Macrophage- and Astrocyte-derived Transforming Growth Factor β as a Mediator of Central Nervous System Dysfunction in Acquired Immune Deficiency Syndrome

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Summary

The multifunctional cytokine, transforming growth factor beta (TGF-β), was identified by immunocytochemistry in the brain tissues of four patients with acquired immune deficiency syndrome (AIDS), but not in control brain tissue. The TGF-β staining was localized to cells of monocytic lineage as well as astrocytes, especially in areas of brain pathology. In addition, the brain tissues from the AIDS patients contained transcripts for human immunodeficiency virus 1 (HIV-1) by in situ hybridization, suggesting a correlation between the presence of HIV-1 in the brain and the expression of TGF-β. However, the expression of TGF-β was not limited to HIV-1-positive cells, raising the possibility of alternative mechanisms for the induction of TGF-β in these AIDS patients' brains. To investigate these mechanisms, purified human monocytes were infected in vitro with HIV-1 and were shown to secrete increased levels of TGF-β. In addition, HIV-1-infected monocytes released a factor(s) capable of triggering cultured astrocytes that are not infected with HIV-1 to secrete TGF-β. The release of TGF-β, which is an extremely potent chemotactic factor, may contribute to the recruitment of HIV-1-infected monocytic cells, enabling viral spread to and within the central nervous system (CNS). Moreover, TGF-β augments cytokine production, including cytokines known to be neurotoxic. The identification of TGF-β within the CNS implicates this cytokine in the immunopathologic processes responsible for AIDS-related CNS dysfunction.

Central nervous system (CNS)[1] dysfunction is a common manifestation of AIDS. CNS dysfunction of the brain in patients with AIDS may result from primary infection with HIV-1 (1–7), opportunistic infections (8–10), and/or neoplasms (10, 11). However, the most common syndrome in AIDS-related CNS disease is HIV encephalopathy, a fatal dementing illness that may occur in the absence of opportunistic pathogens or neoplasms. Although the cellular and molecular events leading to HIV encephalopathy are unclear, a consistent finding in the brains of AIDS patients is infiltration by monocyte-macrophages, cells that appear to be the predominant source of HIV-1 within the brain (1, 2, 12). Since monocytes play a critical role in the pathogenesis of HIV-1 infection and in the evolution of AIDS (13–19), this population of cells may contribute to the neuropathology associated with AIDS-related CNS dysfunction. Consistent with this hypothesis are the recent observations that peripheral blood monocytes infected with HIV-1 constitutively release cytokines such as TNF-α (20, 21), which is reportedly neurotoxic (22, 23) and may contribute to the brain pathology. In addition, the accumulation of monocyte-macrophages in the CNS suggests the presence of a mechanism for recruitment of circulating monocytes into these tissues.

Recently identified as a cytokine with potent chemotactic activity for monocytes, TGF-β can induce migration at femtomolar concentrations (24). To determine whether TGF-β plays a role in recruitment and regulation of monocyte function in the CNS in AIDS patients, we evaluated brain tissue obtained at autopsy or surgery from AIDS patients for the presence of this immunoregulatory cytokine. TGF-β was clearly identified within the AIDS brain tissues in associa-

[1] *Abbreviations used in this paper:* CNS, central nervous system; DTT, dithiothreitol; GFAP, glial fibrillary acidic protein; RT, reverse transcriptase.

tion with the mononuclear phagocytic cells and also cells identified as astrocytes. To determine whether HIV-1 could regulate the production of this polypeptide, we infected peripheral blood monocytes with HIV-1 in vitro and monitored their secretion of TGF-β. The infected monocytic cells secreted TGF-β and released a factor(s) that triggered secretion of TGF-β by cultured, uninfected astrocytes. Identification of TGF-β, a cytokine with numerous immunoregulatory functions (25), in the HIV-1–infected brain tissues suggests a role for this cytokine in the pathobiology of HIV encephalopathy.

Materials and Methods

Patients and Tissue Specimens. Tissue specimens were obtained at autopsy (patients 1–3) and surgery (patient 4) from the brains of four male subjects with AIDS (Table 1). The brains were positive for HIV as determined by electron microscopic evaluation (26) and/or in situ hybridization; in some cases opportunistic pathogens could be detected. The histopathology of two of the AIDS patients (patients 1 and 2) has been described in detail previously (26). Control brain tissue was obtained at autopsy from a person without AIDS or other CNS defects (patient 5) and from a biopsy of a patient with meningioma (patient 6).

Histology and Immunohistochemistry. Brain tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for neuropathologic evaluation. Immunohistochemical staining was performed using the horseradish-peroxidase–labeled avidin-biotin procedure (ABC Vecta Stain Kit; Vector Laboratories Inc., Burlingame, CA) with diaminobenzidine as chromogen (27). Antibodies included one prepared against a synthetic polypeptide identical to the NH_2 terminal residues 1–30 of TGF-β1 (28), a mAb directed at glial fibrillary acidic protein (GFAP; Dakopatts, Carpenteria, CA) that is specific for astrocytes (29), LeuM1, Leu1, and HLE, which recognize monocytes, lymphocytes, and human leukocyte antigens, respectively (Becton Dickinson and Co., Mountain View, CA). After immunoperoxidase staining, the slides were counterstained with methyl green. Additional sections were processed for visualization of the enzyme α-napthyl-acetate-esterase (30) and with a modified Scott's stain for oligodendroglia, microglia, and astrocytes.

In Situ Hybridization. Tissue sections (6–8 μm) were fixed with periodate-lysine-paraformaldehyde-glutaraldehyde onto silanized slides as described (1, 31). After deparaffinization of the tissue sections with xylene, the sections were permeabilized with proteinase K (100 μg/ml), acetylated, dehydrated, and prehybridized. The tissue sections were hybridized to a [^{35}S]-labeled RNA probe encoding pooled portions of the HIV-1 gag/pol/env regions in sense or antisense orientation (10^6 cpm/slide) (Lofstrand, Gaithersburg, MD) in the presence of 1× Denhardt's solution with 10 mM dithiothreitol (DTT), 0.3 M NaCl, 50 mM Tris-HCl, and 10% dextran sulfate in formamide overnight at 48°C (31). The slides were washed sequentially in 2× SSC (1× SSC = 0.15 M NaCl/0.015 M Na citrate, pH 7.0), 2× SSC with 1 mM EDTA, 5 mM DTT, and 0.1% Triton X-100 at 56°C, and 0.1× SSC with EDTA, DTT, and Triton X-100. The slides then were treated with ribonuclease A (40 μg/ml) and T (10 U/ml) (Sigma Chemical Co., St. Louis, MO) in 2× SSC at 37°C for 40 min, washed in 2× SSC, and dehydrated in graded ethanols. Slides were prepared for autoradiography with NTB3 emulsion (Eastman Kodak, Co., Rochester, NY), developed after 3 d and stained with hematoxylin and eosin. Cultured cells were processed and were hybridized to a [^{35}S]-labeled cDNA-TGF-β1 antisense probe (32) as described above and the autoradiography developed after 21 d.

Mononuclear Leukocyte Suspensions. Mononuclear leukocytes were isolated from heparinized peripheral blood of healthy volunteers or from leukapheresis units (National Institutes of Health Blood Bank, Bethesda, MD) by Ficoll-Paque density gradient centrifugation (33). To obtain highly purified populations of monocytes, the mononuclear leukocytes were separated by counterflow centrifugal elutriation in pyrogen-free PBS (B & B/Scott, Fiskville, RI) as described (33).

In Vitro Infection with HIV-1. Purified monocytes (10^7) were cocultured with macrophage tropic HIV-$1_{Ba\text{-}L}$ (5 × 10^5 cpm reverse transcriptase [RT] activity, kindly provided by Drs. S. Gartner and M. Popovic, National Cancer Institute, Bethesda, MD) in suspension or in adherent monolayers as described (14, 16). Monocytes were suspended in RPMI 1640 (Gibco Laboratories, Grand Island, NY) with 50 μg/ml gentamicin, 2 mM glutamine, and 20% FCS with or without virus for 1 h with intermittent shaking at 37°C. The cells were then washed and resuspended (2 × 10^6/ml) in medium containing 10% FCS for suspension cultures in 17 × 100-mm polypropylene tubes (Falcon Labware, Oxnard, CA) or resuspended in medium without serum for plating (10^6/ml) in 24-well tissue culture plates (Falcon Labware). After 2–4 h, the adherent cells were washed, and medium containing 10% FCS was added. Media (50%) were removed and fresh medium added at 3–4-d intervals.

Alternatively, monocytes were cultured as adherent monolayers for 5–7 d and then incubated with HIV-1 for 1–2 h, washed, and fresh serum-supplemented medium was added. Infection was monitored by RT assay, HIV p24 antigen capture ELISA (NEN Research Products, Boston, MA), and/or by electron microscopy (16).

TGF-β Assay. TGF-β in culture supernatants was quantitated by its ability to inhibit thymocyte proliferation (34). Since TGF-β is normally secreted in a latent form, the supernatants were either acid activated (1.5 μl 6 N HCl/200 μl supernatant) for 15 min, followed by neutralization with 6 N NaOH in 1 M Hepes or heated to 80°C for 5 min (34). The treated supernatants then were added in twofold dilutions to cultures of C3H/HeJ mouse thymocytes containing 10 U/ml IL-1 and PHA (1 μg/ml) in parallel with serial dilutions of a TGF-β standard (R & D Systems, Minneapolis, MN). After 68 h, the wells were pulsed for 4 h with 0.5 μCi/ml [^{3}H]TdR (Schwarz-Mann, Orangeburg, NY) (sp act 1.9 Ci/mM), harvested, and processed for determination of [^{3}H]TdR incorporation. In this assay, the inhibitory concentration (IC_{50}) is ~0.05 ng TGF-β/ml and this activity can be blocked with a neutralizing antibody to TGF-β (34).

Northern Blot Analysis. Total RNA was isolated from 1.5 × 10^7 control monocytes or monocytes exposed to HIV-1 for 1 h, washed, and cultured for up to 21 d. Total RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform extraction procedure (35). For Northern blots, 5 μg RNA was fractionated on 1.0% agarose gels containing formaldehyde and transferred to nitrocellulose filters. The blots were prehybridized and then sequentially hybridized with the ^{32}P-labeled cDNA probes: HIV-1 (a 6.5-kb segment of HIV-1 encoding gag/pol/env), human TGF-β1 (provided by Dr. R. Derynck, Genentech Inc., South San Francisco, CA) (32), and He7 (provided by Dr. J. Reed, University of Pennsylvania School of Medicine, Philadelphia, PA) (36). The blots were washed twice in 2× SSC, 0.1% SDS for 15 min at room temperature and for 30 min at 65°C in 0.1× SSC, 0.1% SDS. The filters were exposed to Kodak XAR film with intensifier screens at −70°C for 4–18 h. The relative amounts of hybridizing RNA were determined by scanning the autoradiograms with a laser densitometer (LKB Ultrascan, LKB Instruments, Gaithersburg, MD) and the TGF-β/He7 ratios determined for individual samples.

Astrocyte Isolation and Culture. Primary glial cell cultures were

established from neonatal rat brain hemispheres as previously described (37). Briefly, after removal of meninges, the brain tissue was trypsinized (0.5 mg/ml) in HBSS without Ca^{2+} and Mg^{2+} for 15 min at room temperature. Serum-containing DMEM was added to stop trypsinization, and the cells were mechanically dissociated in 0.5 μg/ml DNase I (Sigma Chemical Co.), washed, and suspended in DMEM with antibiotics, glutamine, and 10% FCS. The cells were plated in 75-cm^2 tissue culture flasks (Nunc) coated with 0.1 mg/ml poly-L-lysine (Sigma Chemical Co.), and cultured at 37°C for 8–10 d. The adherent astrocytes were trypsinized and replated in poly-L-lysine–coated culture dishes. After a second trypsinization, astrocytes were plated at 7.5 × 10^4/well in 24-well tissue culture plates and the cells grown to near confluency. The medium was aspirated and replaced with DMEM with 10% FCS containing dilutions of supernatants obtained from HIV-1–infected or control monocyte cultures. Additionally, 1–20 ng/ml TGF-β was added to astrocyte cultures. After a 4–5-h incubation, medium was removed and discarded, the cells were washed extensively, and fresh DMEM was added for 36 h. At the end of this incubation, the cell-free astrocyte supernatants were harvested and frozen until assayed for TGF-β.

Results

Identification of TGF-β in Brains of Patients with AIDS. The histological alterations of the brain tissues are summarized in Table 1. Compared to normal brain tissue, the brains of the four AIDS patients had varying degrees of cellular infiltrates, primarily mononuclear in composition, as well as areas of tissue damage, multinucleated giant cells, phagocytes with foamy cytoplasm, and gliosis. By immunoperoxidase staining with an antibody that detects primarily intracellular TGF-β1 (27), many cells in each of the AIDS patients' brain stained prominently for TGF-β1 (Fig. 1 *B* and Table 1). Specificity of the antibody for TGF-β was documented by the absence of staining after this IgG preparation had been incubated with Sepharose-coupled TGF-β1 (kind gift of Dr. K. Flanders, National Cancer Institute) as described (38). The presence of TGF-β1 in the AIDS patients' brains was in marked contrast to its absence in the brain tissue sections from the subject without CNS pathology (patient 5, Fig. 1 *E*). Sections of the meningioma from patient 6 were negative for TGF-β1, although a number of cells that had infiltrated around the tumor mass stained positively for TGF-β1 (Table 1).

The TGF-β1 in the AIDS patients' brains appeared to localize preferentially within the white matter. Staining often occurred near the ependyma and frequently exhibited a gradient pattern (Fig. 2 *E*) in which cells adjacent to the site of pathology stained intensely for TGF-β compared to the progressive reduction in intensity and number of positive cells in areas more distant from these foci.

Characterization of Cells Expressing TGF-β. Closer examination of the TGF-β positive brain sections from the AIDS patients allowed for identification of the cellular loci of the TGF-β polypeptide. Many cells with morphologic characteristics of cells in the mononuclear phagocytic lineage were located in perivascular locations and stained positively for TGF-β (Fig. 2 *A*). Among these positively staining cells were multinucleated giant cells (Fig. 2 *B*) and mononuclear phagocytes some of which appeared to contain TGF-β within phagocytic vacuoles (Fig. 2 *C*). In addition, a population of cells with the distribution, morphology, and GFAP staining characteristics of astrocytes were positive for TGF-β1 (Fig. 2 *E* and *F*). Moreover, many of these astrocytes appeared activated as reflected by their enlarged nuclei and cytoplasmic profiles. TGF-β1 staining did not appear in oligodendrocytic, neuronal, or endothelial cell populations. Interestingly, the astrocyte and macrophage populations in the AIDS brains were not universally positive for TGF-β, but positive cells were localized in and around areas of apparent tissue pathology.

Localization of HIV-1. Since mononuclear phagocytes infected with HIV-1 in vitro and in vivo may express increased levels of certain cytokines (20, 21), we next evaluated whether the cells expressing TGF-β also were infected with HIV-1. Sequential brain sections were either probed by in situ hybridization with the [^{35}S]-labeled antisense RNA probe for pooled HIV-1 gag/pol/env regions or stained by the immunoperoxidase procedure for TGF-β1. Grains representing copies of HIV-1-specific RNA were present over multinucleated giant cells and other cells that appeared to be of monocytic lineage, but not over cells identified as astrocytes (Fig.

Table 1. *Pathology and Identification of TGF-β in Brain Tissue of AIDS Patients and Control Subjects*

Patient	TGF-β	Pathology	Infectious agent‡			
			HIV-1	PV	CMV	TOXO
1 (Autopsy)	+ +	PML*	+	+	–	–
2 (Autopsy)	+ +	Lymphoma	+	–	+	–
3 (Autopsy)	+ +	Lymphoma	+	–	–	–
4 (Biopsy)	+ +	Toxoplasmosis	+	–	–	+
5 (Autopsy)	–	Normal	–	–	–	–
6 (Biopsy)	±	Meningioma	–	–	–	–

* PML, progressive multifocal leukoencephalopathy.
‡ PV, papovavirus; CMV, cytomegalovirus; toxo, toxoplasma.

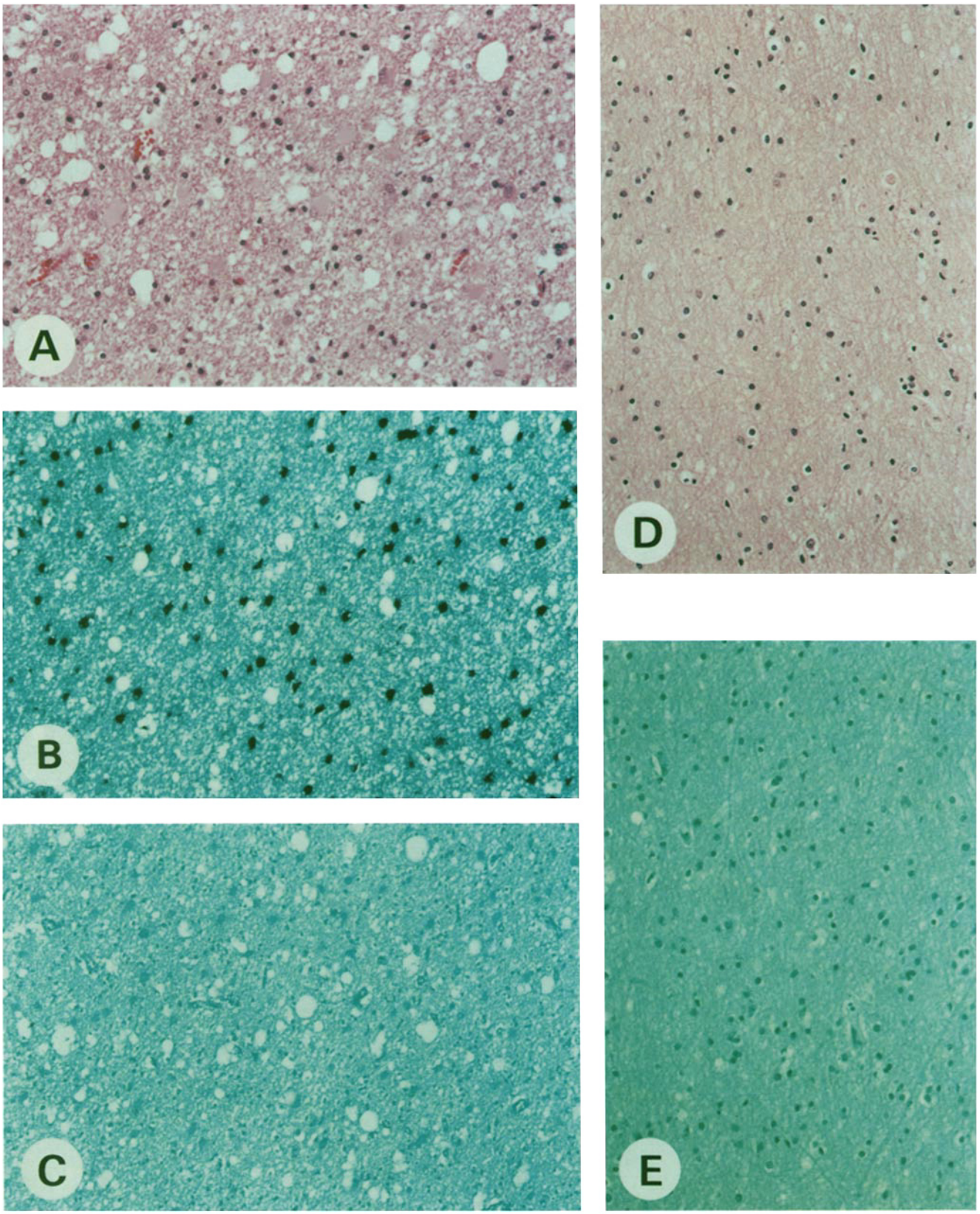

Figure 1. Identification of TGF-β by immunocytochemistry. Formalin fixed brain tissue from an AIDS patient (*A–C*) and a patient without brain pathology (*D* and *E*) were stained with H & E (*A* and *D*) or by immunoperoxidase with an antibody prepared against a synthetic polypeptide identical to the NH_2-terminal residues 1–30 of TGF-β (*B* and *E*). (*C*) Patient tissue treated as in B without primary antibody (original magnification, ×32).

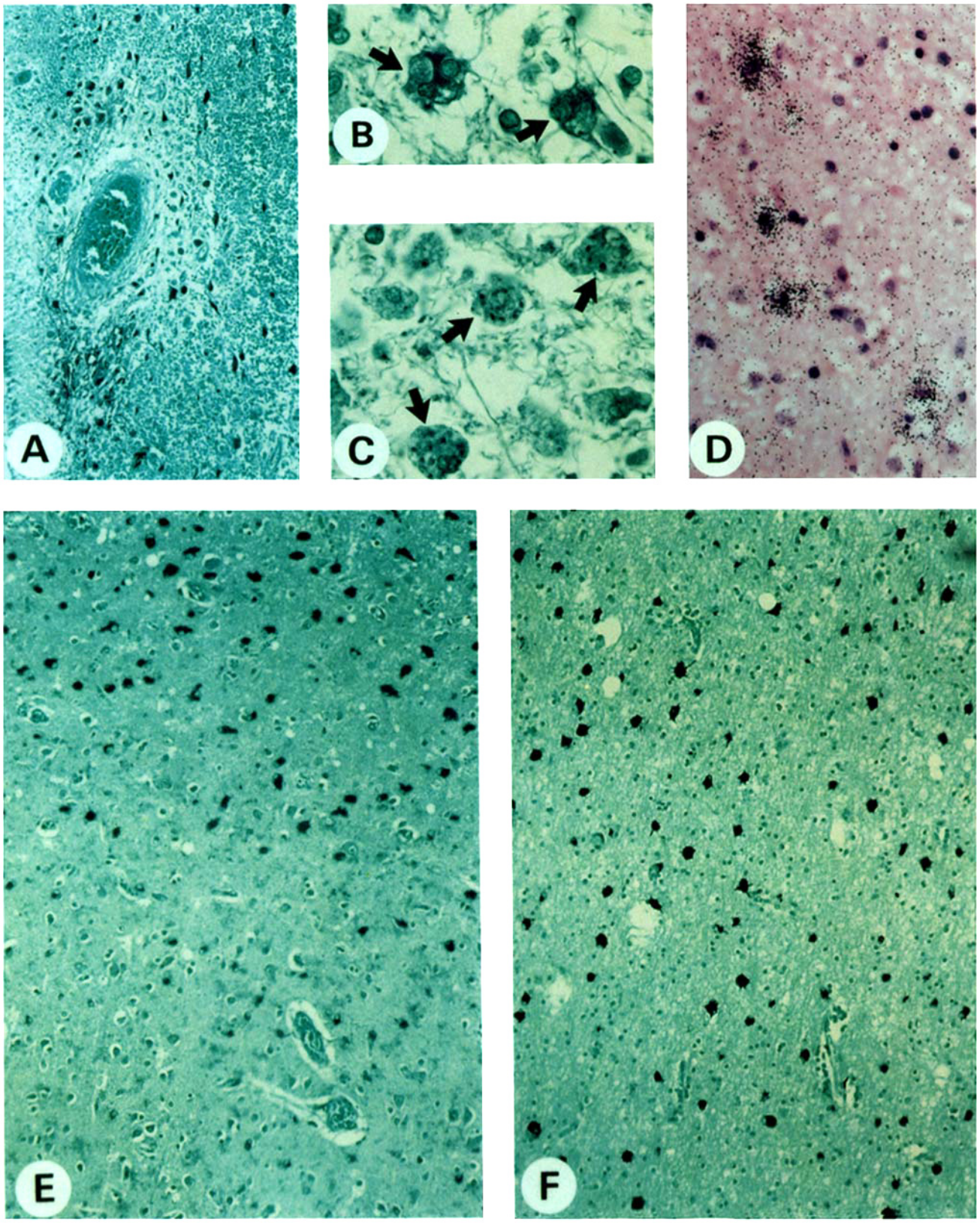

Figure 2. Localization of TGF-β peptide and HIV-1 RNA in AIDS brain tissues. Immunocytochemical staining for TGF-β in (*A*) perivascular regions (original magnification, ×32); (*B*) multinucleated giant cells (original magnification, ×250) and (*C*) vacuolated phagocytic cells (original magnification, ×250). (*D*) In situ hybridization for HIV-1 mRNA. Sections of brain tissue from patient 3 stained with TGF-β (*E*) and GFAP (*F*) (original magnification, ×32).

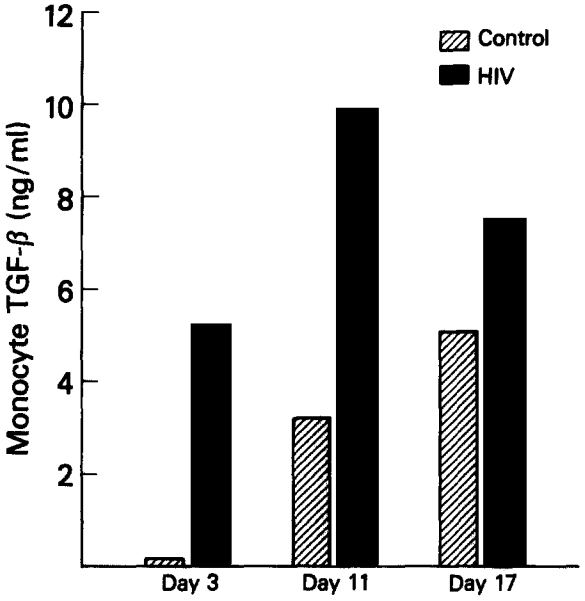

Figure 3. Production of TGF-β by HIV-infected monocytes. Purified monocytes were exposed to HIV-1_{Ba-L} (5×10^5 cpm/10^6 monocytes) for 1 h, washed, and adhered in tissue culture wells. DMEM containing 10% FCS was added and at indicated intervals, 50% of the medium was removed and replaced with fresh DMEM and FCS. TGF-β activity in the harvested supernatants was determined as described in Materials and Methods and is representative of three experiments.

2 *D*). Sections from the same brains using control sense probes were negative. There did not, however, appear to be a direct concordance between the presence of HIV-1 and TGF-β, in that many more cells were TGF-β positive than were HIV-1 positive. Moreover, the TGF-β-positive cells that were astrocytes appeared to be HIV-1 negative. That HIV-1 infection and TGF-β1 production did not exhibit a strong correlation at the cellular level suggested that HIV-1-infected cells may release a factor(s) that induces TGF-β production and/or that other stimuli induce production of TGF-β by the uninfected cells.

HIV-1–Infected Monocytes Produce TGF-β. To determine whether HIV-1 infection of monocyte-macrophages could directly influence TGF-β1 production by these cells, purified peripheral blood monocytes (>95% $CD14^+$) were exposed to HIV-1_{Ba-L} for 1 h, washed, and the cells monitored for secretion of TGF-β1. As shown in Fig. 3, monocyte-macrophages exposed to HIV-1 as compared to control cells secreted increased levels of latent TGF-β1. TGF-β1 bioactivity was detected in acid-activated supernatants within 2–3 d after HIV-1 inoculation and increased further through day 11 of infection before beginning to decline.

To further explore HIV-1 regulation of TGF-β1, monocyte mRNA were isolated and probed for both TGF-β1 and HIV-1. Although monocytes constitutively express the 2.5-kb message for TGF-β1 (39–41), TGF-β1 mRNA expression was increased two- to threefold within 2–6 h after exposure to HIV-1, but before the onset of viral replication (not shown). This increase in TGF-β was reflected by increased levels of secreted TGF-β1 activity (Fig. 3). By day 7 after inoculation of the monocytes with HIV-1, viral transcripts (6.5, 4.3, and 2.0 kb) were detected in the HIV-1–exposed, but not the unexposed, control cells. The intensity of the viral transcripts increased to day 14 and then decreased (Fig. 4 *A*). Coinciding with the period of viral replication, these HIV-1–infected monocytes also expressed increased levels of TGF-β1-specific mRNA (Fig. 4 *A*).

To control for potential differences in RNA levels, the blots were stripped, probed for the constitutively expressed control mRNA for He7, and the ratio of TGF-β to He7 RNA determined. As shown in Fig. 4 *B*, the levels of TGF-β mRNA

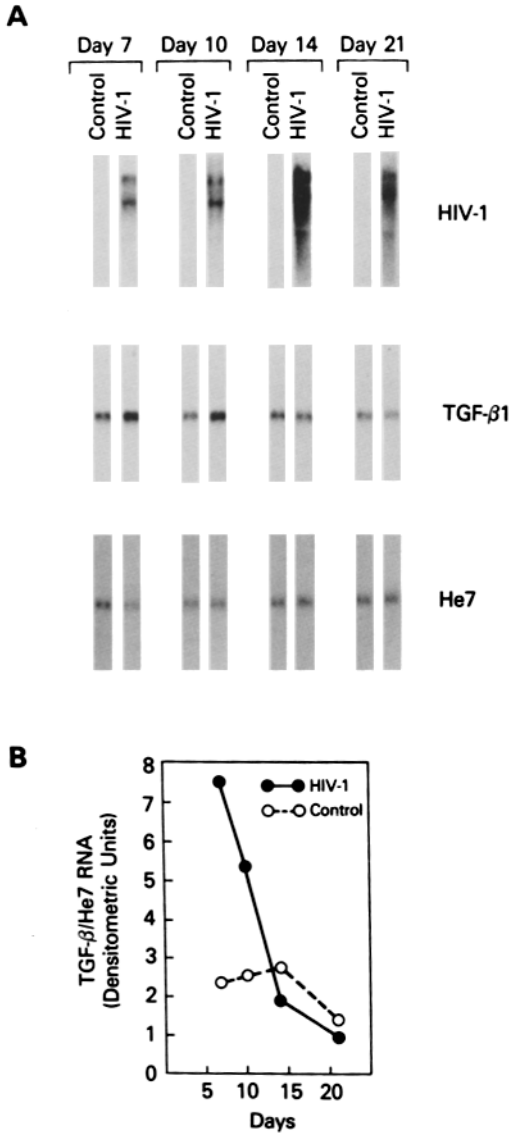

Figure 4. Expression of HIV-1 transcripts and TGF-β mRNA in monocytes exposed to HIV-1. (*A*) Northern blot analysis of mRNA from monocytes infected with HIV-1 and equivalent numbers of control cells at the indicated intervals. RT levels peaked at day 14 ($\sim 9 \times 10^5$ cpm) in the HIV-1-inoculated monocyte cultures. The mRNA were hybridized sequentially with probes for HIV-1, TGF-β, and He7. (*B*) Densitometric analysis of the Northern blots to determine the ratio of TGF-β mRNA to He7 mRNA for each individual sample.

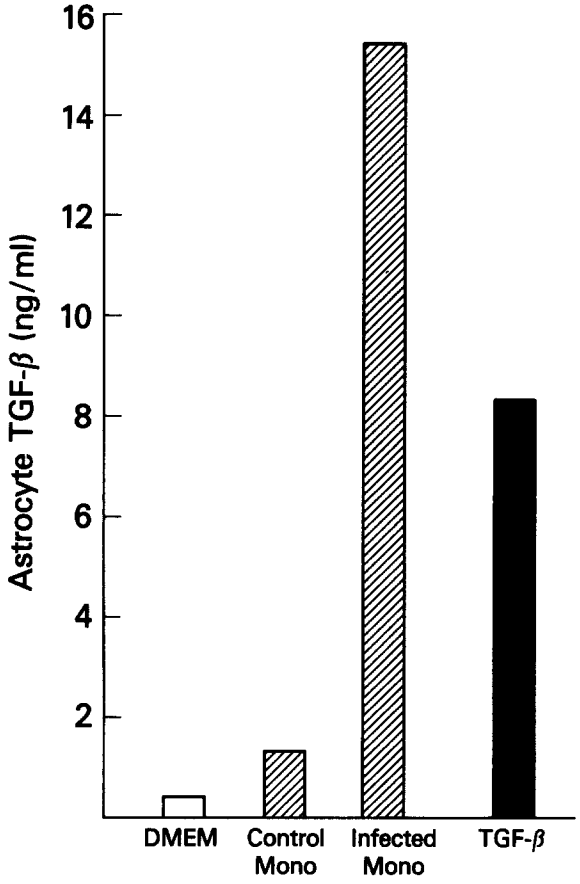

Figure 5. Induction of astrocyte TGF-β production. Rat astrocyte monolayers were cultured in DMEM with FCS only or in supernatants (diluted 1:10) from control or infected monocytes obtained 12 d after inoculation with HIV-1. Astrocytes were also cultured with 20 ng/ml TGF-β. After 4-h incubation with the supernatants or TGF-β, the astrocyte culture medium was aspirated, the cells washed extensively, and fresh medium added. After a 36-h incubation, the cell-free astrocyte culture supernatants were harvested and assayed for TGF-β activity.

compared to He7 mRNA on the same blots were elevated several-fold in the HIV-1–infected monocytes as compared to the control cells. This augmented expression of TGF-β1 mRNA was most apparent at the beginning of viral replication. Thus, as shown for other monocyte products (20, 21, 42, 43), it appears that both the initial interaction of HIV-1 with monocytes and the onset of productive infection stimulate monocytes, likely by distinct signaling pathways, to generate increased levels of TGF-β1.

HIV-1–Infected Monocyte Products Stimulate Cultured Astrocytes to Produce TGF-β. Since astrocytes do not appear to be infected with HIV-1, we investigated whether HIV-1–infected monocyte-macrophages might secrete a factor(s) capable of upregulating TGF-β production by astrocytes. Supernatants from cultures of HIV-1–infected monocytes were added to rat astrocyte monolayers and the secreted astrocyte products monitored for TGF-β. Whereas untreated astrocytes in DMEM and astrocytes treated with control monocyte supernatants produced undetectable or minimal levels of TGF-β, astrocytes treated with HIV-1–infected monocyte supernatants secreted elevated levels of TGF-β (Fig. 5). Moreover, since HIV-1–infected monocytes secreted TGF-β, we also determined whether the addition of exogenous TGF-β1 (10–20 ng/ml) to astrocyte cultures induced these cells to secrete TGF-β. As shown in Fig. 5, astrocytes that were pulsed with TGF-β subsequently secreted nanogram levels of TGF-β into their culture media.

To confirm that it was astrocytes in the cultures that were generating TGF-β, the monolayers were stained with antibodies to GFAP (astrocyte-specific) and to TGF-β1 by the immunoperoxidase technique (Fig. 6). The majority of the astrocyte monolayers were GFAP$^+$ (>90%) and in parallel cultures, similar numbers of cells were positive for TGF-β1 peptide. Although a small percentage of the cells in the cultures stained positively with mAb OX42, which identifies tissue macrophages and microglia (Fig. 6 *B*), the localization of TGF-β peptide by immunoperoxidase and TGF-β mRNA by in situ hybridization in astrocytes (Fig. 6, *C* and *D*) clearly documents that the astrocytes produce TGF-β. These data implicate a link between the presence of HIV-1–infected mononuclear phagocytes in the brain and the apparent activation of astrocytes to secrete TGF-β.

Discussion

In this study we demonstrate that the peptide TGF-β1 is present in the brain tissues of patients with AIDS, but not in normal brain tissue. This potent immunoregulatory cytokine was primarily associated with two populations of cells: cells of the mononuclear phagocytic lineage and astrocytes. Although one of the brain specimens was CMV positive and one was infected with toxoplasma, the only pathogen common to all four tissues appeared to be HIV-1, suggesting an association between HIV-1 infection and the production of TGF-β1. The combination of in situ hybridization for HIV-1 RNA and immunohistochemical staining for TGF-β1 showed that although the frequency of HIV-1–infected mononuclear phagocytes within the brain is high (1, 2), monocytic cells expressing TGF-β1 were not universally HIV-1 positive. These observations suggested that HIV-1–infected cells might be influencing the production of TGF-β1 by uninfected cells.

One population of HIV-1-negative cells found to be TGF-β1 positive was identified as astrocytes. The number of astrocytes positive for TGF-β was usually highest in areas associated with tissue abnormalities, whereas fewer or no TGF-β1-positive astrocytes were identified in areas that appeared normal. Although astrocytes cannot be infected by HIV-1 in culture (44), we infected monocytes with HIV-1 in vitro to explore possible mechanisms of TGF-β regulation in these cells. Exposure of monocytes to HIV-1 induced this population of cells to produce TGF-β. Although the mechanism of HIV-1-induced TGF-β secretion has not been elucidated, it appears similar to HIV-1 upregulation of monocyte-macrophage production of other cytokines, including IL-1, TNF-α, and IL-6 (20, 21, 42, 43). Interestingly, the early increase in TGF-β activity, before evidence of viral replication in the monocytes, appears to mimic the ability of HIV-1 and/or the envelope glycoprotein gp120 to interact with the CD4 mole-

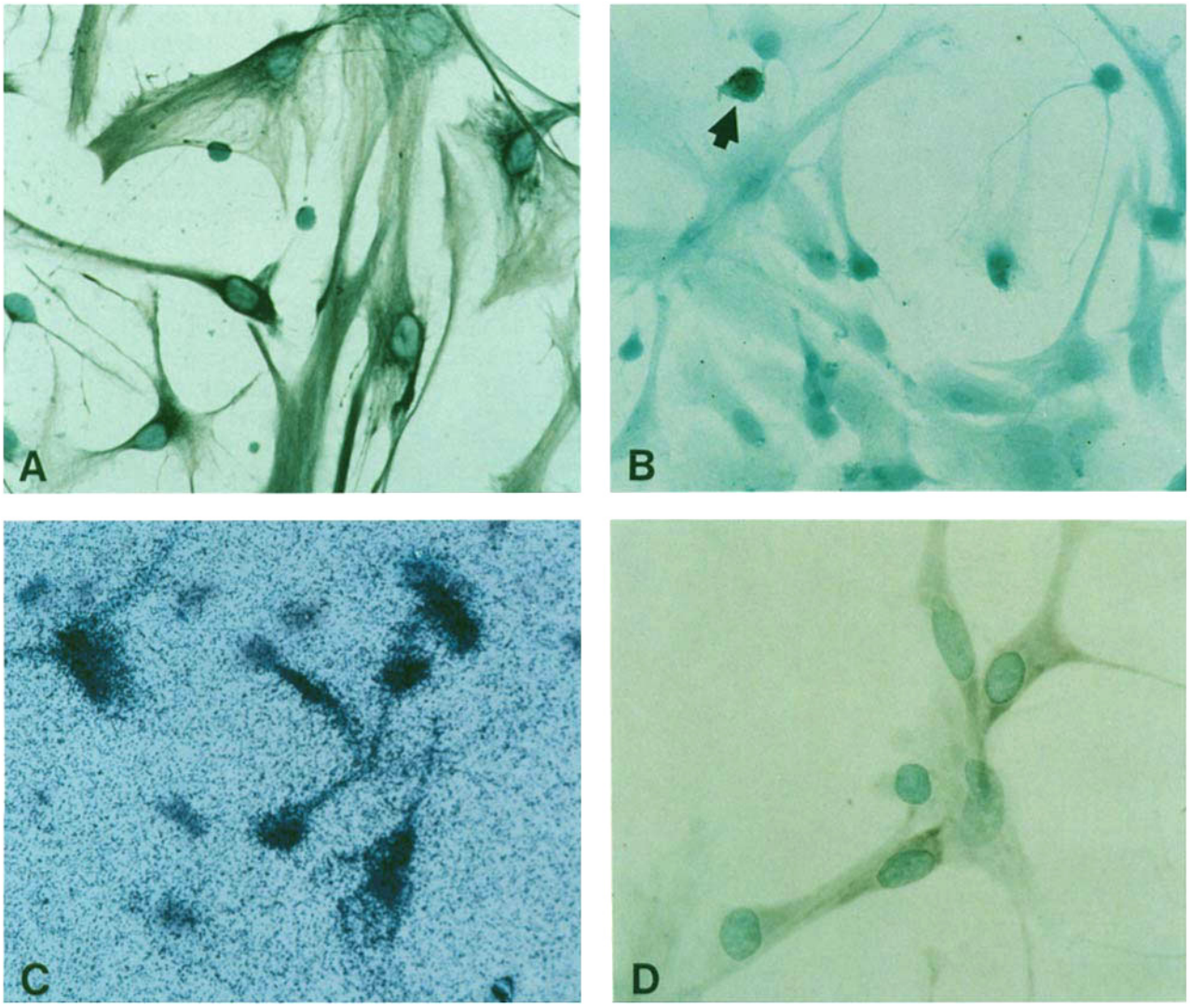

Figure 6. Identification of cells producing TGF-β in astrocyte cultures. Astrocyte cultures prepared as in Fig. 5 were stained by immunoperoxidase with an antibody to GFAP (*A*), mAb OX42 (*B*), and an antibody to TGF-β (*D*). Parallel cultures (*C*) were probed for TGF-β mRNA by in situ hybridization as described in Materials and Methods (original magnification, ×64).

cule and initiate signal transduction leading to transient stimulation of other monocyte functions including cytokine release (16, 17, 20, 21, 45). However, increased TGF-β may occur by other virally dependent mechanisms. Thus, the impact of the virus on monocyte cytokine production appears to be biphasic: an initial response to ligand-CD4 receptor signal transduction, and a subsequent upregulation during viral replication, possibly mediated by products of the viral genome.

Increased TGF-β secretion by HIV-1–infected monocytes in culture is consistent with the elevated levels of constitutively expressed TGF-β1 mRNA in mononuclear cells from AIDS patients (reference 46, McCartney-Francis, unpublished observations) and with the elevated circulating levels of TGF-β recently demonstrated in AIDS patients (47). These observations implicate monocyte-macrophage–derived TGF-β not only locally in the pathogenesis of AIDS-related encephalopathy, but also systemically in the development of immune dysregulation (25, 27, 48) characteristic of AIDS.

Although an association between viral transformation of cells and increased levels of TGF-β mRNA and polypeptide secretion has been observed in previous studies using Harvey or Maloney sarcoma-virus and feline sarcoma virus (49–51), to our knowledge, no one has previously reported that HIV-1 can induce TGF-β secretion. Subsequent to the induction of TGF-β in infected cells, the release of TGF-β by these cells likely promotes additional cytokine production in an autocrine and/or paracrine fashion (25, 39). Consequently, both resident and recruited monocytic populations localized in the brains of the AIDS patients, some infected with HIV-1, some not, may produce TGF-β. The paracrine actions of TGF-β may extend to other cell populations, including astrocytes, thereby amplifying the impact of the virus on surrounding neural cells, even if these neural cells do not support produc-

tive viral infection. Astrocytes also secrete cytokines, including TNF-α, which is neurotoxic and contributes to destruction of oligodendroglial cells and degeneration of the myelin sheath (22, 23, 52, 53). It appears increasingly probable that astrocytes exert an active influence on the pathogenesis of HIV CNS disease. Their ability to secrete TGF-β is in keeping with their other known capabilities, but adds a new dimension to their role in perpetuating the neuropathology associated with AIDS. The strategic location of astrocytes may enable local release of regulatory factors such as TGF-β to initiate recruitment of monocytes and microglia within the brain to local sites of infection and/or injury. The frequent perivascular distribution of HIV-1–infected macrophages is consistent with a migration pattern of the circulating monocytes from the vessels into the brain promoted by potent chemoattractants such as TGF-β.

Although TGF-β1 is not normally found in the adult human brain, it is present in rodent brain during embryogenesis (38) and may or may or not be expressed in the rodent adult brain (54, 55). Interestingly, even though TGF-β1 has a very limited expression in rodent brain tissues, both TGF-β2 and -β3 are abundant, suggesting unique regulatory and/or functional properties for this family of peptides (56). Since neither mRNA for TGF-β1 nor TGF-β2 has been detected by Northern hybridization of poly(A^+) RNA isolated from normal adult human brain (57), it appears that the presence of TGF-β is related to the events associated with AIDS-related neuropathology. After infection and/or injury to the adult brain, production of TGF-β and/or other cytokines, may contribute to inflammation and tissue repair. Moreover, the ability of HIV-1–infected mononuclear phagocyte products to stimulate aberrant astrocyte activities suggests a pivotal role for the monocytic cells once they reach the CNS in the initiation and perpetuation of the intracerebral pathology characteristic of AIDS.

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