# Effects of Transforming Growth Factor-β on Murine Astrocyte Glutamine Synthetase Activity

## Implications in Neuronal Injury

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## Abstract

**Cytokines have been implicated in the pathogenesis of a number of brain diseases in which neurological dysfunction has been attributed to a change in amino acid neurotransmitter metabolism. In the present in vitro study, we investigated the effects of cytokines on astrocyte glutamine synthetase (GS) activity and subsequently on *N*-methyl-D-asparate (NMDA) receptor-mediated neurotoxicity. Proinflammatory cytokines IL-1α, IL-1β, and IL-6 at a concentration of 20 ng/ml did not affect GS activity; however, tumor necrosis factor-α inhibited this activity by 20% in mixed neuronal/astrocyte cultures. Treatment for 24 h with transforming growth factor (TGF)-β1 or -β2 inhibited up to 60% GS activity. TGF-β2 also inhibited GS in enriched astrocyte cultures with an $ED_{50}$ of 10 pg/ml. Antibodies specific to TGF-β2 blocked this effect. Treatment of astrocytes with TGF-β2 (250 pg/ml) resulted in markedly dilated rough endoplasmic reticulum. Since astrocyte GS may play a protective role in NMDA receptor-mediated neurotoxicity, we treated mixed neuronal/astrocyte cultures with TGF-β2 (250 pg/ml) and found a threefold potentiation of NMDA receptor-mediated neurotoxicity. These data suggest that TGF-β impairs astrocyte GS function and enhances neurotoxicity, thus providing insight into understanding one mechanism of cytokine-mediated central nervous system disease. (*J. Clin. Invest.* 1992. 90:1786–1793.) Key words: cytokines • excitotoxicity • *N*-methyl-D-asparate receptors • tumor necrosis factor-α**

## Introduction

Recent studies suggest that cytokines play a pathogenetic role in a variety of central nervous system (CNS) diseases, including certain neurodegenerative disorders (1–3), autoimmune diseases (4, 5), bacterial meningitis (6, 7), and AIDS dementia (8, 9). However, the mechanism by which cytokines mediate neuronal cell dysfunction, injury, and death is unknown. Research in the past decade has indicated that excessive production of the excitatory amino acid neurotransmitter, glutamate, is involved in neurodegeneration (10, 11) and may subsequently impair neurologic function (12). In this context, *N*-methyl-D-asparate (NMDA),[1] a ligand that binds to a specific type of glutamate receptor, has been extensively investigated in an attempt to elucidate the mechanism associated with glutamate receptor-mediated neurodegeneration (13–15).

Recent studies have demonstrated that astrocytes protect neurons against glutamate-induced neurotoxicity, although the mechanism is unknown (16, 17). In the brain, astrocytes uniquely contain glutamine synthetase (GS) (18, 19), which is known to play a central role in the metabolism of glutamate to glutamine (20, 21). Glutamine is used by gamma-amino butyric acid (GABA)-ergic neurons to synthesize GABA, an inhibitory neurotransmitter (14, 15), and by glutamatergic neurons to synthesize the excitatory transmitter glutamate. Other investigators have proposed that loss of GS with an associated increase in brain glutamate may be a critical factor in the neurotoxicity produced by cerebral ischemia (22, 23). If certain cytokines selectively suppress GS activity, this would provide a potential mechanism among many others responsible for glutamate-induced neurotoxicity.

In the present study, we investigated the effects of several cytokines, tumor necrosis factor-α (TNF-α), IL-1α, IL-1β, IL-6, and transforming growth factor-β (TGF-β), on astrocyte GS activity in mixed murine neuronal/astrocyte cultures and, more specifically, in enriched astrocyte cultures. Furthermore, we tested the hypothesis that cytokine-mediated suppression of GS would be associated with an enhancement of NMDA receptor-mediated neurotoxicity. Our findings indicate that TGF-β potently inhibits GS function and causes ultrastructural abnormalities in astrocytes and potentiates NMDA receptor-mediated neurotoxicity.

## Methods

### *Reagents*

Recombinant human TGF-β1 and -β2 and murine (m)IL-1α, mIL-1β, and mIL-6 were provided by R&D Systems, Inc. (Minneapolis, MN). mTNF-α was purchased from Genzyme (Boston, MA). Polyclonal rabbit antibodies specific to TGF-β1 and goat antibodies to TGF-β2 were provided by R&D Systems. Rabbit antibodies to mTNF-α were

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1. *Abbreviations used in this paper:* GABA, gamma-amino butyric acid; GFAP, glial fibrillary acid protein; GS, glutamine synthetase; LDH, lactate dehydrogenase; NMDA, *N*-methyl-D-asparate; RER, rough endoplasmic reticulum; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha.

purchased from Genzyme. Control rabbit and goat sera were provided by R&D Systems.

### *Primary astrocyte and mixed neuronal/astrocyte cultures*

The primary murine astroglial cultures were established after dispersion of BALB/c neonatal (< 24 h of birth) cerebral cortices with trypsin (0.25%) for 30 min, as previously described (24). Briefly, cells ($2.4 \times 10^5$/300 μl) were plated into wells of 24-well plates with DME containing 10% heat-inactivated FCS (Hyclone Laboratories Inc., Logan, UT) and antibiotics, and were then incubated at 37°C with 10% $CO_2$. On the following day, the culture medium was replaced with DME (500 μl) and incubated with medium containing 10% heat-inactivated FCS but no antibiotics. The culture medium was changed again 24 h later and every 3 d thereafter. On day 9, there were > 95% astrocytes, as determined by glial fibrillary acid protein staining (Sigma Chemical Co., St. Louis, MO), and 2–3% microglial cells, as determined by anti-MAC-1 antibodies (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Mixed neuronal/astrocyte cultures were established after dispersion of fetal (day 16 of gestation) cerebral cortices with trypsin, as previously described (25). Briefly, cells ($5 \times 10^5$/500 μl) were plated into collagen-coated wells of 24-well plates with DME containing 10% heat-inactivated FCS and antibiotics. On day 5, the culture medium was replaced with DME containing 10% heat-inactivated FCS, 10% heat-inactivated horse serum (Hyclone Laboratories Inc.), uridine (33.6 μg/ml; Sigma Chemical Co.), and fluorodeoxyuridine (13.6 μg/ml; Sigma Chemical Co.) to suppress glial cell growth. After 24 h, media was replaced with DME containing 10% heat-inactivated horse serum. Culture medium was changed every 4 d thereafter. On day 9 of cultures, most neurons were growing in clusters and some in single cells, with a confluent supporting layer of astrocytes. In mixed neuronal/astrocyte cultures, ~ 40% of the total cells are astrocytes (stained with glial fibrillary acid protein antibodies), < 2% are microglia (stained with anti-MAC-1 antibodies), and ~ 60% are cortical neurons with extensive processes and birefringent cell bodies.

### *Experimental protocol*

*Effects of cytokines on GS activity in the mixed neuronal/astrocyte cultures.* On day 10, cell cultures were treated for 48 h with cytokines (mIL-1α, mIL-1β, mIL-6, mTNF-α, and TGF-β) at indicated concentrations under an atmosphere with 10% $CO_2$ at 37°C. Cell cultures were then washed three times with DME without FCS and frozen at −20°C before determination of GS activity.

*Effect of TGF-β on GS activity in enriched astrocyte cultures.* On day 9, astrocyte cultures were treated with TGF-β2 (from 1 pg/ml to 5 ng/ml) for 3, 8, 24, and 48 h before harvest for determination of GS activity. $ED_{50}$ of TGF-β inhibition of GS activity was determined at 24 h based on these doses. To determine the specificity of the effects of TGF-β, GS activity was assessed after pretreatment with antibodies (10 μg/ml) specific to TGF-β1 or TGF-β2 for 30 min before exposure to TGF-β2 (250 pg/ml) for 24 h.

*GS activity.* GS was assayed as previously described (25). In brief, cell monolayers were washed three times with DME at 4°C followed by incubation for 30 min with 0.4 ml of 1 mM imidazole buffer (pH 6.2) to lyse the cells. Cells were then incubated for 1 h at 37°C with buffer containing the following (mM): 120 glutamine, 0.1 ATP, 20 $Na_2HAsO_4$, 50 imidazole, 30 hydroxylamine, 0.3 $MgCl_2$, with a final pH of 6.2. To terminate the reaction, the contents of each well were transferred to a tube containing 0.2 ml of a mixture of 15% $FeCl_3$ (wt/vol), 2.5 N HCl, and 25% trichloroacetic acid (wt/vol). After incubating for 10 min at room temperature, samples were centrifuged at 1,000 *g* for 15 min to sediment the cell debris, and absorbance of the supernatant was determined at 500 nm and compared with a standard curve prepared with different concentrations of glutamyl-γ-hydroxamic acid. 1 U of GS activity was defined as the amount of enzyme that produces 1 μmol of glutamyl-γ-hydroxamic acid/h at 37°C. The sensitivity of this assay was < 0.125 μmol. Findings were recorded as specific activity (U/mg protein) or percent of control values.

*Protein and cell viability determinations.* After treatment for 48 h with cytokines, cell cultures were harvested and protein was determined (26). Cell viability was assessed by the trypan blue exclusion method. Trypan blue solution (Sigma Chemical Co.) was added to cell cultures, and dye exclusion (determined under light microscopic examination) was used to define cell viability.

*Effect of TGF-β2 on ultrastructure of astrocytes.* Astrocytes maintained in six-well plates were treated with TGF-β2 (250 pg/ml). After 24 h of exposure to TGF-β2, the cells were examined under light microscopy for changes in morphology. Cells were then harvested and pelleted. The cell pellet was fixed and processed as described below for electron microscopy. To test the specificity of TGF-β2, antibodies specific to TGF-β2 (10 μg/ml) were applied simultaneously with TGF-β2 (250 pg/ml) for 24 h before harvest for transmission electron microscopic examination.

Trypsinized cultures of astrocytes were centrifuged at 1,000 *g* to form a pellet and fixed for 2 h at 4°C in 3% glutaldehyde buffered with 0.1 M phosphate. Specimens were then dehydrated in graded alcohols and embedded in epoxy resin. Ultrathin sections were prepared with a diamond knife, mounted on uncoated copper grids, and triple-stained with lead citrate and uranyl acetate. Specimens were then examined in an electron microscope (JEOL 100CX; JEOL USA, Peabody, MA). Cells were examined by an electron microscopist who was unaware of the treatment assignment.

*Effect of TGF-β2 on NMDA receptor–mediated neurotoxicity.* On day 12, mixed neuronal/astrocyte cultures were treated for 48 h with 250 pg/ml of TGF-β or culture medium (control) before a 5-min exposure to varying concentrations of NMDA. Cell cultures were then washed thoroughly and maintained in DME culture medium for an additional 24 h before measurement of lactate dehydrogenase (LDH) activity in culture supernatants. Increased LDH activity indicates neuronal loss due to NMDA exposure (27). Morphological changes of neuronal cell cultures treated with TGF-β2 (250 pg/ml) and NMDA (30 μM) were examined under light microscopy.

To test the effect of supplemental astrocytes on TGF-β–potentiated neurotoxicity, purified astrocytes ($5 \times 10^4$) were added to each well of mixed neuronal/astrocyte cultures that had been treated with TGF-β (250 pg/ml) for 24 h before exposure of the cell cocultures to NMDA (30 μM) for 5 min. Both LDH and GS were determined 24 h after NMDA exposure. To test the effect of supplemental glutamine on NMDA receptor–mediated neurotoxicity in mixed neuronal/astrocyte cultures treated with TGF-β, glutamine (1 mM) was added to these cultures.

LDH activity (U/liter) was determined according to the instructions supplied with a commercial diagnostic assay kit (Sigma Chemical Co.). One unit of LDH activity is defined as that amount of enzyme that will catalyze the formation of 1 μmol of NADH/min per culture at 30°C.

*High affinity glutamate uptake.* To assess astrocyte uptake of glutamate, enriched astrocyte cultures were studied after treatment with cytokines at the concentrations indicated above. [$^3$H]Glutamate uptake was determined by a previously described procedure (25). In brief, after a 48-h treatment of cells with TGF-β1 and -β2 (1 ng/ml) and other cytokines tested in this study (20 ng/ml), cell cultures were preincubated for 10 min at 37°C in $KH_2PO_4$–$K_2HPO_4$ buffer (5 mM, pH 7.4) adjusted to 330 mosM with NaCl or choline chloride for determination of total uptake or blank values, respectively. [$^3$H]Glutamate (5 nM; New England Nuclear/Du Pont, Boston, MA) was added for 10 min. Cell cultures were then washed three times with $KH_2PO_4$–$K_2HPO_4$ buffer, and cell-associated radioactivity was determined after cell lysis with NaOH (1 N).

### *Statistical analysis*

Where appropriate, data are presented as mean±SEM or as percent of control values. Statistical significance among multiple means was determined by analysis of variance followed by Scheffe's test. To compare two means, Student's *t* test was used. Fisher's exact test was also used to compare the two treatment groups.

## Results

*Effects of cytokines on GS activity in mixed neuronal/astrocyte cultures.* The effects of cytokines on GS activity in mixed neuronal/astrocyte cultures are shown in Fig. 1 *A*. Both TGF-β1 and -β2 (1 ng/ml) significantly ($P < 0.05$) inhibited GS activity by ~ 60 and 50%, respectively, whereas mTNF-α (20 ng/ml) inhibited GS by ~ 20%. Neither IL-1 nor IL-6 (20 ng/ml) affected GS activity (Fig. 1 *A*). To further confirm these effects, IL-1 and IL-6 at concentrations between 20 pg/ml and 20 ng/ml did not significantly affect GS activity in mixed neuronal/astrocyte cultures (data not shown). In contrast, TGF-β and mTNF-α inhibited ($P < 0.05$) GS activity in a dose-dependent manner after a 24-h exposure (Fig. 1 *B*). A kinetic study also demonstrated that TGF-β (250 pg/ml) and mTNF-α (20 ng/ml) inhibited GS activity within 3 h, with maximal effect reached by 24 h of incubation. No further suppression of GS activity was observed after 24 h treatment (data not shown).

*Effect of TGF-β on GS activity in enriched astrocyte cultures.* Since GS is located exclusively in astrocytes (18), we examined the direct effect of TGF-β on GS activity in enriched astrocyte cultures. TGF-β, in a dose- and time-related manner, inhibited GS activity in the astrocyte-enriched cultures by a maximum of 60% with an $ED_{50}$ of ~ 10 pg/ml as determined at 24 h (Fig. 2 *A*). The inhibitory effect on astrocyte GS activity was equally well observed following treatment with TGF-β1 and -β2. Antibodies to TGF-β2 blocked the inhibitory effect of TGF-β2 but not of TGF-β1 (Fig. 2 *B*).

*Effects of cytokines on protein levels and cell viability.* Treatment with cytokines tested at the indicated concentrations above for 3, 8, 24, and 48 h did not significantly affect the Lowry protein levels in either enriched astrocyte cultures or mixed neuronal/astrocyte cultures (data not shown), suggesting that general metabolism is not significantly altered by these cytokines. Also, cell viability, assessed by the trypan blue exclu-

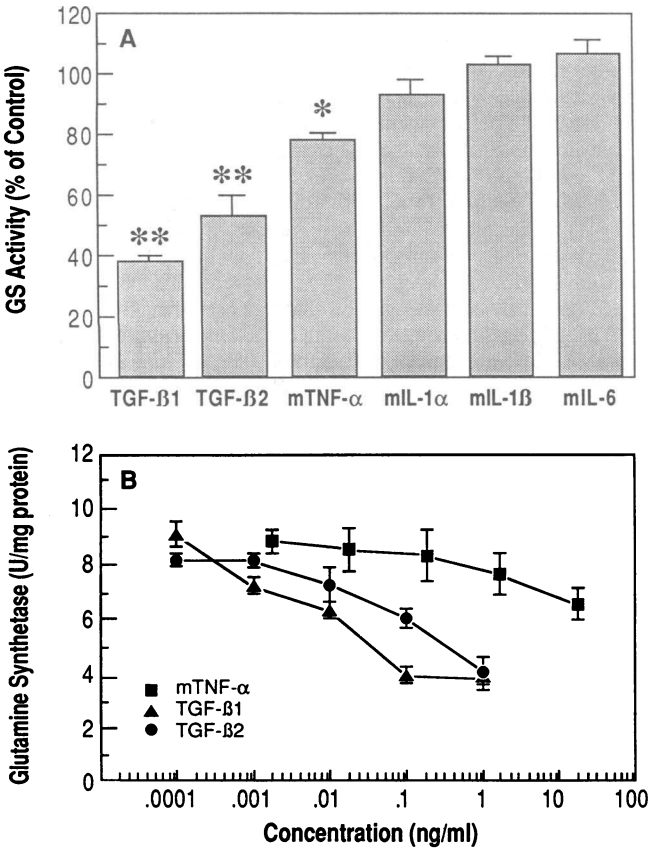



*Figure 1.* Effects of cytokines on GS activity in mixed murine neuronal/astrocyte cultures. Mixed neuronal/astrocyte cultures were treated with (*A*) mIL-1α, mIL-1β, mIL-6, and mTNF-α at a concentration of 20 ng/ml and TGF-β1 or -β2 at a concentration of 1 ng/ml for 24 h or (*B*) TGF-β1, TGF-β2, and mTNF-α at the indicated concentrations for 24 h before harvest of cells for determination of GS activity. Data are presented as GS Activity (% Control) in (*A*) and U/mg protein (mean±SEM of triplicate values) in (*B*) from one experiment and are representative of three separate experiments. $*P < 0.05$, $**P < 0.01$ vs. control values.

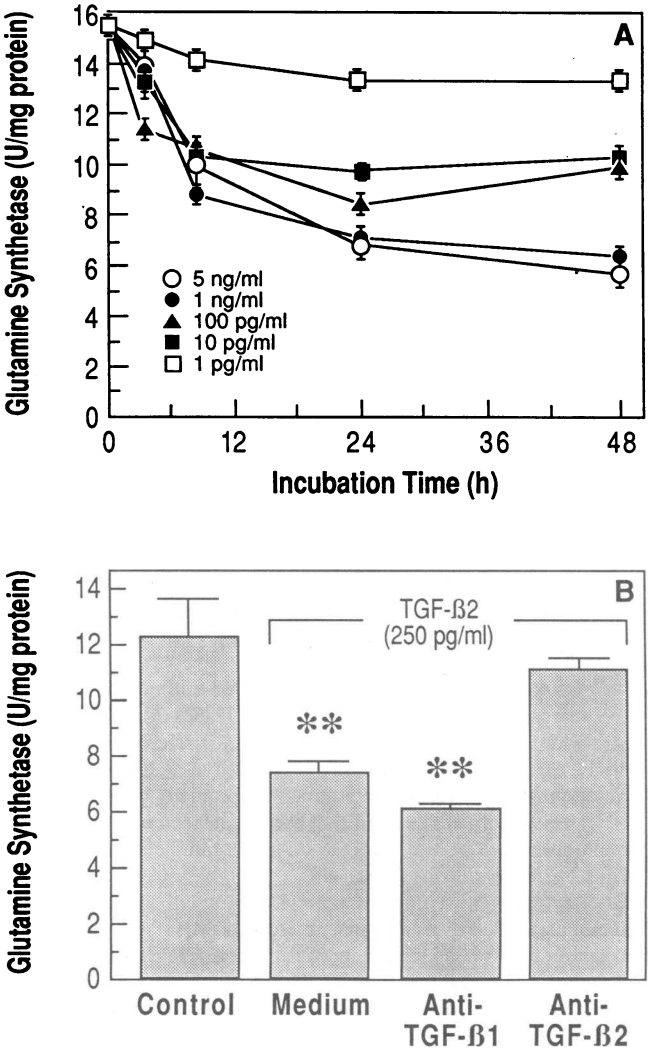
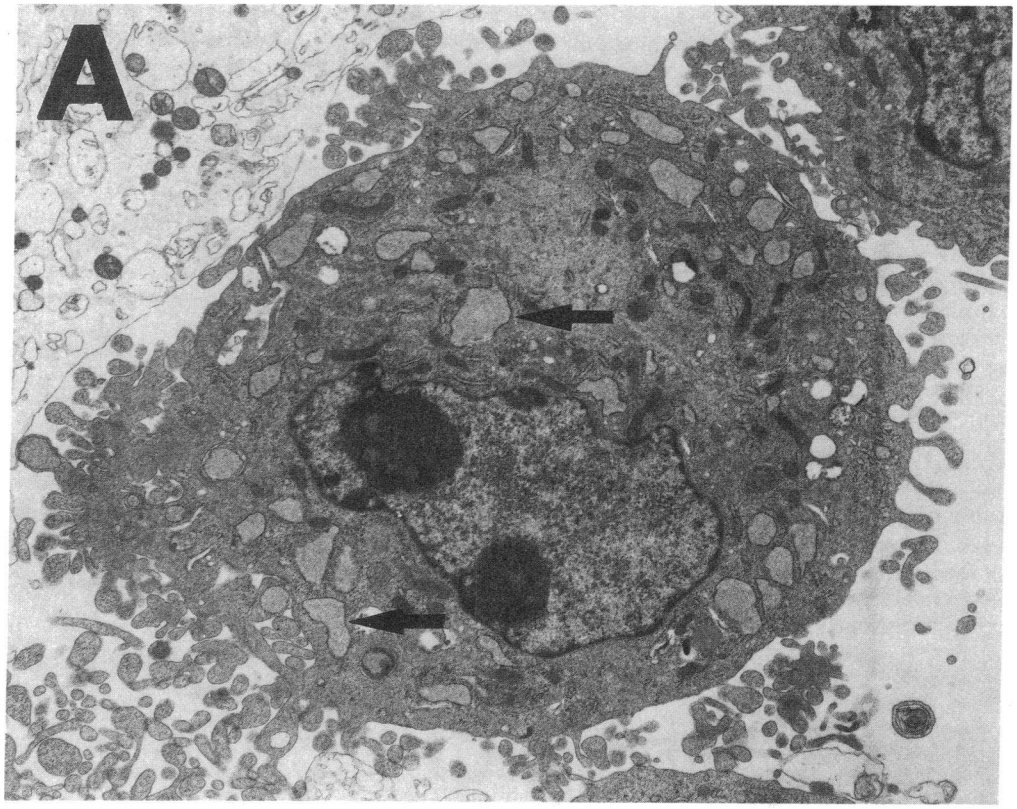



*Figure 2.* Effect of TGF-β2 on GS activity in primary murine astrocyte cultures. (*A*) Astrocyte cultures were treated with TGF-β2 at indicated concentrations for varying incubation periods. Cells were then harvested for assessment of GS activity. (*B*) Astrocyte cultures were pretreated with antibodies (10 μg/ml) specific to TGF-β1 or to TGF-β2 for 30 min before incubation with TGF-β2 (250 pg/ml) for 24 h. Cells were then harvested for determination of GS activity. Data are presented as mean±SEM of triplicate values from one experiment and are representative of three separate experiments. $**P < 0.01$ vs. control group.



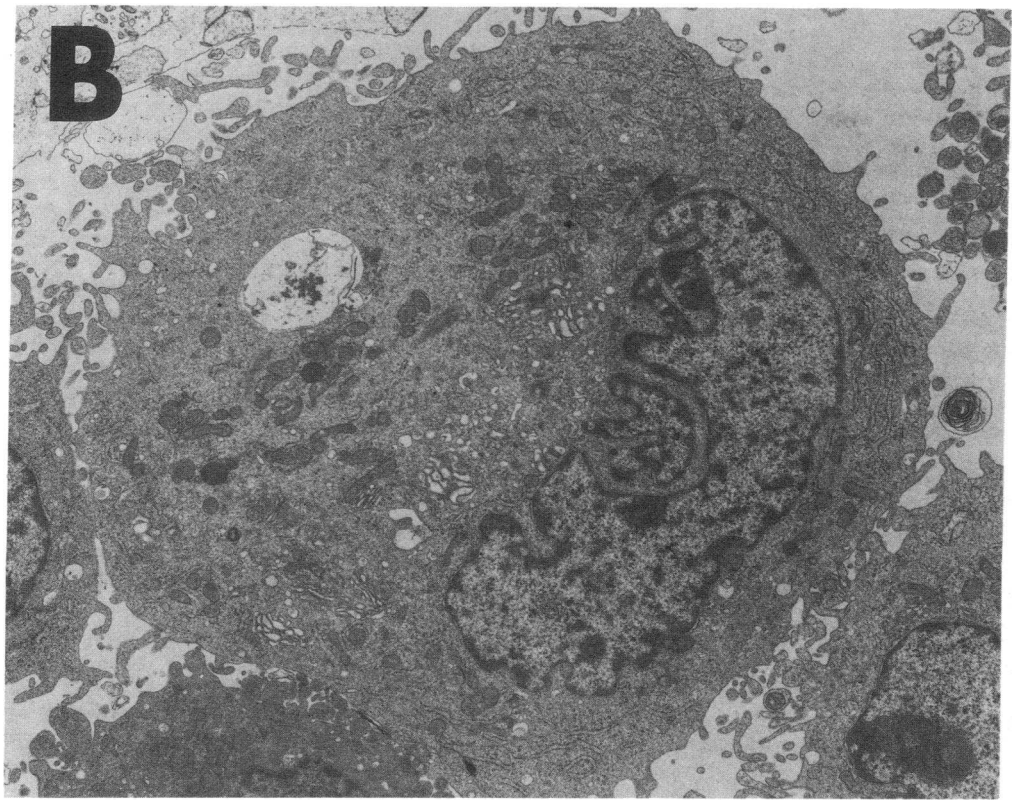




*Figure 3.* Effect of TGF-β2 on ultrastructure of astrocytes. Astrocyte cultures at day 12 of dispersion were treated with (*A*) TGF-β2 (250 pg/ml) or (*B*) culture medium for 24 h before fixing for transmission electron microscopy. Markedly dilated RER (*arrow*) with accumulation of intracanalicular flocculent material was apparent in TGF-β2–treated cells. ×7,000.

sion method, was not affected after exposure to these cytokines at the concentrations indicated above for 48 h.

*Effect of TGF-β2 on ultrastructure of astrocytes.* Since TGF-β significantly inhibited GS activity after a 24-h exposure, we also looked for ultrastructural changes in astrocytes that would correlate with this functional defect. Many astrocytes exposed to TGF-β2 for 24 h (Fig. 3 *A*) displayed markedly dilated rough endoplasmic reticulum (RER) compared with astrocytes in control medium (Fig. 3 *B*). The findings were similar for astrocytes exposed to TGF-β1 (data not shown). Although astrocytes responded heterogeneously to TGF-β treatment, the number of astrocytes displaying dilated RER was significantly ($P < 0.05$) higher in the TGF-β2–treated group (9 of 30 cells) than in the control group (1 of 30 cells). Simultaneous treatment with antibodies to TGF-β2 (10 μg/ml) and TGF-β2 (250 pg/ml) blocked TGF-β2–induced changes in ultrastructure (data not shown). The number of cells showing dilated RER in the antibody-treated group (2 of 32 astrocytes) was smaller ($P < 0.05$) than in the TGF-β2–treated group (10 of 33 cells), indicating the specificity of the TGF-β2 effect.

*Effect of TGF-β on NMDA receptor–mediated neurotoxicity.* To assess neuronal injury, release of LDH was used as an indication of the death of neurons in cultures (27). Exposure of mixed neuronal/astrocyte cultures for 5 min to NMDA induced, in a dose-related manner, an increase in LDH release (Fig. 4). After a 24-h pretreatment with TGF-β2 (250 pg/ml), a shift in the NMDA receptor–induced neurotoxicity curve to the left was observed, and the $LD_{50}$ of NMDA was decreased threefold (15 and 45 μM for TGF-β–treated and control groups, respectively). This finding indicates an enhancement of NMDA receptor–mediated neurotoxicity by exposure of mixed neuronal/astrocyte cultures to TGF-β at a relatively low concentration of this cytokine. Microscopic evaluations also demonstrated that fewer neurons survived in mixed neuronal/astrocyte cultures treated for 24 h with TGF-β2 (250 pg/ml) before exposure to NMDA (30 μM) compared with the non-treated control group exposed to NMDA (Fig. 5).

The GS activity in TGF-β2–treated cultures was inhibited 43% (0.81±0.05 vs. 1.42±0.12 U/mg protein in control cultures). Exposure of neurons to NMDA (30 μM) did not further inhibit GS in TGF-β2 (1 ng/ml)–treated cultures (0.88±0.10 U/mg protein); however, the same treatment suppressed GS by 26% in control cultures (1.05±0.01 U/mg protein). Exposure to higher concentrations of NMDA did not further inhibit GS in non-TGF-β2–treated cultures (data not shown).

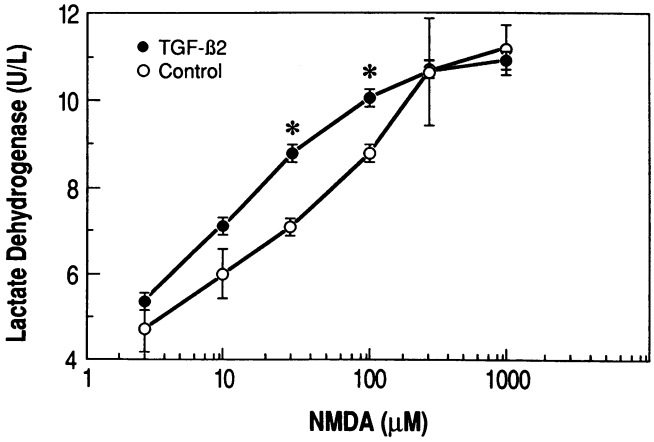



*Figure 4.* Effect of TGF-β2 on NMDA receptor–mediated neurotoxicity. Treatment for 24 h with TGF-β2 (250 pg/ml) promoted NMDA-stimulated LDH release. Data are mean±SEM of triplicate values and are representative of three separate experiments. *$P < 0.05$ vs. corresponding control values.

The findings that TGF-β suppresses astrocyte GS activity and enhances NMDA receptor-mediated neurotoxicity in mixed neuronal/astrocyte cultures, suggest that the enhanced neurotoxicity is related to the impairment of GS function by TGF-β. To test this possibility, just before NMDA exposure, mixed neuronal/astrocyte cultures were supplemented with astrocytes to provide additional GS to TGF-β–treated cultures. Supplementation of the mixed neuronal cell cultures with astrocytes resulted in a partial reversal (52%) of TGF-β enhancement of NMDA receptor-mediated neurotoxicity (Table I). The neuroprotective effect afforded by additional astrocytes was associated with increased GS levels (Table I), suggesting that astrocyte/GS plays a role in protecting against NMDA receptor–mediated neurotoxicity possibly by catabolizing glutamate to a nontoxic form, i.e., glutamine. Supplementation of the mixed neuronal/astrocyte cultures with 1 mM glutamine did not significantly alter TGF-β enhancement of NMDA neurotoxicity (11.99±0.22 vs. 12.28±0.25 U/liter LDH in glutamine-supplemented vs. nonsupplemented, TGF-β–treated cultures, respectively; mean±SEM of triplicate values from a representative of three separate experiments), suggesting that this precursor for glutamate synthesis by neuronal cells is not playing a role in the effects observed in this study.

*Effects of cytokines on high affinity glutamate uptake activity.* One possible mechanism whereby TGF-β could amplify NMDA receptor–mediated neuronal injury in mixed neuronal/astrocyte cultures would be by decreasing glutamate uptake by astrocytes, which has been suggested to play a protective role in excitotoxicity (28). We investigated whether high affinity glutamate uptake is altered by cytokine exposure in enriched astrocyte cultures. After a 48-h exposure to TGF-β2 (1 ng/ml), glutamate uptake by purified astrocytes was not affected (103±5% of control glutamate uptake). Thus, the cytokines tested did not seem to affect this function in cultured astrocytes.

## Discussion

In this study, our chief observations were that certain cytokines inhibited GS activity in mixed neuronal/astrocyte and enriched astrocyte cultures. TGF-β in particular suppressed astrocyte GS activity and did so in a dose-dependent manner with an $ED_{50}$ of 10 pg/ml. Antibodies specific to TGF-β2 blocked this inhibitory effect. Exposure to TGF-β2 also altered ultrastructure in cultured astrocytes, resulting in markedly dilated RER. These findings indicate that TGF-β targets astrocytes and affects both the function and the structure of these cells. One of the biological consequences of exposure of astrocytes to TGF-β suggested by findings in this study is enhanced glutamate-induced neurotoxicity. Although the mechanism of TGF-β enhancement of NMDA receptor–mediated neurotoxicity is unknown and is potentially complex, TGF-β inhibition of astrocyte GS with an attendant reduction in metabolism of glutamate could be involved.

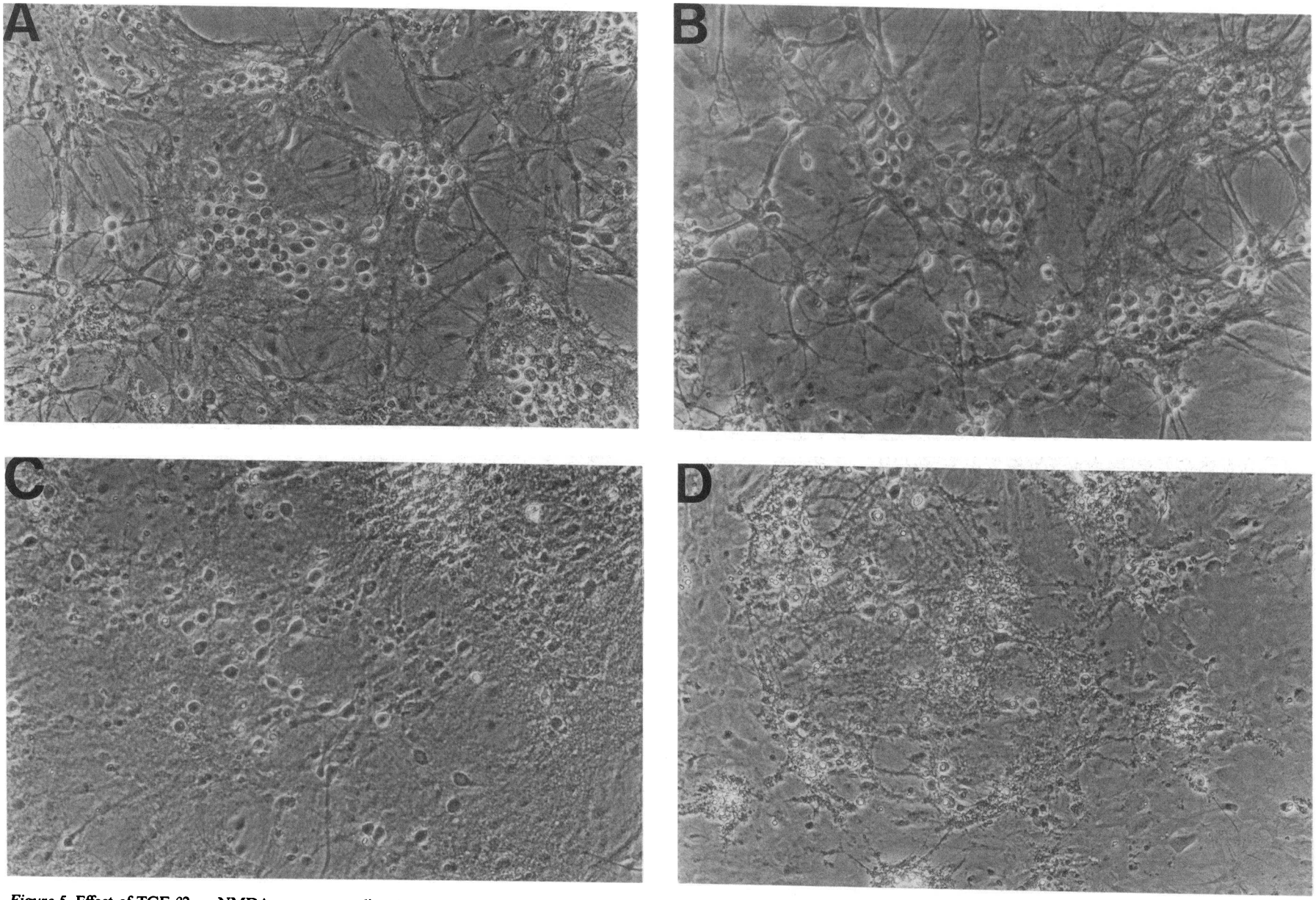



*Figure 5.* Effect of TGF-β2 on NMDA receptor–mediated neuronal loss. Mixed neuronal/astrocyte cultures were treated with medium or TGF-β2 (250 pg/ml) for 24 h followed by a 5-min exposure to 30 μM of NMDA. Microscopic photographs were taken at 24 h after NMDA exposure. (*A*) Control, non-NMDA–exposed cells: multiple neurons, single cells, and clusters; (*B*) TGF-β–treated, non-NMDA–exposed cells: cell number similar to *A*; (*C*) Control, NMDA-exposed cells: ~50% neuronal loss; and (*D*) TGF-β–treated, NMDA-exposed cells: >85% neuronal loss. ×200.

*Table I. Protective Effects of Astrocyte/GS on NMDA Receptor-mediated Neurotoxicity*

| Treatment | Astrocytes | LDH | GS |
|---|---|---|---|
| | | *U/liter* | *U/mg protein* |
| NMDA (30 μM) | − | 9.65±0.25 | 1.59±0.07 |
| TGF-β+NMDA | − | 12.28±0.18* | 0.99±0.08* |
| TGF-β+NMDA | + | 10.92±0.10 | 1.73±0.02 |

Mixed neuronal/astrocyte cultures were treated for 24 h with TGF-β or medium alone. Purified astrocytes ($5 \times 10^4$) containing 0.98±0.12 U GS activity/mg protein were added to indicated (+) mixed neuronal/astrocyte cultures just before exposure to NMDA for 5 min. Data, derived from measurements of supernatants (LDH) and cellular GS 24 h after NMDA exposure, are mean±SEM of triplicate values from one experiment and are representative of three separate experiments. * $P < 0.05$ vs. the other groups in each column.

Cytokines produced during infection play an important role in inflammation and host defense (1–9). In the peripheral immune system, the immunologic functions of the proinflammatory cytokines (e.g., TNF-α, IL-1, and IL-6) are in many cases antagonized by the antiinflammatory cytokine TGF-β (29–31). Similarly, in the CNS, TGF-β has been shown to play a regulatory role in inflammation and to antagonize the hyperinduction of astrocyte MHC class II antigen expression by interferon-γ and TNF (32, 33). It would be reasonable to assume that these cytokines have opposite effects on CNS functions. To our surprise, however, both TGF-β and TNF-α significantly inhibited GS activity in mixed neuronal/astrocyte cultures, suggesting that the neurological effects of cytokines may be distinct from their immunologic activities.

Neurological symptoms are common in certain immunologic diseases and during infection. Since peripheral immune cells are activated during a number of infectious diseases, an abnormal production of cytokines would result from the activation of these immunocytes. The role of these cytokines in host defense is complex; however, the neurological symptoms found in patients during infection could partly result from an abnormally high level of cytokines (8). Disruption of the blood–brain barrier has been observed and contributes to the entrance of serum cytokines into cerebrospinal fluid (34). Activated monocytes and neutrophils synthesize and release TGF-β (35, 36), which may result in altered astrocyte function and subsequent neuronal injury.

The CNS itself may be another site (besides the peripheral immune system) of cytokine production. Increased cytokine gene expression or cytokine production has been detected in brain tissue and in cerebrospinal fluid after certain infections and brain diseases (8, 37, 38). TGF-β has been found in glioblastoma patients (39, 40) and in the brain tissues of AIDS patients (41, 42). It is possible that the local production of cytokines plays a pivotal role in CNS inflammation and altered neuronal function via a paracrine or an autocrine mechanism.

CNS inflammation may affect brain cell biochemistry and ultrastructure. Astrocyte GS activity is inhibited by TGF-β exposure when cells are stimulated with hydrocortisone (43). Since GS is found exclusively in astrocytes (18, 19), inhibition of GS activity may in turn increase the vulnerability of cultured neurons to glutamate neurotoxicity. Indeed, a 100-fold increase in neuronal susceptibility to glutamate toxicity has been observed when mixed neuronal/astrocyte cultures contain relatively few astrocytes (16, 17). Although the mechanisms involved in NMDA receptor–mediated neurodegeneration are complex, the selective suppression by certain cytokines of astrocyte GS activity in mixed neuronal/astrocyte cultures could play a role in potentiation of NMDA receptor–mediated neurotoxicity. Indeed, TGF-β suppresses astrocyte GS and potentiates NMDA neurotoxicity. Supplementation of mixed neuronal/astrocyte cultures with astrocytes to provide additional GS resulted in partial but significant protection against TGF-β enhancement of NMDA neurotoxicity. Although other unidentified mechanisms have not been excluded, nonetheless, the present studies lend support to the hypothesis that the suppressive effect of TGF-β on GS is involved in the enhancement of NMDA receptor–mediated neurotoxicity.

Although the exact intracellular mechanism for altering astrocyte GS activity is unknown, TGF-β has been reported to trigger phosphoinositol metabolism and translocation of protein kinase C in cultured astrocytes (44). It is unknown whether this secondary messenger system is involved in the inhibition of astrocyte GS by TGF-β.

Besides the functional changes after cytokine exposure, we found that TGF-β also dramatically affected astrocyte intracellular structure. The biological impact of ultrastructural abnormalities after exposure to TGF-β is unknown. We also noted, through electron microscopy, the heterogeneous ultrastructural response of astrocytes to TGF-β; whether a more homogeneous response would be detected during infectious disease and whether a more severe injury would result are also unknown. In any event, the ultrastructural abnormalities we observed did indeed correlate with the functional impairment seen in mixed neuronal/astrocyte cultures. Further studies are necessary to elucidate, at the ultrastructural level, the biological meaning of dilated RER and, at the molecular level, the synthesis of GS in astrocytes.

The neuronal injury observed in the present study was not due to a toxic effect of TGF-β, since this molecule did not significantly alter protein synthesis or the viability of astrocytes or neurons after a 48-h exposure. Nor is DNA synthesis affected by exposure of astrocytes to TGF-β for 1 to 3 d (43). Interestingly, both TGF-β1 and -β2 exerted similar inhibitory effects on GS activity, suggesting that CNS TGF-β receptors bind equally well to these highly conserved molecules. Though the role of TGF-β in the CNS is unknown (45), the cellular source for CNS TGF-β has been located in astrocytes (41) and endothelial cells (46). Since TGF-β is synthesized and released as a latent, inactive form, the production of bioactive TGF-β in the CNS needs to be investigated further.

In conclusion, the inhibition of astrocyte GS activity by TGF-β demonstrated in this study has implications not only for the integrity of astrocytes but also indirectly for neuronal cell survival. Our findings are consistent with the proposed neuroprotective role of astrocytes in glutamate-induced neurotoxicity (16, 17), although the precise mechanism whereby TGF-β potentiates NMDA receptor–mediated neurotoxicity is not yet established. Cytokines such as TGF-β may have many influences on astrocytes and neuronal cells, and yet unidentified effects may be operative in the neurotoxicity model used in this study. Given the increasing evidence that TGF-β plays a pathogenetic role in diseases such as AIDS dementia (41, 47, 48), more work is needed to determine if and how this cytokine is involved in a wide range of immune-mediated CNS diseases.

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