Human T-cell lymphotropic virus type 1 (HTLV-1) is the causative agent of a chronic progressive myelopathy called tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM). This neurological syndrome is a chronic progressive encephalomyelopathy characterized by corticospinal attack (9, 36). To date, the precise mechanisms causing TSP/HAM remain largely undetermined. Nevertheless, several studies have emphasized the prime role of the high number of circulating HTLV-1-infected T lymphocytes (viral load) in the appearance of TSP/HAM (46, 63). Such viral load has been considered a consequence of inefficient immune response to HTLV-1 (26). In TSP/HAM patients, marked infiltration of the CNS by infected T cells is consistently observed (33, 59), particularly in demyelinating lesions. These T cells harboring provirus and expressing the viral protein Tax-1 (33, 42, 43) may cause bystander effects damaging neural cells or affecting their functions (25). Possible implication of direct infection of neural cells is not well documented in TSP/HAM, as viral products can hardly be detected in neural cells (34).

One important notion when considering the effects of the virus on the CNS is that certain impairments in actually infected cells may be perpetuated via indirect effects of the virus on neural cells. Such impairment may persist and eventually pervade the entire neuraxis. In the case of HTLV-1, whether or not they produce virus (19). Such activated astrocytes may prolong and amplify the deleterious effects produced by invading T cells, given the crucial roles of astrocytes in brain homeostasis (production of energetic metabolites for neurons and oligodendrocytes, neurotransmitter catabolism, and ionic homeostasis [24]). One of the major roles of astrocytes is the control of the CNS excitability (13) by regulating the extracellular concentration of neurotransmitters, especially the major excitatory (glutamate) and inhibitory (γ-aminobutyric acid) amino acids. Astrocytes scavenge glutamate from the synaptic cleft and terminate its action via high-affinity sodium-dependent glutamate transporters specific to glia. These are the excitatory amino acid transporters 1 and 2 (EAAT1 and EAAT2 in humans, the rat counterparts being GLAST and GLT-1, respectively) (6, 29). Glutamate taken up by astrocytes is converted to glutamine by glutamine synthetase and decreased for glutamate dehydrogenase, the magnitudes of these effects being correlated with the level of Tax-1 transcripts. In conclusion, Tax-1 and cytokines produced by HTLV-1-infected T cells impair the ability of astrocytes to manage the steady-state level of glutamate, which in turn may affect neuronal and oligodendrocytic functions and survival.

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Human T-cell lymphotropic virus type 1 (HTLV-1) (49) is the etiological agent of an inflammatory demyelinating pathology of the central nervous system (CNS) known as tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM). This neurological syndrome is a chronic progressive encephalomyelopathy characterized by corticospinal attack (9, 36).
pend on metabolic precursors provided by astrocytes (48) and are highly sensitive to excessive concentrations of extracellular glutamate (7, 37, 54).

We have previously shown that transient exposure of human or rat astrocytes to cell lines of T lymphocytes chronically infected with HTLV-I induces GS expression in these astrocytes (1). This altered catabolism of glutamate in astrocytes is mediated by the viral transactivator Tax-1 and has suggested that the deleterious effects of HTLV-I infection may be caused by a compromised management of glutamate by astrocytes. In this study, we investigated glutamate uptake by astrocytes since this step is crucial in the clearance of glutamate from the extracellular space and, subsequently, in the provision of metabolic precursors to neurons and oligodendrocytes. We show that glutamate accumulation and expression of mRNAs encoding glial glutamate transporters are significantly reduced in astrocyte culture after transient contact with HTLV-I-infected T lymphocytes. These effects result at least partly from paracrine effects of the viral protein Tax-1 via tumor necrosis factor alpha (TNF-α). Such bystander effects of Tax-1-producing cells emphasize the importance of the interaction between astrocytes and HTLV-I-infected T cells in the physiopathology of TSP/HAM.

MATERIALS AND METHODS

Unless otherwise noted, all reagents were obtained from Sigma (L’Isle d’Abeau, France).

Cell cultures. Primary cultures of astrocytes were obtained by mechanical disaggregation of microdissected cortices from 1-day-old rat pups or of sensory motor cortices from the human fetus (embryonic day 116). The dissociated cells were diluted to a density of 2 × 106 cells/ml in Dulbecco’s modified Eagle essential medium (DMEM) Glutamax (Life Technologies, Cergy Pontoise, France) containing 25 mM glucose, supplemented with 20% heat-inactivated fetal calf serum (FCS) and gentamicin (1 mg/ml). Cells were seeded in 35-mm diameter culture dishes precoated with poly-L-lysine (3 μg/ml) and cultured in RPMI 1640 Glutamax medium (Life Technologies) supplemented with 20% heat-inactivated fetal calf serum (FCS) and gentamicin (1 mg/ml). The medium was changed 2 days after plating (DMEM Glutamax, 25 mM glucose, 10% FCS) and every 3 days thereafter until confluence. At that point, the FCS concentration was progressively decreased to 2% over 1 week. Using this procedure, we obtained 3-week-old cultures which more than 85% expressed astrocytic phenotype. This was systematically determined by detection of glial fibrillary acidic protein (GFAP; a specific astrocytic marker) using immunocytochemistry (data not shown).

The human cell line Dev, established from a primitive neuroectodermal tumor (27), is similar to neural stem cells (38) and retains the capacity to differentiate toward astrocytes (21). We obtained various Dev cell lines after cloning and characterized by reverse transcription-PCR (RT-PCR) and of CD4+ T cells from astrocyte culture after transient contact (day 4) was verified by the presence of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and TNF-α (by reverse transcription-PCR [RT-PCR]) and of CD4+ T lymphocytes (by flow cytometry using a CD3-PE conjugated antibody). Transient contact with the C91PBL cell line led to astrocyte infection; the proportions of infected cells were around 2% for primary rat astrocytes, 20% for human Dev cells, and 90% for human fetal primary astrocytes. To test the effect of soluble factors produced by chronically infected T cells, such as Tax-1 and cytokines, the C91PBL and C8166/45 T-cell lines were seeded in cell culture inserts (0.4-μm pore size; Becton Dickinson, La Pont de Clai, France) and placed for 96 h over astrocyte cultures, supernatants were collected at different days posttreatment without specific antibodies, were made under the same conditions. In treated cultures and assayed for TNF-α using human- or rat-specific kits (CytoScreen ultrasensitive TNF-α detection kit; BioSource International, Camarillo, Calif.). In Tsp1-treated cultures, the samples were collected at 24 h posttreatment.

DNA-RNA purification and RT-PCR analysis. Total RNAs from astrocyte cultures (control or treated, at different days posttreatment) were prepared by solubilization and extraction with RNAzol (Bioprobe, Montreuil/Bois, France) according to the protocol adapted from that of Chomczynski and Sacchi (10). The concentration and purity of the extracted RNA were determined spectrophotometrically (Beckman DU-640 instrument). RNA integrity was checked by electrophoresis on denaturing agarose gels, subsequently stained by ethidium bromide.

The primers were designed using the Wisconsin Package version 9.1 (Genetics Computer Group; Madison, Wis.). Primer sequences are shown in Table 1. PCR conditions were 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, and a final extension step of 10 min at 72°C. The specificity of the primers and internal probes was verified using the GenBank database (European Molecular Biology Laboratory, Heidelberg, Germany). The amplified DNA products were detected by agarose gel electrophoresis. The amplified DNA products were confirmed by DNA sequencing performed by the Molecular Biology Laboratory, Heidelberg, Germany. The identity of the amplified DNA fragments was confirmed by DNA sequencing.

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for GS, GDH, GLAST, GLT-1, Tax-1, NF-κB, GFAP, cyclophilin A (CyP-A), and Tax-1.

mRNAs (1 μg of total denatured RNA, 10 min at 70°C) were reverse transcribed into sense-strand cDNAs using 100 ng of oligo(dT)12–18 (Pharmacia) and 80 U of murine leukemia virus reverse transcriptase (Life Technologies). The reaction proceeded at 42°C for 1.75 h in a 20-μL reaction mix (1× buffer from Life Technologies, 0.5 mM each deoxynucleoside triphosphate, 10 mM dithiothreitol, 40 U of RNAsin from Promega). The RT products (1/20 of total volume for each sample, corresponding to 50 ng of extracted total RNA) were subjected to PCR with total volume of 50 μL (1× buffer, 3 mM MgCl2, 0.2 mM each deoxynucleoside triphosphate, 0.4 mM each specific couple of primers, 2 U of Taq DNA polymerase [Promega]) according to the criteria for semiquantification of mRNAs as defined by Mohler and Butler (41). The PCR was run on a PTC-200 thermocycler (MJ Research, Watertown, Mass.) and consisted of a denaturation step (10 min at 95°C) followed by 25 to 35 cycles of 45-s denaturation (95°C), 45-s annealing (62°C), and 1.75-min elongation (72°C). The optimal number of cycles for each cDNA was 25 for GFAP, 27 for CyP-A, and 28 for GS, GDH, GLAST, GLT-1, and Tax-1. The PCR products were quantified by Southern blotting (separation by 1.8% agarose gel electrophoresis and electrob-
trocytes, once transported from culture medium. We first determined the optimal conditions for glutamate uptake in naïve astrocyte cultures obtained from rat cortex (n = 4). The uptake velocity was linear for at least 20 min for extracellular glutamate concentrations of 100 to 200 μM. The glutamate concentration in the culture medium under investigation can be considered constant, as less than 3% of radiolabeled glutamate was cleared from the extracellular space. Therefore, we determined the kinetic properties of tritiated l-glutamate uptake with respect to the extracellular concentration of glutamate at 10 min by measuring the rate of radiolabeled glutamate accumulation in astrocytes. Curve fitting and subsequent statistical analyses showed that glutamate transport was compatible with a hyperbolic model characterized by a Km of 72 ± 16 μM and a Vmax of 121 ± 12 nmol of glutamate/min/ng of DNA (mean ± SEM, n = 3, P < 0.05) (Fig. 1A). Hill plot analysis using linear regression gave Hill coefficients of 0.9 to 1, which is consistent with a single-site transport system. The specificity of glutamate transport was verified by evaluating (i) the passive diffusion of glutamate (accumulation at 4°C) into the cellular compartment (1.8% ± 0.1% of the mean value obtained at 37°C, n = 3, extracellular glutamate concentration of 100 μM) and (ii) the degree of inhibition of glutamate transport by the competitive inhibitor, β-THA (1 mM at 37°C; 97.1% ± 0.1% inhibition, n = 6, P < 0.005). These results are in agreement with those published for cultured astrocytes obtained from rat cortices (see review by Robinson and Dowd [51]). Thereafter, glutamate uptake was measured with extracellular concentration of 100 μM glutamate unless otherwise specified. In addition, in situ autoradiography of tritiated d-aspartate uptake indicated that all cultured astrocytes had the capacity to uptake glutamate (not shown). We also determined the affinity and maximal velocity of glutamate uptake in human Dev cell cultures containing around 20% of cells of astrocytic phenotype (Km = 14.6 ± 2.7 μM and Vmax = 5.1 ± 0.2 nmol glutamate/min/ng of DNA; n = 3).

Transient contact of rat primary astrocytes with HTLV-1-infected T cells (C91PL) reduced astrocytic accumulation of tritiated l-glutamate in all cases examined (68.5% ± 1.8% of control value, n = 9, P < 0.05) (Fig. 1B). This reduction was consistently observed within 1 week after contact with C91PL T cells. Contact with noninfected T lymphocytes (CEM cell line) did not significantly affect glutamate accumulation (97.7% ± 17.2% of control value, n = 3) (Fig. 1B). A reduction in the intracellular accumulation of glutamate generally reflects decreased uptake, rather than increased catabolism of glutamate by GS or GDH, the resulting glutamate metabolites being released into the extracellular space. To discount the latter possibility, tritiated d-aspartate was used instead of tritiated l-glutamate; this structural analogue of l-glutamate is transported in the same way but is not catabolized by GS and GDH. Using d-aspartate, we observed a reduction similar to that seen for l-glutamate (61.1% ± 9.9% of control value, n = 9, P < 0.05) (Fig. 1B), showing that the reduced glutamate accumulation is not due to increased intracellular catabolism of l-glutamate by GS or GDH. The relevance of the decrease in glutamate accumulation in rat astrocytes after contact with infected T cells was confirmed by the decrease observed in human astrocytic cultures established from fetal brain and Dev cell line (30% ± 4% and 70% ± 3% of control values, respectively, n = 3 for both) (Fig. 1B). In control experiments, we used a noninfected CD4+ T-cell line (CEM) with or without prior activation (phorbol myristate acetate and ionomycin). Such contact had no effect on glutamate uptake in human Dev cells (104% ± 2% of control value with inactivated CEM cells, n = 3; 97% ± 6% with CEM cells activated by phorbol my-
The possible implication of Tax-1 was assessed using hybridoma B cells secreting antibodies against Tax-1. In these experiments, human Dev cells were directly exposed to HTLV-1-infected T cells (C91PL), while hybridoma cells were seeded in culture inserts (10^6 cells) above the Dev cell monolayer. As shown in Fig. 2A, coculture with anti-Tax-1 hybridoma completely reversed the effect of infected T cells on glutamate uptake in Dev cells. Note that glutamate uptake in human Dev cells was more dramatically decreased for extracellular concentrations of glutamate smaller than 100 μM.

**Tax-1 protein decreases glutamate uptake by astrocytes via TNF-α.** The potent transactivator protein Tax-1 may be one of the soluble factors (3, 5, 11) responsible for the effect of HTLV-1-infected T lymphocytes on glutamate uptake by astrocytes. To determine the effect of extracellular Tax-1 on glutamate transport, rat astrocytes were treated with the recombinant protein Tax-1–GST (3). The control was the immediate rinsing with the medium containing recombinant Tax-1 (25 nM, i.e., 1.5 μg of Tax-1–GST/ml, 0-h treatment), which showed no difference in glutamate uptake compared to naive astrocytes (96% ± 7% of control value, n = 3) (Fig. 2B). Glutamate uptake was decreased by treatment with 25 and 50 nM Tax-1 (2, 4, 8, 16, and 24 h) but not with the protein GST alone (113% ± 7% of control value, n = 4). The maximal reduction (63% ± 4% of control value, n = 9, P < 0.01) (Fig. 2B) in glutamate transport was obtained after 4 h of treatment with 50 nM Tax-1 (3 μg of Tax-1–GST/ml). The specificity of this effect was confirmed using anti-Tax-1 antibodies (NIH 467, diluted 1/100), which completely neutralized the effect of Tax-1 protein (106% ± 6% of control value, n = 3) (Fig. 2B) at its optimal concentration (Tax-1–GST at 3 μg/ml, 4 h). The specificity of the blockade by anti-Tax-1 antibody was checked using irrelevant antibodies (63% ± 6% of control value, n = 3, P < 0.01).

We then examined whether the effect of Tax-1 may be mediated by TNF-α secreted by astrocytes, as Tax-1 has been shown to induce cultured astrocytes to secrete inflammatory cytokines, including TNF-α (11, 40). Treatment of rat astrocytes with 50 nM Tax-1 (3 μg of Tax-1–GST/ml, 4 h) induced a secretion of TNF-α in these astrocytes (2.6 ± 0.2 pg/ml, n = 3), whereas TNF-α was not secreted with the protein GST alone (n = 6). Treatment (for 4 h) of rat astrocytes with exogenous rat recombinant TNF-α (10 ng/ml; Diaclone Research) reduced glutamate uptake by 25% ± 5% (n = 3, P < 0.01; range, 20 to 55%) (Fig. 2B). The specificity of the effect was shown using anti-TNF-α antibodies, which prevented the decrease (101% ± 8% of control value, n = 3). Finally, Tax-1 seems to decrease glutamate uptake in astrocytes via TNF-α, as anti-TNF-α antibodies virtually suppressed (103% ± 5% of control value, n = 3) the effect of Tax-1 on glutamate transport.

### FIG. 2. (A) Implication of Tax-1 in the decrease in glutamate uptake induced by HTLV-1-infected T cells. Glutamate uptake was consistently decreased in human Dev cells after transient contact with infected T cells (C91PL) compared to naive cells. C91PL + anti-Tax-1, Dev cells cocultured with inserts containing hybridoma B cells secreting anti-Tax-1 antibodies. (B) Tax-1 reduces glutamate uptake by rat astrocytes via TNF-α. Treatment by recombinant rat TNF-α (grey bars) or Tax-1–GST (black bars) significantly reduced glutamate uptake. The effects of recombinant proteins were specific, as they were abolished by pretreatment with their corresponding antibodies (Ab). Moreover, the reduction in glutamate uptake induced by Tax-1 was suppressed by pretreatment with anti-TNF-α antibody (rightmost column). Controls show the lack of effect of GST protein, anti-TNF-α antibody, or anti-Tax-1 antibody when tested alone on glutamate uptake. Tritiated i-glutamate uptake by treated astrocytes was expressed as percentage of tritiated i-glutamate uptake by naive astrocytes. **, P < 0.01.
The metabolic fate of glutamate taken up by astrocytes largely depends on the catabolic enzymes, GS and GDH (see the introduction). Therefore, the effect of HTLV-1-infected T cells (C91PL) on the expression of GS and GDH was investigated by RT-PCR in rat astrocytes transiently exposed to HTLV-1-infected T cells at a period when glutamate uptake was reduced (n = 3). GS and GDH mRNAs were upregulated and downregulated, respectively (Table 2). The magnitudes of these opposite changes were inversely correlated. These changes were also correlated with the level of Tax-1 transcripts expressed by cultured cells (note that 2 to 5% of astrocytes were expressing Tax-1 immunoreactivity, as shown below).

**Prolonged expression of Tax-1 and TNF-α.** As we observed a long-term decrease in glutamate uptake induced by infected T cells (79.4% ± 4.1% of control value, n = 4, day 22, P < 0.05), we examined the expression of Tax-1 and TNF-α in primary astrocytes and the Dev cell line over the month following contact with the T-cell line C91PL. mRNA encoding Tax-1 (Fig. 3A) and Tax-1 protein were detected as early as 2 days postcontact and were persistently expressed throughout the period of observation, but without production of viral particles (55a). We found that 2 to 5% of rat astrocytes and about 20% of human Dev cells showed Tax-1 immunoreactivity during long-term cultures. Such persistent expression of Tax-1 was closely associated with a sustained secretion of the proinflammatory cytokine TNF-α. Within the first week following transient contact with the HTLV-1-infected T-cell line C91PL, TNF-α (Fig. 3B) was secreted in the culture supernatants from the human Dev cell line (460 pg/ml) and human primary astrocytes (628 pg/ml), and TNF-α mRNA was detected in Dev cells until 30 days postcontact (Fig. 3A). In rat primary astrocytes, TNF-α secretion was observed within the first week postcontact (71 pg/ml), and TNF-α transcripts were detected at least until 22 days postcontact. Stimulation of rat astrocyte cultures by HTLV-1-infected T lymphocytes also expressing Tax-1 (C8166/45, placed for 96 h in cell culture inserts over the astrocyte monolayer) induced sustained secretion of TNF-α by astrocytes at least for 3 weeks postcontact (8 pg/ml) (Fig. 3B). Control cultures of rat and human astrocytes with noninfected CD4+ T lymphocytes (CEM cell line) did not result in TNF-α secretion. These data indicate that TNF-α secretion is associated with the presence of Tax-1-expressing cells, regardless of their lymphocytic or astrocytic phenotype.

**DISCUSSION**

Examination of TSP/HAM patients has consistently revealed invasion of the CNS by T cells harboring provirus and expressing the viral protein Tax-1 (42, 43). These data have led to the hypothesis that Tax-1-expressing cells play a critical role in the pathogenesis of TSP/HAM. In this study, we have shown that Tax-1-expressing T cells can interact with astrocytes and induce a variety of cellular responses, including changes in glutamate uptake and expression of catabolic enzymes.

**TABLE 2. Expression of GS, GDH, and Tax-1 transcripts in astrocytes after contact with infected T-cells (C91PL)**

<table>
<thead>
<tr>
<th>Expt</th>
<th>% Change with respect to naive cultures</th>
<th>Tax mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GS mRNA</td>
<td>GDH mRNA</td>
</tr>
<tr>
<td>1</td>
<td>+20</td>
<td>-10</td>
</tr>
<tr>
<td>2</td>
<td>+22</td>
<td>-33</td>
</tr>
<tr>
<td>3</td>
<td>+37</td>
<td>-52</td>
</tr>
</tbody>
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*Expressed in arbitrary units after normalization with CyP-A mRNA expression.

(Tax-1–GST [3 μg/ml] and anti-TNF-α antibody [1/100], 4 h) (Fig. 2B).

FIG. 3. (A) Tax-1 is persistently expressed in HTLV-1-infected cultures of astrocytes, at least until 22 and 30 days postinfection (dpi) in rat and human cells, respectively. Tax-1 mRNA was detected in astrocyte cultures after transient contact with HTLV-1-infected T lymphocytes (Southern blotting of RT-PCR products hybridized with a specific internal radiolabeled probe). CyP-A was used as the quality control for RT-PCR. (B) The proinflammatory cytokine TNF-α was secreted following transient treatment of rat and human astrocyte cultures with infected T cells (direct contact or use of insert allowing only soluble factors to diffuse). Note that TNF-α was not detected in control cultures (naive astrocytes and astrocytes transiently exposed to noninfected CD4+ T lymphocytes [CEM cell line]. TNF-α was assayed using rat- or human-specific EIA kits.

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to a consensus on the importance of CNS infiltration by HTLV-1-infected T cells in the physiopathology of TSP/HAM, although many subsequent events remain unaddressed. In this study, we used transient coculture of astrocytes with HTLV-1-infected T lymphocytes to mimic CNS invasion by infected T lymphocytes and examined the functional consequence on astrocytes. Indeed, an effective operation of astrocytes is critical in brain homeostasis and neural cell survival. These glial cells are essential managers of a variety of metabolic pathways involved in energy storage, ionic equilibrium, and the control of extracellular concentrations of various neurotransmitters. In particular, the main excitatory amino acid glutamate is specifically taken up and then catabolized by astrocytes. Our data show that glutamate management is significantly impaired in astrocytes following transient contact with infected T cells, whether or not they produce virus. Such astrocyte impairments triggered by HTLV-1 may have deleterious effects on neighboring cells by affecting their electrical activity and energy metabolism (55). In human astrocytes, the effects were even greater than rat cells, confirming the clinical relevance of our model.

**Activation of astrocytes by infected T lymphocytes.** Transient contact of cultured astrocytes with infected T cells did not affect the amount of DNA or the number of cells. There was even an increase in the expression of astrocyte-specific proteins, such as GFAP and GS, indicating that the presence of HTLV-1-infected T cells did not promote a general shut off, but rather induced factors targeting specific astrocytic functions. Marked activation of astrocytes was observed after transient contact with HTLV-1-infected T lymphocytes (19). This activation was characterized by the sustained expression of the proinflammatory cytokine TNF-α and upregulation of the glial filament protein GFAP. These changes in astrocytes produced by infected T lymphocytes are typical of astrocytes engaging a variety of regulatory processes in response to several types of insults (4).

**Glutamate uptake by astrocytes.** Glutamate uptake by astrocytes is crucial in the regulation of its extracellular concentration and intracellular metabolism (glutamine synthesis, ammonia detoxification, and energy/cell respiration). Glutamate accumulation was consistently reduced in astrocyte cultures treated with HTLV-1-infected T lymphocytes. This decrease was due to a change in the net transport activity rather than to an accelerated turnover of glutamate catabolism (e.g., by increased GS activity), as a similar decrease was observed using tritiated d-aspartate, which is not metabolized by GS and GDH.

Glutamate is mainly transported into astrocytes via two high-affinity, sodium-dependent transporters (6, 28), GLAST/EAA1 and GLT-1/EAA2. Analysis of the expression of their messengers shows a significant decrease in both, occurring as early as 3 days after contact with HTLV-1-producing T lymphocytes. The decreased glutamate transport probably results from the downregulation of genomic expression of glial glutamate transporters, as glutamate uptake was found to correlate with the expression of the transporters’ messengers (16, 52). At a more functional level, such decreased glutamate uptake by astrocytes should increase the extracellular level of glutamate, which in turn may perturb neuronal transmission (e.g., a decreased signal-to-noise ratio or temporal detuning) or even exert an excitotoxic effect. Another retrovirus, feline immunodeficiency virus, has also been shown to decrease glutamate uptake in cultured astrocytes. In this model, the effect was interpreted as a result of direct infection of astrocytes (67), whereas with HTLV-1, the decreased glutamate uptake was observed even after contact with a noninfectious HTLV-1-infected T-cell line.

**Implication of soluble factors.** The possible involvement of soluble factors in the effects of HTLV-1-infected T cells was verified using transient cocultures with astrocytes and HTLV-1-infected T lymphocytes in two distinct compartments, which resulted in a reduction of glutamate uptake similar to that observed with single compartment coculture (direct cell-to-cell contact). This raised the possibility that infected T lymphocytes may impair glutamate uptake and catabolism by bystander effects via soluble factors, such as the viral protein Tax-1 and cytokines. In our model, we clearly showed that human as well as rat astrocytes secrete TNF-α after transient contact with infectious or noninfectious HTLV-1-infected T-cell lines (C91PL or C8166/45), both expressing Tax-1. The addition of recombinant Tax-1 protein to the culture medium was also able to induce TNF-α secretion. Involvement of Tax-1 and TNF-α in the effects of HTLV-1 infection on glutamate uptake was further substantiated by the decreased glutamate uptake after application of recombinant Tax-1 or TNF-α. Coincubation with both Tax-1 and anti-TNF-α antibody abolished the effect of Tax-1 on glutamate transport, providing evidence that TNF-α acts as the mediator of the Tax-1-induced decrease in glutamate uptake. These findings are in agreement with the induction of TNF-α expression by Tax-1 (11) and the decreased glutamate uptake induced by TNF-α in astrocytes (14). Thus, we can assume that the signaling cascade leading to the decreased uptake of glutamate in astrocytes successively involves two paracrine mediators, Tax-1 and TNF-α.

The critical role of TNF-α in the physiopathology of TSP/HAM is also suggested by the presence of this cytokine in astrocytes and T lymphocytes within CNS lesions of TSP/HAM patients (57). The molecular downstream of TNF-α are not precisely known for its effects on glutamate uptake. But TNF-α may affect other molecular or cellular processes, such as migration and activation of lymphocytes, be toxic for oligodendrocytes, and alter the expression of other cytokines (2, 45). The present work underscores the importance of examining nonclassical effects of TNF-α (17), other than its well-documented ability to enhance inflammatory processes. Glutamate catabolism and energy metabolism. Effective inactivation of glutamate uptake by astrocytes is achieved by the glial enzymes GS and GDH. Therefore, the functional outcome of the decreased glutamate accumulation induced by HTLV-1-infected T lymphocytes must be considered by taking into account the ability of astrocytes to metabolize glutamate via GS and GDH. In this regard, we show that transient contact with HTLV-1-infected T cells expressing Tax-1 (C91PL and C8166/45) led to imbalanced expression of glutamate-glutamine cycle enzymes in rat (this study) and human (1) astrocytes (increase for GS and decrease for GDH). We have previously shown that Tax-1 protein transactivates the GS gene promoter (1). In the present study, we further show that the magnitude of these opposite changes correlates with the level of viral Tax-1 mRNA, demonstrating the critical role of Tax-1-expressing T lymphocytes and astrocytes in the effects observed. The Tax-1-induced imbalance between GS and GDH is expected to preferentially drive glutamate catabolism toward the formation of glutamine, rather than toward the TCA cycle. Decreased GDH expression may result in insufficient energetic stores in astrocytes, as the mitochondrial enzyme GDH primarily catalyzes the metabolic pool of glutamate to generate α-ketoglutarate, which passes into the TCA cycle (48). Such effect on the energy store may be enhanced by the decreased intracellular pool of glutamate following its impaired uptake. Thus, astrocytes represent an important target for HTLV-1-
infected T lymphocytes infiltrating the CNS, possibly depleting energy precursors for the surrounding cells, in particular neurons and oligodendrocytes, which need to be continuously fed with essential metabolites (lactate and \( \alpha \)-ketoglutarate) released by astrocytes (24).

**Functional significance.** The present study demonstrates that HTLV-I-infected T cells impair the uptake and metabolism of glutamate by astrocytes. Although the in vivo significance of our results must be evaluated cautiously, these data suggest that Tax-1 initiates a divergent and high-gain transduction cascade which may pervade the entire CNS. Indeed, at each step of this cascade, the input signal can affect several targets which, in turn, can amplify the output signal. The magnitude of the resulting bystander effects is probably enhanced by factors secreted by infected T cells, but also by astrocytes (cytokines, chemokines, integrins, and metalloproteinases and their endogenous inhibitors). These factors probably affect the number of infected/activated T cells infiltrating the CNS and the extent of migration of these cells through the CNS parenchyma (61, 62, 64).

Once in the CNS, Tax-expressing T lymphocytes may persist and expand within the CNS, as Tax is able to prevent T cells from undergoing apoptosis (44) that may be stimulated by neighboring astrocytes (32). In conclusion, our results demonstrate a great susceptibility of astrocytes vis-à-vis HTLV-I-infected T cells, which significantly alters the metabolism of an important excitatory-amino acid, glutamate. Clinically, current knowledge on glutamate neurotransmission and metabolism suggests that astrocytic glutamate metabolism may be an effective therapeutic target in TSP/HAM.

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