurological complications (Weiser et al., 1990) strongly suggests that HIV-1 is directly involved in central nervous system pathology. Since evidence of a direct infection of neuronal cells in vivo is still lacking, it is conceivable that neuronal cell functions can be affected indirectly by cellular and viral proteins released by infected microglial cells. In this respect, it is noteworthy that the regulatory Tat protein of HIV-1 can be actively secreted by infected cells (Ensoli et al., 1990) and affects the proliferation of different cell types (Visci & et al., 1989; Ensoli et al., 1990), acting as a viral growth factor.

In this study, we evaluated the influence of Tat on the proliferation and differentiation of the rat pheochromocytoma PC12 cell line. Several tat-positive PC12 cell clones showed a significantly increased proliferation rate with respect to mock-transfected PC12 cells, and this increase in cell proliferation correlated well with the level of Tat expression by the different tat-positive PC12 cell clones. A similar increase in cell proliferation was also observed when low doses of recombinant Tat (5 to 25 ng/ml) were added to PC12 cells.

Our data on PC12 neuronal cells expand previous observations of Ensoli et al. (1990, 1993), demonstrating that the growth-promoting activity of low doses of Tat is not confined to Kaposi's sarcoma cells but also involves a completely different cell type of neuronal origin. Moreover, it is remarkable that Tat has the ability to induce a partial morphological differentiation of PC12 cells into sympathetic-like neurons, although in tat-positive PC12 cell clones proliferation never stopped and eventually a confluent monolayer was reached.

To our knowledge, HIV-1 Tat is the first molecule to be found that can stimulate cell proliferation and induce morphological differentiation of PC12 cells at the same time. Typically, when PC12 cells are induced to differentiate by addition of cellular growth factors, such as NGF or IL-6, a parallel progressive decrease in cell proliferation has always been reported (Greene, 1978; Rudkin et al., 1989; Satoh et al., 1988). Conversely, cellular growth factors that are able to increase the proliferation rate of PC12 cells, such as EGF (Huff & Guroff, 1979), never induce morphological changes characteristic of differentiated sympathetic-like neurons, similar to those observed in the presence of Tat.

However, our data suggest that Tat could influence proliferation and differentiation of PC12 cells through different pathways. In fact, the addition of recombinant Tat to PC12 cells significantly stimulated cell proliferation but had little effect on neuronal differentiation, whereas the addition of anti-Tat monoclonal antibody to the culture medium of tat-positive PC12 cell clones almost completely blocked their increased proliferation but did not affect their differentiated morphology. A possible explanation for our findings is that Tat, continuously produced and released in low amounts by tat-positive cell clones, could promote cell growth by signal transduction after interaction with specific integrin receptors, whereas it could induce cell differentiation by direct trans-activation of strategic cellular genes. Were this the case this could greatly improve our understanding of the effect of Tat on neuronal functions.

**Table 4. Comparative analysis of the percentage of cells incorporating BrdU in PC12 cells, tat-positive PC12 cell clone no. 1 and NGF-treated PC12 cells, at different days of culture**

<table>
<thead>
<tr>
<th>Time in culture (days)</th>
<th>1</th>
<th>5</th>
<th>10</th>
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<tbody>
<tr>
<td>PC12 cells</td>
<td>32.5±3.1</td>
<td>25.2±2.8</td>
<td>18.5±3.2</td>
</tr>
<tr>
<td>Tat-positive PC12 clone no. 1</td>
<td>48.6±3.5</td>
<td>37.3±4.2</td>
<td>30.2±3.5</td>
</tr>
<tr>
<td>NGF-treated PC12 cells</td>
<td>28±3.2</td>
<td>9.4±4.5</td>
<td>3.4±1.2</td>
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</tbody>
</table>

* The data represent the percentage of cells in S-phase as detected by BrdU incorporation, and are expressed as mean ± S.D. of three separate experiments performed in duplicate.
and the process of neuronal differentiation. The recent report of Taylor et al. (1992), showing the ability of Tat to activate the expression of genes coding for extracellular matrix proteins in glioblastoma cells, is particularly noteworthy since the process of neuronal differentiation involves profound changes of the cytoskeleton. Moreover, it has been previously demonstrated that Tat stimulates human B lymphocyte cell lines (Rezai et al., 1991) and normal monocytes (Zauli et al., 1993) to produce IL-6, which is able to induce neuronal differentiation of PC12 cells (Satoh et al., 1988). Tat-positive PC12 cell clones described in this study represent a unique model for studying the complex influences of Tat on cell proliferation and differentiation.

In conclusion, our data demonstrate that Tat protein can markedly influence the functions of neuronal cells and suggest that an active release of Tat by infected cells in the central nervous system could be involved in the pathogenesis of the different neurological manifestations frequently observed in HIV-1-infected individuals.

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References


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