Published in final edited form as:
Brain Res. 1992 August 7; 587(2): 216–225.

Enhanced expression of transforming growth factor β1 in the rat brain after a localized cerebral injury

Ann Logan[a,b], **Sally A. Frautschy**[a], **Ana-Maria Gonzalez**[a], **Michael B. Sporn**[c], and **Andrew Baird**[a]

[a]Department of Molecular and Cellular Growth Biology, The Whittier Institute for Diabetes and Endocrinology, La Jolla, CA 92037 (USA)

[b]Department of Clinical Chemistry, University of Birmingham, Edgbaston, Birmingham (UK)

[c]Laboratory of Chemoprecention, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 (USA)

Abstract

It is becoming clear that transforming growth factor β (TGFβ) may be a key factor regulating inflammatory and tissue specific wound responses. Because the formation of a glial-collagen scar at CNS lesion sites is thought to contribute to the pathology associated with penetrating CNS injuries, and because in the periphery TGFβ1 stimulates fibroblast deposition of scar tissue, we used in situ hybridization and immunohistochemistry to investigate the effect of a defined cerebral lesion on the local expression of TGFβ1. Induction of TGFβ1 mRNA and protein is relatively diffuse in the neuropile around the margins of the lesion at 1, 2 and 3 days, but becomes localized to the region of the glial scar at 7 and 14 days. The signal intensity for TGFβ1 mRNA and protein is maximal between 2 and 3 days and decreases between 7 and 14 days after lesion. The predominant cell types in the neuropile localizing TGFβ1 mRNA and protein have the morphological characteristics of astrocytes, although macrophages are also detected. An induction of TGFβ1 mRNA was also observed in endothelial cells of the meninges, hippocampal fissure and choroid plexus, at 2 and 3 days, However, this is dramatically reduced by 7 days and has disappeared by 14 days. These results suggest a role for TGPβ1, not only in inflammation, but also in the tissue-specific glial scar formation that occurs in the CNS. Furthermore, they suggest a potential therapeutic use of TGFβ1 antagonists in the CNS to help limit the pathogenesis associated with matrix deposition in the wound.

Keywords

Transforming growth factor β1; mRNA; Injury; Central nervous system; Glial scar; Astrocyte

Correspondence: A. Logan, Department of Clinical Chemistry. The Wolfson Research Laboratories, Queen Elizabeth Medical Centre, Edgbaston, Birmingham B15 2TH, UK, Fax: (44) (021) 472 0499.

Preliminary results of this investigation were presented at the Keystone Symposia entitled ‘FGF, endothelial cell growth factors and angiogenesis’ and ‘Wound healing’ in April 1991 and published in abstract form in the J. of Cell. Biochem., Suppl 15F.

INTRODUCTION

Injury to the adult mammalian central nervous system (CNS) generates a cascade of cellular events including recruitment of inflammatory cells into the lesion, transient abortive regeneration of damaged neurones, astrocyte proliferation and angiogenesis. The blood-brain barrier is reformed by the tight association of astrocyte end-feet at the wound margins, to make a glia limitans externa which encloses a core of matrix molecules laid down by fibroblasts recruited from the meninges[24]. The glial-mesodermal scar provides a physical barrier that any regenerating neurones have to penetrate in order to re-establish functional connections with their target organs. The limited ability of the adult CNS to recover function after injury relates to unsuccessful nerve regeneration and deposition of a dense, permanent fibrotic scar [5,21].

Although the mechanisms responsible for post-traumatic CNS degeneration are not defined, interacting growth factors are thought to play crucial roles in determining the outcome of CNS injury. Indeed, there have been a number of recent reports suggesting the involvement of several growth factors, including basic fibroblast growth factor (FGF) [11,22], acidic FGF [20], transforming growth factor β1 (TGFβ1)[17,28], and interleukins 1 and 2 [13,29]. Some of these factors may play important neurotrophic roles [1,6,30,43,49] but their role in scar formation has not been studied. While it remains unclear which components of the trophic and cellular responses are helpful and which harmful to functional recovery of the CNS, the agents which promote scar formation presumably impede neuronal regeneration and the establishment of new synaptic connections [5,21,35].

In addition to the reported effects of TGFβ1 as an inflammatory mediator [51], this factor also influences endothelial cells [4,9,31], neurones [6] and glia [7,18,39,41]. TGFβ1 is a potent desmoplastic agent in peripheral tissues [37]. It is an important regulator of extracellular matrix deposition by fibroblasts, increasing collagen, fibronectin and proteoglycan expression [14,34] and stimulating collagen-matrix contraction [27]. It also increases integrin expression [14], decreases the synthesis of proteases which degrade extracellular matrix components such as collagenase [8] and transin [23] and increases the expression of protease inhibitors [8]. Thus, many of the activities of TGFβ1 are directed towards the production and deposition of fibrous scar tissue within peripheral wounds.

Normally, there are low levels of TGFβ1 in the CNS [26,46,50], but since TGFβ1 is released from platelets at the site of injured blood vessels [2] and is also expressed by macrophages [15], it is likely that breakage of the blood-brain barrier will allow entry of TGFβ1 protein into the CNS. We reasoned that after a penetrating CNS lesion, in addition to its action as a mediator of the inflammatory response, TGFβ1 may influence gliosis and deposition of the extracellular matrix of the scar and predicted that this factor may be expressed locally in neural tissue following a penetrating wound. Of direct relevance, TGFβ1 mRNA has been found to increase in the ipsilateral hippocampus following entorhinal cortex lesions [28] and after ischaemic injury [16] supporting our hypothesis that locally-produced TGFβ1 may regulate some of the tissue-specific responses of microvessels, fibroblasts, microglial/macrophages and neurones after injury. However, precisely which cells in the CNS synthesize TGFβ1 after injury and how the response differs depending on the type of injury

have not been established. Therefore, we used immunohistochemistry and in situ hybridization in a rat model of penetrating CNS injury to investigate the possibility that TGFβ1 mRNA and protein are induced locally at wound sites by injury-responsive cells.

MATERIALS AND METHODS

Materials

All reagents not specified were analytical grade from Sigma Chemical Co., St. Louis, MO. Radioisotopes were obtained from Amersham International, Arlington Heights, IL.

Animals and surgery

Surgical and animal care procedures were carried out with strict adherence to the guidelines set out in the 'NIH guide for the care and use of laboratory animals', National Institutes of Health Publications no. 80–23.

Groups of adult, male Sprague–Dawley rats (200 g) were anaesthetized (i.m.) with a mixture of acepromazine (1.875 mg/kg), ketamine (3.75 mg/kg) and xylazine (1.9 mg/kg). A stereotactically defined, 4-mm deep rostro-caudal knife-wound incision was made vertically into the occipital cortex, corpus callosum and presubiculum [22]. At 1, 2, 3, 7 and 14 days after surgery, animals (n = 4/group) were put under deep anaesthesia with the same anaesthetic and perfused transcardially with 300 ml of 0.9% (w/v) saline, 250 ml of 4% (w/v) paraformaldehyde (PFA) in 0.1 M acetate buffer, pH 6.5, followed by 500 ml of 4% (w/v) PFA plus 0.05% (w/v) glutaraldehyde in 0.1 M borate buffer, pH 9.5, using the pH shift method [45].

Histology

For in situ and immunocytochemical studies, the perfusion-fixed brains were post-fixed overnight at 4°C in 4% (w/v) PFA in 0.1 M borate buffer containing 10% (w/v) sucrose. They were then rapidly frozen on powdered dry ice in Tissue Tek OCT compound (Miles Laboratories, Napeville, IL) and stored at −80°C. Frozen sections (20 μm) were mounted on poly-L-lysine coated slides, air-dried and stored at −80°C until use.

In situ hybridization

The methods used for the cloning of rat TGFβ1 are described elsewhere [33]. In situ hybridization of TGFβ1 used the *Hind* III-*Xba*I fragment of 0.985 kbp, derived from the major coding region of the rat TGFβ1 precursor, which was subcloned into pBluescript SK^+ (Stratagene, San Diego, CA). The antisense strand of the coding sequence was transcribed using T7 polymerase. A [^{35}S]UTP-labelled RNA probe encoding the sense strand of 5′ non-coding sequence was prepared with T3 RNA polymerase and used for control tissue sections.

Mounted frozen sections of brain, prepared as described in the histology section of Materials and Methods, were digested with 10 μg/ml of proteinase K in 0.1 M Tris (pH 8.0) containing 50 mM EDTA at 37°C for 30 min. Sections were rinsed in deionized water followed by incubation in 0.1 M triethanolamine (TEA), pH 8.0, for 3 min. Sections were then acetylated

for 10 min with 0.25% (w/v) acetic anhydride in 0.1 M TEA, rinsed in 2 ×SSC, dehydrated through a graded series of ethanol washes and air-dried under vacuum for 2 h before hybridization.

Hybridizations with labelled TGFβ1 antisense or sense probes (1 × 10 cpm/ml) were performed at 55°C overnight in 10 mM Tris (pH 8.0) containing 50% (w/v) formamide, 0.3 M NaCl, 1 mM EDTA, 10 mM dithiothreitol (DTT), 1 × Denhardt's solution and 10% (w/v) dextran sulphate. After hybridization, sections were rinsed for 1 h in 4 ×SSC and treated with 25 μg/ml ribonuclease A in 10 mM Tris (pH 8.0) containing 0.5 M NaCl and 1 mM EDTA at 37°C for 30 min. This was followed by increasing high stringency washes of SSC containing 1 mM DTT, finishing with 0.1 ×SSC at 65°C for 30 min.

Slides were then dehydrated through a graded series of ethanol, dried under vacuum and then exposed to βmax hyperfilm (Amersham) for 5 days to examine gross changes in mRNA. For microscopic analysis, slides were exposed to Kodak NTB-2 liquid autoradiographic emulsion for 3 weeks at 4°C, processed with Kodak D19 developer, rinsed and fixed with Kodak rapid fixer. The slides were rinsed for 30 min in tap water, counterstained with Harris' haematoxylin and examined by dark field and bright field microscopy.

Immunoperoxidase staining

The primary antiserum raised against TGFβ1 has been previously described and characterized [10]. It is an IgG fraction of a rabbit polyclonal, raised against amino acids 1-30 of rat TGFβ1, which was purified by passage over a Protein A-Sepharose column.

Immunoperoxidase staining for TGFβ1 in 20-μm frozen sections of brain used the ABC Vectastain Elite kit (Vector Laboratories, Burlingame, CA). The tissue sections were washed in PBS and the endogenous peroxidase quenched by incubating with 0.3% (v/v) hydrogen peroxide in PBS for 30 min. The sections were rinsed in PBS and incubated in 1.5% (v/v) goat serum, diluted in PBS containing 0.3% (v/v) Triton X-100, for 30 min to reduce non-specific staining.

After a 24-h incubation at 4°C with the protein-A purified primary antibody (0.015 mg/ml), diluted in PBS supplemented with 0.3% (v/v) Triton X and 1% (v/v) BSA, the sections were treated with a 1:200 dilution of biotinylated goat anti-rabbit IgG (Vector) for 1 h. This was followed by a 30-min incubation with a biotin-avidin-peroxidase complex (Vector). Finally, the sections were treated for 5 min with 0.5 mg/ml diaminobenzidine in PBS containing 0.01% (v/v) hydrogen peroxide. All steps were separated by buffer washes consisting of PBS with 0.3% (vol/vol) Triton X-100.

The sections were finally washed in PBS. counterstained with Harris' haematoxylin, dehydrated, cleared and mounted. Sections incubated with anti-TGFβ1 antiserum preincubated with recombinant TGFβ1 or without primary antisera were used as controls. Sections processed with the control procedures failed to stain.

RESULTS

General properties of the lesion

Haematoxylin staining of sections through the lesion site at different days after surgery shows the typical transformation of the wound from an open cavity to a closed glial/mesodermal scar (Fig. 1) in both the rostral (Fig. 1A, B) and caudal (Fig. 1C, D) portions of the lesion. The incision is seen to have penetrated the cortex and the corpus callosum and passed through the ventricle, medial to the hippocampal formation. After 7 days, the migration and proliferation of glial cells has produced a well-defined glia limitans which defines the wound margin, and surrounds a core of mesodermal, fibrous elements. Glial proliferation through the neuropile appears especially extensive in the corpus callosum. The contraction of the scar tissue is particularly evident at 7 days (Fig. 1B).

TGFβ1 mRNA and the margin of the lesion

The TGFβ1 probe recognizes the expected major 2.5-kb band when the injured brains are processed for Northern blot hybridization (not shown). The time course of the TGFβ1 mRNA response in the rostral region of the wound was assessed by in situ hybridization and is shown in Fig. 2. In this study signal for TGFβ1 mRNA is very low or negligible in sections through similar regions of the unlesioned adult rat brain (not shown). A clear increase in TGFβ1 mRNA signal is observed in sections from lesioned animals at 1, 2, 3, 7 and 14 days post-lesion (Fig. $2A_1$–E_1). This increase is apparent when the level of signal in sections from lesioned animals is compared to the signal observed in sections taken through the same region of unlesioned brain and processed simultaneously and identically (not shown), or in alternate lesion sections hybridized with the TGF β1 sense strand probe (Fig. $2A_2$–E_2).

By in situ hybridization, the TGFβ1 mRNA signal is most intense at 2 and 3 days post-lesion (Fig. $2B_1$–C_1). Interestingly, while the signal is relatively diffuse at 1, 2 and 3 days, it becomes focal when the glia limitans is formed after 7 days. The meninges and presumptive astrocytes (by morphological criteria) adjacent to the site of incision, show intensive TGFβ1 mRNA hybridization at 1, 2 and 3 days but the signal has diminished at 7 and 14 days.

The response is very similar in the caudal region of the wound and is presented in Fig. 3. A marked induction of TGFβ1 mRNA in the choroid plexus is observed in these sections at 1, 2, and 3 days, and has diminished by 7 days (Fig. $3A_1$–E_1). When adjacent sections are hybridized with sense probe, the signal is negligible (Fig. $3A_2$–E_2), As in the rostral part of the wound, the signal for mRNA has localized to the glia limitans at 7 days. Signal in all caudal regions of the wound has diminished by 14 days post-lesion.

The time course of the TGFβ1 mRNA response in cells on the margin of the wound is shown with high magnification bright field micrographs in Fig. 4. At 1 and 2 days post-lesion, cells at the wound margin are almost ubiquitously labelled, and these include some neurones (large pale nuclei) (Fig. 4A, B). Cells of glial origin (small dark nuclei) are densely labelled with silver grains 2 days after lesion (Fig. 4C-E). By 14 days few cells are labelled at the margin (Fig. 4E). Adjacent sections hybridized with the sense strand show minimal signal (Fig. $4A_3$–E_3).

TGFβ1 mRNA and the corpus callosum

At low magnification in bright field view, an extensive distribution of dense signal for TGFβ1 mRNA is seen through the corpus callosum after 7 days (Fig. 5A), but no labelling is detected with the sense probe (Fig. 5B, D). Based on morphology, location and staining properties, higher magnification suggests that the labelled cells may be of glial origin (Fig. 5C).

TGFβ1 mRNA and microvasculature

In situ hybridization of TGFβ1 mRNA following the cortical wound demonstrates a clear association between TGFβ1 mRNA and cells of the microvasculature. Low or negligible message is detected in the normal adult rat brain in this experiment. At 2 and 3 days after injury, TGFβ1 mRNA is detected in the meninges and in the endothelium of the hippocampal fissure and choroid plexus (Fig. 6); this signal is significantly diminished by 7 and 14 days post lesion (Fig. 3). *Meninges.* In the meninges, sections hybridized with the antisense probe to TGFβ1 mRNA show signal in cells containing darkly stained round nuclei (Fig. $6A_1$). No signal is present when sections are hybridized with sense probe (Fig. $6A_2$).

Hippocampus—While no TGFβ1 mRNA is present in the hippocampus of unlesioned animals, TGFβ1 mRNA is visible in the endothelial cells of the hippocampal fissure but not in adjacent sections hybridized with the sense strand (Fig. 6B).

Choroid plexus—Low to negligible levels of TGFβ1 mRNA are present in the choroid plexus of unlesioned animals (Fig. 6C). This is in striking contrast to the lesioned rats in which a dramatic and extensive induction of TGFβ1 mRNA is observed in the choroid plexus (Fig. 6D). Labelled arteriole endothelial cells are seen in sections hybridized with antisense probe but not in sections hybridized with the control sense probe (Fig. 6E).

TGFβ1 immunohistochemistry

Immunocytochemical localization of TGFβ1 demonstrates a distribution pattern similar to that of its mRNA (Fig. 7). In this study no immunoreactivity is apparent in the normal rat brain (not shown). The predominant cell type that appears to contain TGFβ1 after injury has the appearance of astrocytes that are limited to the tissue bordering the glia limitans (Fig. 7). However, immunopositive macrophage-like cells are visible at all time points examined. The time course of the TGFβ1 protein response is very similar to that of its mRNA, immunoreactivity reaches a relative peak 3 days after lesion and is considerably diminished by 14 days. At this time point the staining is effectively restricted to the residual cells that have, at higher magnification (not shown), all the morphological characteristics of macrophages.

DISCUSSION

These results confirm the observations of others [26,46,50] that, in vivo, TGFβ1 expression is normally low in the adult CNS. However, there is an immediate and dramatic induction of TGFβ1 expression locally within the neuropile after a penetrating cerebral injury, evidenced

by increased TGFβ1 mRNA and immunoreactive protein. Furthermore, the results demonstrate that TGFβ1 mRNA expression in the choroid plexus and meninges is also markedly increased after CNS lesion.

Enhanced signal for TGFβ1 mRNA is detected in the neural tissue at the margins of the lesion throughout the 1–14-day response period examined, which peaks at 2 days but appears much reduced at 14 days. This transient response is paralleled by immunocyto-chemical localization of TGFβ1 protein, which may arise both from the local release of TGFβ1 by platelets and macrophages recruited into the wound, and from de novo synthesis of TGFβ1 by neural tissues. A recent report by others demonstrated a similar transient elevation of TGFβ1 mRNA (measured by northern analysis) in the hippocampus after an entorhinal cortex lesion, with maximal levels occurring 4–6 days after lesion [28]. The differences in timing of the peak levels may be a result of differences in the time courses of the cellular responses to a discrete cortical wound compared to those that occur by deafferentation.

Based on the known functions of TGFβ1 and on the cellular responses to this type of cortical lesion, the results suggest that TGFβ1, released into the marginal tissues of a CNS wound, may have a number of potential activities. Thus, TGFβ1 released by platelets, neutrophils, activated monocytes, macrophages and lymphocytes may initially regulate the inflammatory process by actions on the recruitment, priming and activation of these cells in an autocrine or paracrine manner (reviewed in ref. 52). The observations of immunopositive cells of the monocyte/macrophage lineage within the lesion site at all post-injury time-points examined in this experiment supports this immunoregulatory role for TGFβ1. The ability of TGFβ1 to up-regulate both its own gene expression and the expression of other growth factor genes suggests a mechanism for the initiation and amplification of its signal within target tissues [25]. This response may underlie the local induction of TGFβ1 expression that is observed in damaged CNS tissue.

The many possible roles for TGFβ1 after CNS injury include chemoattraction of microglia [53], which rapidly migrate to the site of CNS injury [13]. Some of these microglia may originate from cells of the monocyte/macrophage lineage [19]. The migration and proliferation of microglia appears critical for subsequent proliferation of astrocytes, and both these cell types appear to secrete multiple growth factors that regulate the many responses to injury, including neuronal sprouting and endothelial cell proliferation [22]. TGFβ1 may also stimulate the release or synthesis of neurotoxic cytokines from macrophages or microglia [49].

The predominant cell-type localizing TGFβ1 and its mRNA in the wound margin has the features of the astrocyte, suggesting that this factor could act in a paracrine or autocrine manner to stimulate astroglia after injury. Astrocytes proliferate and migrate in injury-responsive neural tissue and eventually form a glia limitans around the wound margins [24]. However, TGFβ1-expressing glial cells are not confined to the wound borders, but also coincide with the medio-lateral proliferation of astrocytes in the corpus callosum. Gliomas are reported to express TGFβ1, supporting the notion that glia are capable of TGFβ1 synthesis[41]. The action of TGFβ1 on astrocytes in vitro is very much dependent on the interaction with other growth factors and on the dosage used [7,17]. For example, in the

absence of other growth factors TGFβ1 inhibits growth, but will potentiate the mitogenic effect of basic FGF [17]. TGFβ1 may stimulate NGF synthesis in the CNS [18] and NGF is mitogenic for astrocytes [12] as well as neurotrophic [39]. TGFβ1 also stimulates glial fibrillary acidic protein [39] and cystatin C mRNA synthesis in astrocyte-like SFME (serum-free mouse embryo) cells [45], consistent with a role in glial differentiation. The association of TGFβ1 with glial cells and its actions as a potent inhibitor of glutamine synthetase implies that it may also impair some glial functions, interfering with their ability to support neurones [43] and thus contributing to the pathology observed after CNS injury.

The temporal and spatial change in TGFβ1 localization in the neuropile may also initiate the migration of fibroblasts into the wound, a phenomenon which occurs 4 days after injury [24]. TGFβ1 is a potent chemoat- tractant for fibroblasts to the site of injury [32] as well as controlling fibroblast deposition of extracellular matrix proteins and the synthesis of degradative enzymes (reviewed in ref. 36). A key role for TGFβ1 in the regulation of fibrosis at CNS wound sites is strongly suggested. The contraction of the scar tissue associated with the CNS injury observed here and in other studies after lesioning [5,24] is consistent with the role TGFβ1 is purported to play in the periphery [27].

In peripheral tissues, TGFβ1 also plays an important role in regulating angiogenesis [37] and in the CNS TGFβ1 may also regulate the neovascularization induced by injury in the damaged neuropile and in the deposited scar tissue [3]. This activity is supported by our observation of increased levels of TGFβ1 mRNA in vascular endothelial cells of the microvasculature of the tissue around the lesion site.

The transient increase in TGFβ1 mRNA observed in the meninges is of particular interest and may also relate to changes in the local microvasculature. This region is outside the blood-brain barrier and consists of blood vessels, fibroblasts and macrophages. The local induction of TGFβ1 in the meninges following injury may additionally play a role in promoting the early repair of the blood-brain barrier, by stimulating fibroblast migration and deposition of extracellular matrix proteins. The effect of TGFβ1 may depend on its interaction with other growth factors, especially basic FGF [4,9,31]. Therefore the absence of basic FGF in the meninges but not in the margin of the wound after injury [11,22] may signify different functions of TGFβ1 in these two regions.

As in the meninges, endothelial cells in the choroid plexus and hippocampal fissure also appear to transiently and strongly express TGFβ1 mRNA. The role of TGFβ1 in the choroid plexus is unknown, although it may involve attracting microglia which have phagocytized debris and are clearing it into the ventricle or vessels. Clearly, the CSF may serve as a route for the wider distribution of newly synthesized TGFβ1. TGFβ1 is present in the choroid plexus on both the ipsilateral and contralateral sides of the lesion. Whether the extensive bilateral distribution of TGFβ1 mRNA throughout the lateral ventricles is due to an injury-as-sociated soluble substance which circulates in the CSF and induces TGFβ1 mRNA in the ventricle or due to other factors (for example, interaction with microglia or nervous stimulation) is unclear.

Because the increase in TGFβ1 synthesis following a cortical knife wound is an early response to CNS injury, the physiological implications of these findings are particularly interesting. A role for TGFβ1 in the acute inflammatory response to injury is certainly indicated. Although TGFβ1 mRNA is induced in endothelial cells of the local neuropile, meninges, choroid plexus and hippocampal fissure, its function on the microvasculature in these situations remains to be defined, although an autocrine angiogenic activity could be implied. Of major importance, the distribution of TGFβ1 and its mRNA in the wound and the known roles of TGFβ1 in peripheral tissues are indicative of a role in formation of the glia limitans externa and the associated fibrous matrix of the CNS scar. Several investigators have suggested that the presence of a glial scar after CNS injury can have profound deleterious effects on neuronal survival and neuritic extensions. If the formation of the glial/mesodermal scar is dependent on the well-described ability of TGFβ1 to stimulate astroglia and matrix deposition, then the findings described here point to the therapeutic value of developing TGFβ1 antagonists to block matrix deposition and enhance the functional recovery from CNS injury and trauma.

Acknowledgments

Our thanks are particularly due to Kathleen Flanders for providing and characterizing excellent TGFβ1-related reagents. We also thank Rich Dahl. Mike Ong and James Farris for technical assistance. This work was supported by grants to A.L. from The International Spinal Research Trust. The Royal Society and the Wellcome Trust. Support was also provided hy NIH Grants DK-18811 and NS-28121, and the Whittier/Erbamont Angiogenesis Program to A.B.

References

1. Anderson KJ, Dam D, Lee S, Cotman CW. Basic fibroblast growth factor prevents death of lesioned cholinergic neurons in vivo. Nature. 1988; 332:360–361. [PubMed: 3352734]
2. Assoian RK, Komoriya A, Myers CA, Miller DM, Sporn MB. Transforming growth factor-β in human platelets. J Biol Chem. 1983; 258:7155–7160. [PubMed: 6602130]
3. Beck DW, Hart MN, Cancilla PA. The role of the macrophage in microvascular regeneration following brain injury. J Neuropathol Exp Neurol. 1983; 42:601–614. [PubMed: 6631454]
4. Bensaid M, Malecaze F, Bayard F, Tauber JP. Opposing effects of basic fibroblast growth factor and transforming growth factor-β on the proliferation of cultured bovine retinal capillary endothelial (BREC) cells. Exp Eye Res. 1989; 48:791–799. [PubMed: 2731575]
5. Berry M, Maxwell WL, Logan A, Mathewson A, McConnell P, Ashhurst DE, Thomas GH. Deposition of scar tissue in the central nervous system. Acta Neurochir Suppl. 1983; 32:31–35. [PubMed: 6581703]
6. Chalazonitis A, Kessler JA, Morrison RS. Transforming growth factors α and β in contrast to epidermal growth factor stimulate survival of sensory neurons in vitro. Soc Neurosci Abstr. 1989; 15:1361.
7. Eccleston PA, Jessen KR, Mirsky R. Transforming growth factor-β and G-interferon have dual effects on growth of peripheral glia. J Neurosci Res. 1989; 24:524–530. [PubMed: 2513415]
8. Edwards DR, Murphy G, Reynolds JJ, Whiham SE, Docherly AJP, Angel P, Heath JK. Transforming growth factor β modulates the expression of collagenase and metalloproteinase inhibitor. EMBO J. 1987; 6:1899–1904. [PubMed: 2820711]
9. Fafeur V, Terman BI, Blum J, Böhlen P. Basic FGF treatment of endothelial cells down-regulates the 85-kDa TGF β receptor subtype and decreases the growth inhibitory response to TGF-β1. Growth Factors. 1990; 3:237–245. [PubMed: 2173937]
10. Flanders KC, Thompson NL, Cissel DS, Obberghen-Schilling EV, Baker CC, Kass ME, Ellingsworth LR, Roberts AB, Sporn MB. Transforming growth factor-β1: histochemical

localization with antibodies to different epitopes. J Cell Biol. 1989; 108:653–660. [PubMed: 2465297]

11. Frautschy SA, Walicke PA, Baird A. Localization of basic FGF and its mRNA after CNS injury. Brain Res. 1991; 553:291–299. [PubMed: 1933286]
12. Furukawa S, Furukawa Y, Satoyoshi E, Hayashi K. Synthesis and secretion of nerve growth factor by mouse astroglial cells in culture. Biochem Biophys Res Commun. 1986; 136:57–63. [PubMed: 2423082]
13. Giulian D, Chen J, Ingeman JE, George JK, Noponen M. The role of mononuclear phagocytes in wound healing after traumatic injury to adult mammalian brain. J Neurosci. 1989; 9:4416–4429. [PubMed: 2480402]
14. Ignotz RA, Massague J. Transforming growth factor-β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem. 1986; 261:4337–4345. [PubMed: 3456347]
15. Khalil N, Bereznay O, Sporn M, Greenberg AH. Macrophage production of transforming growth factor β and fibroblast collagen synthesis in chronic pulmonary inflammation. J Exp Med. 1989; 170:727–737. [PubMed: 2475572]
16. Klempt ND, Williams C, Sirimanne E, Gluckman PD. Transforming growth factor β expression in the rat brain is markedly increased after a hypoxic-ischemic insult. Proc 73rd Ann Mtg, The Endocr Soc. 1991:1809.
17. Labourdette G, Janet T, Laeng P, Perraud F, Lawrence D. Transforming growth factor type β1 modulates the effects of basic fibroblast growth factor on growth and phenotypic expression of rat astroblasts in vitro. J Cell Physiol. 1990; 144:473–484. [PubMed: 1975257]
18. Lindholm D, Hengerer B, Zafra F, Thoenen H. Transforming growth factor-β1 stimulates expression of nerve growth factor in the rat CNS. NeuroReport. 1990; 1:9–12. [PubMed: 2129862]
19. Lindsay, RM. Astrocytes cell biology and pathology of astrocytes. Academic Press; London:
20. Logan A. Increased levels of acidic fibroblast growth factor mRNA in the lesioned rat brain. Mol Cell, Endocrinol. 1988; 58:275–278. [PubMed: 2463193]
21. Logan A. Growth factors in the central nervous system. Br J Hosp, Med. 1990; 43:428–437. [PubMed: 2194623]
22. Logan A, Frautschy SA, Gonzalez AM, Baird A. A time course for the focal elevation of synthesis of basic fibroblast growth factor and one of its high affinity receptors (fig) following a localized cortical brain injury. J Neurosci. in press.
23. Matrisian LM, Leroy P, Ruhlmann C, Gesnel M-C, Breathnach R. Isolation of the oncogene and epidermal growth factor-induced transin gene: complex control in rat fibroblasts. Mol Cell Biol. 1986; 6:1679–1686. [PubMed: 2431284]
24. Maxwell WL, Follows R, Ashhurst DE, Berry M. The response of the cerebral hemisphere of the rat to injury, I. The mature rat. Philos Trans R Soc London Ser B. 1990; 328:479–499. [PubMed: 1974074]
25. McCartney-Francis N, Mizel D, Wong H, Wahl L, Wahl S. TGF-β regulates production of growth factors and TGF-β by human peripheral blood monocytes. Growth Factors. 1990; 4:27–35. [PubMed: 1707635]
26. Miller DA, Lee A, Matsui Y, Chen EY, Moses HL, Derynck R. Complementary DNA cloning of the murine transforming growth factor β3 (TGFβ3) precursor and the comparative expression of TGFβ3 and TGFβ1 messenger RNA in murine embryos and adult tissues. Mol Endocrinol. 1989; 3:1926–1934. [PubMed: 2628730]
27. Montesano R, Orci L. Transforming growth factor β stimulates collagen-matrix contraction by fibroblasts: implications for wound healing. Proc Natl Acad Sci USA. 1988; 85:4894–4897. [PubMed: 3164478]
28. Nichols NR, Laping NJ, Day JR, Finch CE. Increases in transforming growth factor-β mRNA in hippocampus during response to entorhinal cortex lesions in intact and adrenalectomized rats. J Neurosci Res. 1991; 28:134–139. [PubMed: 2041055]
29. Nieto-Sampedro M, Chandy KG. Interleukin-2-like activity in injured rat brain. Neurochem Res. 1987; 12:723–727. [PubMed: 3114659]

30. Otto D, Frotscher M, Unsicker K. Basic fibroblast growth factor and nerve growth factor administered in gel foam rescue medial septal neurons after fimbria fornix transection. J Neurosci Res. 1989; 22:83–91. [PubMed: 2926842]
31. Pepper MS, Belin D, Montesano R, Orci L, Vassalli JD. Transforming growth factor-β1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells in vitro. J Cell Biol. 1990; 111:743–755. [PubMed: 1696269]
32. Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor β. J Exp Med. 1987; 165:251–256. [PubMed: 3491869]
33. Qian SW, Kondaiah P, Roberts AB, Sporn MB. cDNA cloning by PCR of rat transforming growth factor β-1. Nucleic Acids Res. 1990; 18:3059. [PubMed: 2349108]
34. Raghow R, Postlethwaite AE, Keski-Oga J, Moses HL, Kang AH. Transforming growth factor-β increases steady state levels of type I procollagen and fibronectin messenger RNAs posttranscriptionally in cultured human dermal fibroblasts. J Clin Invest. 1987; 79:1285–1288. [PubMed: 3470308]
35. Reier, PJ.; Stensaas, LJ.; Guth, L. The astrocyte scar as an impediment of regeneration in central nervous system. In: Kan, CG.; Bunge, RP.; Reier, PJ., editors. Spinal Cord Reconstruction. Raven; New York: 1983. p. 163-195.
36. Roberts, AB.; Sporn, MB. The transforming growth factor-βs. In: Sporn, MB.; Roberts, AB., editors. Peptide Growth Factors and Their Receptors. I. Handbook of Experimental Pharmacology. Vol. 95. Springer; Berlin: 1990. p. 419-472.
37. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS. Transforming growth factor type-β: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci USA. 1986; 83:4167–4171. [PubMed: 2424019]
38. Rosenberg MB, Friedmann T, Robertson RC, Tsuszynski M, Wolff JA, Breakefield XO, Gage FH. Grafting genetically modified cells to the damaged brain: restorative effects of NGF expression. Science. 1988; 242:1575–1578. [PubMed: 3201248]
39. Sakai Y, Rawson C, Lindberg K, Barnes D. Serum and transforming growth factor β regulute glial fibrillary acidic protein in serum-free-derived mouse embryo cells. Proc Natl Acad Sci USA. 1990; 87:8378–8382. [PubMed: 1700431]
40. Samuels V, Barrett JM, Bockman S, Pantazis CG, Allen MB Jr. Immunocytochemical study of transforming growth factor expression in benign and malignant gliomas. Am J Pathol. 1989; 134:895–902.
41. Schluesener HJ. Transforming growth factors type β1 and β2 suppress rat astrocyte autoantigen presentation and antagonize hyperinduction of class II major histocompatibility complex antigen expression by interferon-gamma and tumor necrosis factor-α. J Neuroimmunol. 1990; 27:41–47. [PubMed: 2108188]
42. Schwartz M, Cohen A, Stein-Izak C, Belkin M. Dichotomy of the glial cell response to axonal injury and regeneration. FASEB J. 1989; 3:2371–2378. [PubMed: 2676680]
43. Sievers J, Hausmann B, Unsicker K, Berry M. Fibroblast growth factors promote the survival of adult rat retinal ganglion cells after transection of the optic nerve. Neurosci Lett. 1987; 76:157–162. [PubMed: 3587749]
44. Simmons DM, Arriza JL, Swanson LW. A complete protocol for in situ hybridization of messenger RNAs in brain and other tissues with radiolabeled single-stranded RNA probes. J Histotechnol. 1989; 12:169–181.
45. Solem M, Rawson C, Lindburg K, Barnes D. Transforming growth factor β regulates cystatin C in serum-free mouse embryo (SFME) cells. Biochem Biophys Res Commun. 1990; 172:945–951. [PubMed: 2241983]
46. Unsicker K, Flanders KC, Cissel DS, Marascalo S, Layfyatis R, Roberts AB, Sporn MB. TGF-β isoforms in the central and peripheral nervous system. Soc Neurosci Abstr. 1990; 16:823.
47. Wahl SM, Allen JB, McCartney-Francis N, Morganti-Kossman MC, Ellingsworth L, Mai UE, Mergenhagen SE, Orenstein JM. Macrophage- and astrocyte-derived transforming growth factor β as a mediator of central nervous system dysfunction in acquired immune deficiency syndrome. J Exp Med. 1991; 173:981–991. [PubMed: 2007861]

48. Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Roberts AB, Sporn MB. Transforming growth factor β (TGF-β) induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci USA. 1987; 84:5788–5792. [PubMed: 2886992]

49. Walicke P, Cowen WM, Ueno N, Baird A, Guillemin R. Fibroblast growth factor promotes the survival of dissociated hippocampal neurones and enhances neurite extension. Proc Natl Acad Sci USA. 1986; 83:3012–3016. [PubMed: 3458259]

50. Wilcox JN, Derynck R. Developmental expression of transforming growth factors alpha and beta in mouse foetus. Mol Cell Biol. 1988; 8:3415–3422. [PubMed: 3211146]

51. Wong, HL.; Wahl, SM. Inflammation and repair. In: Sporn, MB.; Roberts, AB., editors. Peptide Growth Factors and Their Receptors, II, Handbook of Experimental Pharmacology. Vol. 95. Springer; Heidelberg: 1990. p. 509-548.

52. Yao J, Harvath L, Gilbert DL, Colton CA. Chemotaxis by a CNS macrophage, the microglia. J Neurosci Res. 1990; 27:36–42. [PubMed: 2254955]

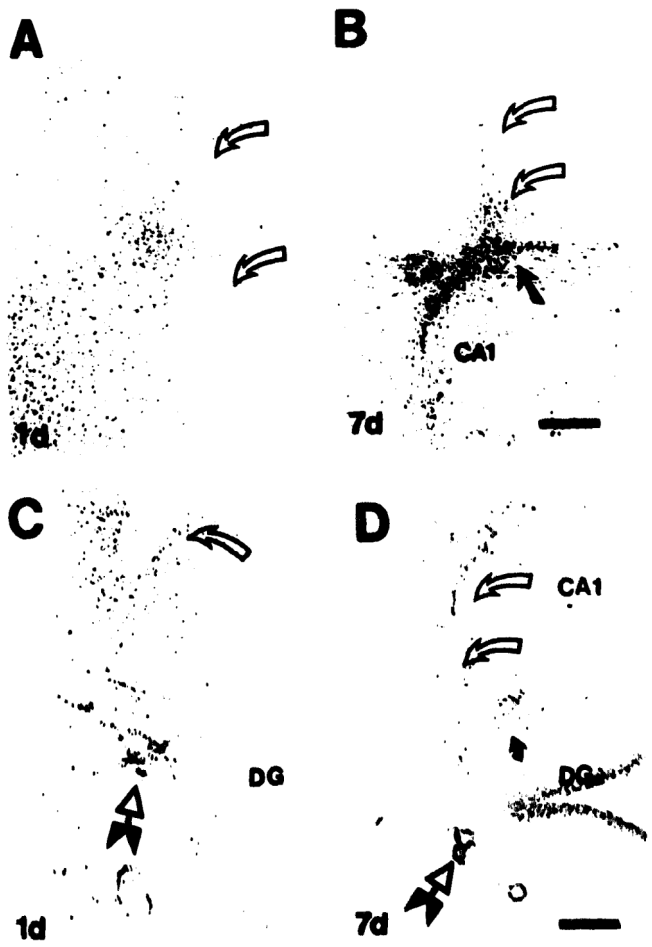

Fig. 1.

Bright field micrograph of the brain 1 day and 7 days after injury. The caudal (panels A and B) and rostral (panels C, D) portions of the lesion are shown. The curved open arrows show the region of the lesion in the cortex at 1 day (panels A, C) and 7 days (panels B and D) after the lesion. Bar = 250 μm. Note that the glial scar is well established by 7 days. The corpus callosum (large closed arrow), hippocampal fissure (closed curved arrow), the choroid plexus (straight open arrow with tail), dentate gyrus (DG) and CA1 region of the hippocampus are shown for orientation.

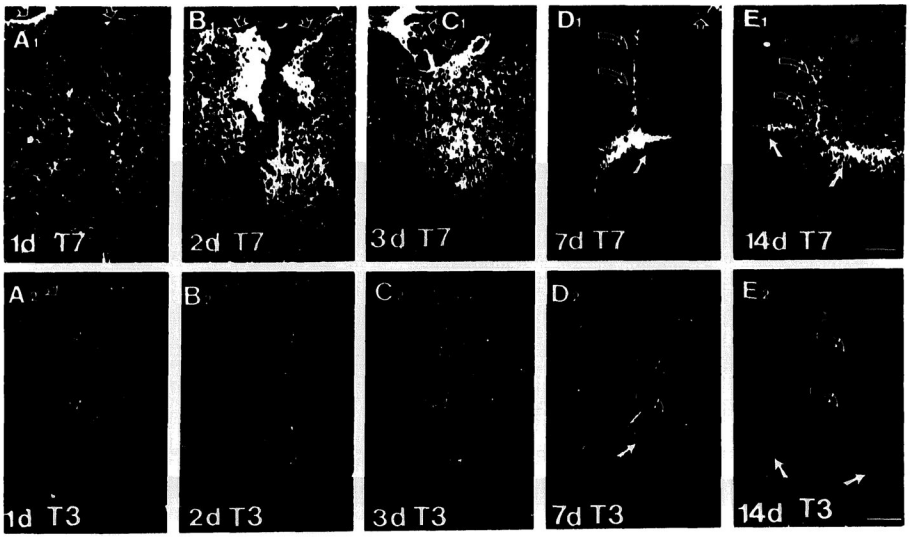

Fig. 2.

Dark field micrographs showing TGFβ1 mRNA in the caudal region of the wound. TGFβ1 mRNA (T7) is observed at 1 day (panel A_1), 2 days (panel B_1), 3 days (panel C_1), 7 days (panel D_1) and 14 days (panel E_1) following the lesion. When sections are hybridized with the sense strand (T3) the signal is minimal (panels A_2–E_2). The curved open arrows show the margin of the lesion. TGFβ1 mRNA, but not the control signal, is also observed in the corpus callosum (panels D_1 and D_2) as shown by the closed straight arrows and in the meninges (panels A_1–D_1 and A_2–E_2) as shown by straight open arrows. The signal is most intense at 2 and 3 days after lesion. At 7 days and 14 days the signal is less diffuse and only present within the boundary of the well defined glial scar. Bar = 250 μm.

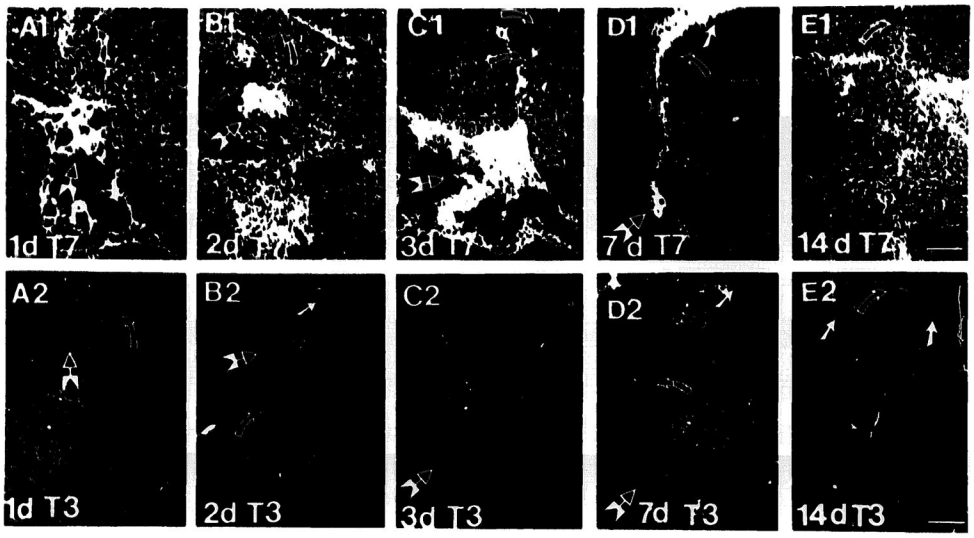

Fig. 3.

Dark field micrographs showing TGFβ1 mRNA in the rostral region of the wound. TGFβ1 mRNA (T7) is observed at 1 day (panel A_1), 2 days (panel B_2). 3 days (panel C_1). 7 days (panel D_1)and 14 days (panel E_1) following the lesion. When sections are hybridized with the sense strand (T3) the signal is minimal (panels A_2–E_2). The curved open arrows show the margin of the lesion. TGFβ1 mRNA, but not the control signal, is also observed in the corpus callosum (panels B_1, D_1, E_1 and B_2, D_2, E_2) as shown by the closed straight arrows and in the choroid plexus (panels A_1–D_1 and A_2–E_2) as shown by straight open arrows with tails. The signal appears considerably diminished by 14 days.

Bar = 250 μm.

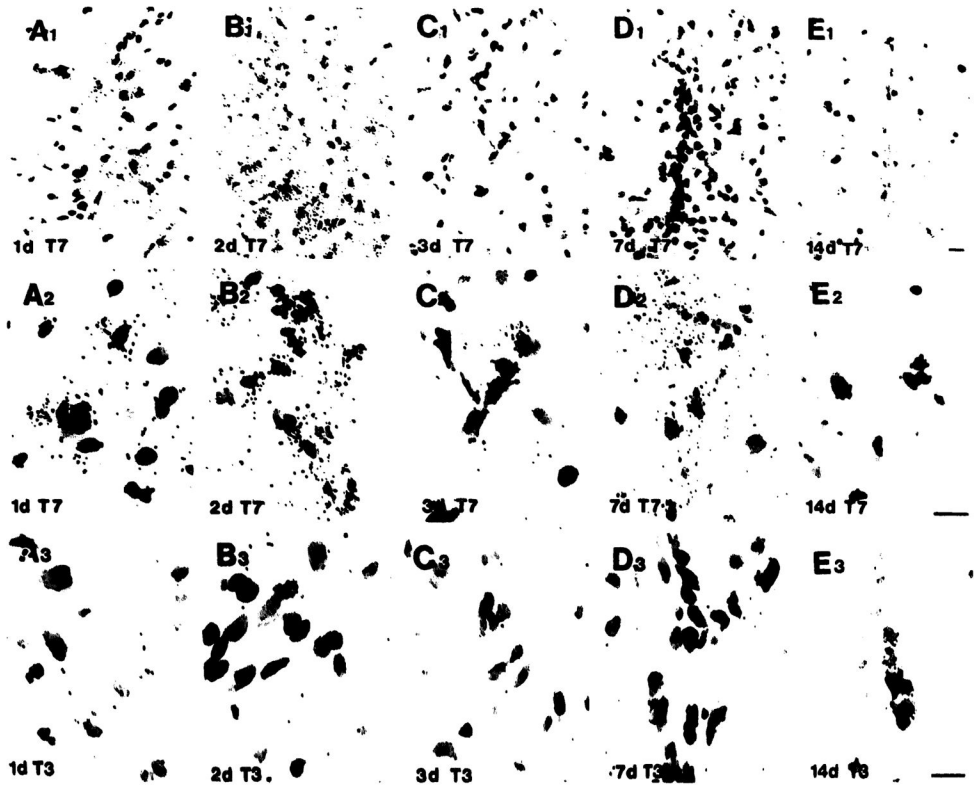

Fig. 4.

High magnification bright field micrographs of cells in the margin of the wound. TGFβ1 mRNA (T7) is observed associated with selective cells in the margin of the lesion at 1 day, (panel A) 2 days (panel B), 3 days (panel C), 7 days (panel D) and 14 days (panel E) after the lesion but not in cells hybridized with the sense strand (T3, row 3). Initial signs of the glial scar are seen at 3 days and are well defined by 7 days, at which time the mRNA appears less diffuse. High magnification reveals that these cells are typically glial cells (row 2). Bar = 10 μm.

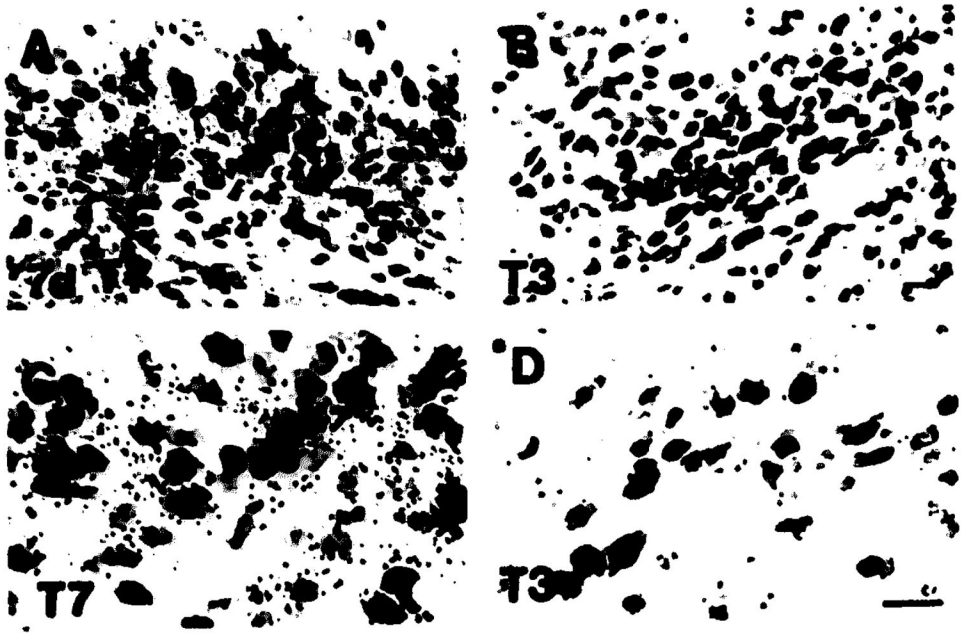

Fig. 5.

The presence of TGFβ1 mRNA in the corpus callosum 7 days following a defined lesion. There is an intense signal for TGFβ1 mRNA (Panels A and C) in the corpus callosum at the point of lesion penetration. Control sections show no signal (T3, panels B and D). Bar = 10 μm.

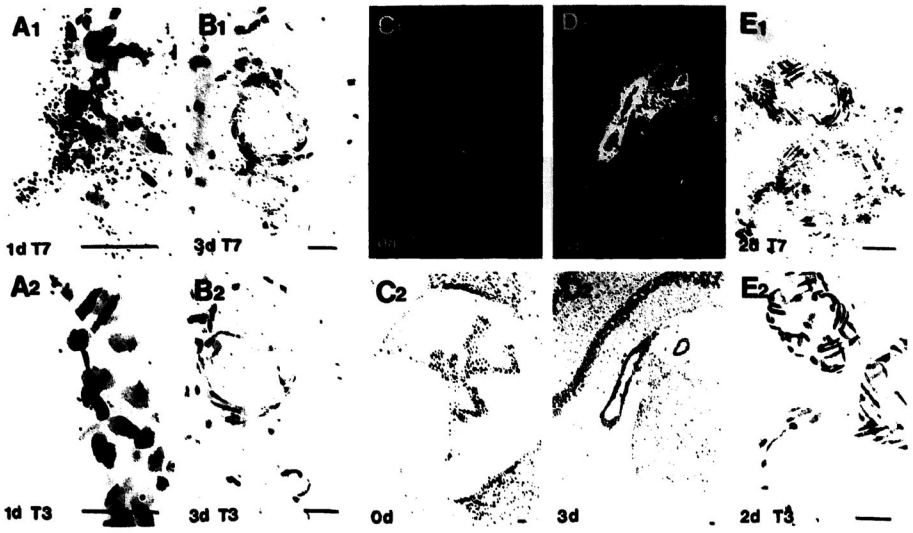

Fig. 6.

TGFβ1 mRNA in the meninges, hippocampal fissure and choroid plexus after injury. Intense TGFβ1 mRNA (T7) is observed in the meninges (panel A) and hippocampal fissure (panel B) but not in the control sections hybridized with the sense strand (T3). In the choroid plexus, TGFβ1 mRNA is almost non-detectable in uninjured animals (0 day, C1) but very intense following injury (panels D_1, E_1). Control sections show no signal (T3). Bright field of low-power micrographs are shown in panels C_2 and D_2. Bar = 25 μm.

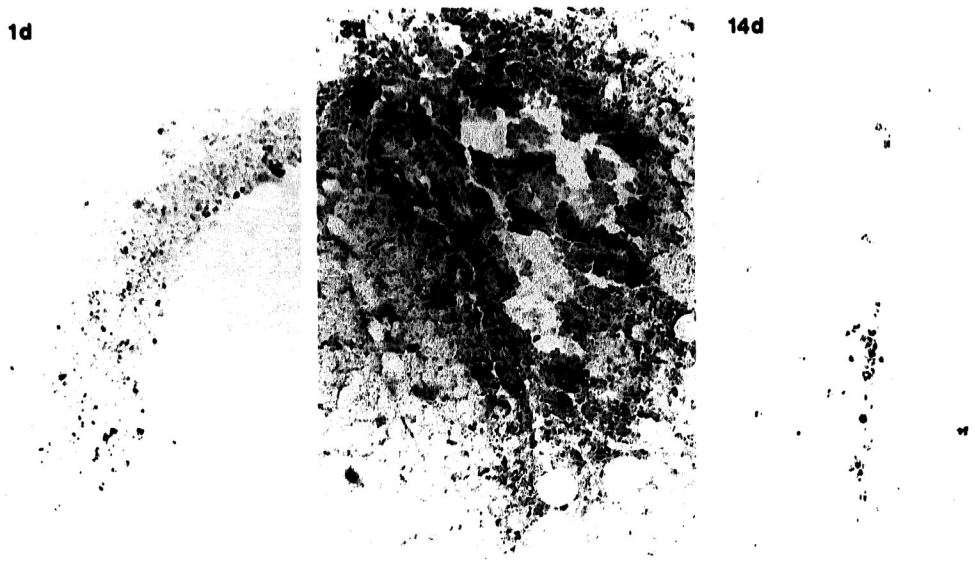

Fig. 7.

Immunolocalization of TGFβ1 after injury. Immunoreactive TGFβ1 is seen diffusely in the neuropile along the borders of the lesion after 1 day and this increases at 3 days. Staining is residual by 14 days and mostly confined to the macrophages remaining in the center of the wound.

Bar = 10 μm.