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ORIGINAL ARTICLE

TGF-β1 and TGF-β2 expression after traumatic human spinal cord injury

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Study design: Immunohistochemical investigation in control and lesioned human spinal cords.
Objectives: To assess the spatial and temporal expression patterns of transforming growth factor-β1 and -β2 (TGF-β1 and TGF-β2) in the human spinal cord after traumatic injury.
Setting: Germany, Aachen, Aachen University Hospital.
Methods: Sections from human spinal cords from 4 control patients and from 14 patients who died at different time points after traumatic spinal cord injury (SCI) were investigated immunohistochemically.
Results: In control cases, TGF-β1 was confined to occasional blood vessels, intravascular monocytes and some motoneurons, whereas TGF-β2 was only found in intravascular monocytes. After traumatic SCI, TGF-β1 immunoreactivity was dramatically upregulated by 2 days after injury (the earliest survival time investigated) and was detected within neurons, astrocytes and invading macrophages. The staining was most intense over the first weeks after injury but gradually declined by 1 year. TGF-β2 immunoreactivity was first detected 24 days after injury. It was located in macrophages and astrocytes and remained elevated for up to 1 year. In white matter tracts undergoing Wallerian degeneration, there was no induction of either isoform.
Conclusion: The early induction of TGF-β1 at the point of SCI suggests a role in the acute inflammatory response and formation of the glial scar, while the later induction of TGF-β2 may indicate a role in the maintenance of the scar. Neither of these TGF-β isoforms appears to contribute to the astrocytic scar formation in nerve fibre tracts undergoing Wallerian degeneration.
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Keywords: spinal cord injury; Wallerian degeneration; scar; inflammation

Introduction

In contrast to the peripheral nervous system, lesioned axons within the adult mammalian central nervous system (CNS) do not undergo regeneration. The loss of function following traumatic spinal cord injury (SCI) is often permanent and results in a serious limitation of the patients' quality of life. Despite considerable progress in recent years, the underlying mechanisms responsible for the failure of axon regeneration after SCI remain only partially understood.

Following SCI, the initial parenchymal damage is followed by a complex cascade of secondary events including a strong inflammatory reaction. The early inflammatory cascade is at least partially responsible for secondary tissue destruction and additional loss of function.[1] The early phase of secondary parenchymal damage is followed by the removal of tissue debris. Finally, severe lesions become dominated by the deposition of scar tissue composed of connective tissue and fluid-filled cysts, surrounded by a dense astroglial scar.[2] Multiple studies in experimental animals demonstrate that this scar represents a physical and molecular barrier that is important for isolating the lesion site from adjacent, intact spinal cord[3] but is also crucial for the lack of axonal regrowth after SCI.[2]

Transforming growth factor-β (TGF-β) belongs to a family of cytokines with important functions in inflammation, extracellular matrix formation and scar formation in wound healing.[4,5] In a number of CNS pathologies of experimental animals and humans (including inflammatory disorders, degenerative diseases and traumatic injuries) TGF-β1 and TGF-β2 are rapidly and abundantly expressed.[6–9] In experimental SCI, TGF-β isoforms were shown not only to be

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involved in the early inflammatory response but also to play a central role in the formation of the scar at the lesion site.[8,10] However, the extent to which experimental SCI induced changes of TGF-β expression reflect the events taking place following human SCI remains unknown. Thus, the present investigation was undertaken to determine the spatio-temporal pattern of TGF-β1 and TGF-β2 expression in samples of post-mortem human spinal cord, taken from patients who died at a range of survival times following severe traumatic SCI.

Materials and methods

Post mortem, the spinal cords and brains were removed from 4 control patients who had not suffered from any neurological disease and from 14 patients who died at a range of time points after severe traumatic SCI (for details see Table 1). None of the control patients received any immunosuppressive/immunomodulatory medication for at least 3 months before death. Patients with traumatic SCI had been diagnosed as having 'complete' injuries and presented with paraplegia or tetraplegia. The study was approved by the Aachen University Ethics Committee. The spinal columns were removed at autopsy, approximately 15–48 h after death. Following incision of the dura mater, the spinal cord was fixed in 4% buffered formaldehyde for at least 2 weeks. Thereafter, blocks of approximately 1 cm thickness were prepared from the lesion epicentre, the intermediate zone, the peri-lesional area and from cervical, thoracic and lumbar levels and were embedded in paraffin wax.

Peroxidase immunohistochemistry

Sections were de-waxed in xylene, rehydrated and endogenous peroxidase activity was blocked by incubation in 0.1 M phosphate-buffered saline containing 0.7% H_2O_2 for 30 min. Microwave treatment in 10 mM citrate buffer (pH 6) for 3×3 min was followed by blockade of nonspecific binding by incubation in 10% defatted milk powder for 20 min and subsequent incubation with the primary antibody, overnight at room temperature. The following antibodies were used in the investigation: monoclonal mouse anti-TGF-β1 (1:300, Serotec MCA797, Oxford, UK), polyclonal rabbit anti-TGF-β2 (1:50, Santa Cruz sc-90, Santa Cruz, CA, USA) and polyclonal rabbit anti-glial fibrillary acidic protein (1:2500, Code Z-0334, DAKO, Hamburg, Germany). Following extensive rinsing steps in 0.1 M phosphate-buffered saline, sections were incubated in biotinylated horse anti-mouse or anti-rabbit antibody (diluted 1:500, Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature. For visualization, sections were treated with the Vector ABC system for 30 min at room temperature, followed by incubation in diaminobenzidine. For negative controls the primary antibody was omitted. The specificity of both anti-TGF antibodies on sections was also confirmed by preincubating the primary antibody with excess immunogenic peptide (Santa Cruz, CA, USA).

Table 1 Patients who served as the control group (C1–4) and who died after traumatic injury to the spinal cord (P1–14)

Case number	*Age*	*Cause of death*	
C1	64 years	Pulmonary embolism	
C2	47 years	Pneumonia	
C3	35 years	Myocardial infarction	
C4	83 years	Myocardial infarction	
		Injury level	*Injury–death interval*
P1	21 years	T12	2 days
P2	51 years	C1	4 days
P3	84 years	C3–4	5 days
P4	65 years	C5	8 days
P5	63 years	C6	11 days
P6	18 years	T6	12 days
P7	72 years	T11–12	24 days
P8	85 years	C3	4 months
P9	76 years	T8–9	10 months
P10	80 years	C5–6	1 year
P11	44 years	L1	8 years
P12	71 years	C3–4	20 years
P13	47 years	T5	26 years
P14	57 years	T3–4	30 years

Double immunofluorescence

Sections were de-waxed in xylene and rehydrated. Microwave treatment in 10 mM citrate buffer (pH 6) for 3×3 min was followed by blockade of nonspecific binding by incubation in 3% normal goat serum in 0.5% Triton X-100 in 0.1 M phosphate-buffered saline for 30 min and subsequent incubation for 3 h at room temperature with the primary antibodies anti-TGF-β1 (diluted 1:50) or anti-TGF-β2 (diluted 1:30) with either polyclonal rabbit anti-GFAP (diluted 1:1000, Code Z-0334, DAKO), monoclonal mouse anti-GFAP (diluted 1:250; Sigma, Munich, Germany, clone G-A-5), monoclonal mouse anti-CD68 (diluted 1:30; DAKO, clone PG-M1), monoclonal mouse anti-CD31 (diluted 1:20; DAKO, clone JC70A), mouse monoclonal anti-smooth muscle actin (diluted 1:200; DAKO, clone 1A4) or monoclonal mouse anti-βIII tubulin (diluted 1:10 000; Promega, Madison, WI, USA). Following extensive rinsing steps in 0.1 M phosphate-buffered saline, sections were incubated with Alexa 594 (red-fluorescence)-conjugated goat anti-mouse and Alexa 488 (green fluorescence)-conjugated goat anti-rabbit secondary antibodies (diluted 1:500, Molecular Probes, Carlsbad, CA, USA) for 1 h at room temperature. The tyramide signal amplification kit (TSA Cyanine 3 system, NEL704A, PerkinElmer Life Sciences, Waltham, MA, USA) was used for double immunofluorescence requiring primary antibodies from the same species, for example, anti-TGF-β1 and anti-CD68, CD31 or smooth muscle actin double staining. Following the blockade of endogenous tissue peroxidase, sections were rinsed in the tyramide signal amplification block buffer (prepared as recommended by the manufacturers) and incubated with the anti-TGF-β1 antibody (1:500) overnight. Incubation with a biotinylated horse anti-mouse antibody (1:500, BA2000, Vector Laboratories, Burlingame, CA, USA) for 1 h, rinsing in 0.05% Tween-20 and blocking with the provided reagent for 30 min was followed by streptavidin–horseradish peroxidase (1:500) in blocking reagent for 30 min and cyanine 3-tyramide working solution

(1:100) for 10 min. After rinsing, the slides were incubated with the anti-CD68, the CD31 or the smooth muscle actin antibody overnight, followed by Cy2-conjugated goat anti-mouse (1:100, Jackson Laboratories) for 3 h at room temperature. Finally, nuclei were stained for 5 min with 4′,6-diamidino-2-phenylindole (1:1000, Sigma).

For a semiquantitative description of the amount of TGF-β1 and TGF-β2 immunoreactive cells at the various survival times, an arbitrary rating scale for the number of labelled cells was chosen, ranging from 0 to + + + + with 0 indicating no immunopositive cell and + + + + indicating the presence of large numbers of labelled cells.

Results

Distribution of TGF-β1, TGF-β2 and GFAP in control human brain and spinal cords

In the control, non-lesioned human brains, TGF-β1 staining was detected in sub-populations of neurons (that is, cortical neurons) as well as in intravascular monocytes (Figure 1a) and some blood vessel walls (Figure 1b). TGF-β2 immunoreactivity within control brains was also found in sub-populations of neurons (Figure 1c). Furthermore, TGF-β2 was detected in ramified microglia (Figure 1d). Similarly, TGF-β1 immunoreactivity in the control spinal cords was confined to occasional motoneurons (Figure 1e) as well as to scattered blood vessels and rare intravascular monocytes (Figures 1f and g, and 3a and b). In the blood vessel walls, TGF-β1 was colocalized with the endothelium (Figures 3a and b). TGF-β2 immunoreactivity was restricted to rare intravascular monocytes (not shown). This distribution pattern was identical in all four control cases, indicating that, in the normal human CNS, there were no age-related changes in the distribution for either TGF-β isoform. Glial fibrillary acidic protein (GFAP) immunohistochemistry revealed a homogeneous network of astrocytic processes with cell bodies spread over white and grey matter (Figure 1h).

Distribution of TGF-β1 and TGF-β2 at the lesion site following SCI

For an overview of the temporal expression of TGF-β1 and TGF-β2 at and around the lesion site, see Table 2. At the lesion site, a dramatic upregulation of TGF-β1 staining was detected 2 days after traumatic injury, the earliest survival time investigated. Immunoreactivity for TGF-β1 was associated with macrophages that invaded the lesion epicentre (Figures 2a and 3c), an area characterized by the complete destruction of cytoarchitecture to the extent that it was difficult, if not impossible, to distinguish grey and white matter regions. The number of invading macrophages remained high in all cases with survival times up to 24 days after SCI. In the intermediate zone, which included the extremities of the lesion site and their interface with the adjacent damaged, but non-degenerating, CNS parenchyma, both macrophages and astrocytes demonstrated TGF-β1 immunoreactivity already 2 days after SCI. Furthermore, most neurons were immunoreactive and showed a cytoplasmic and sometimes nuclear distribution of TGF-β1 immunoreactivity at this early survival time (Figures 2b, 3d and e). This staining

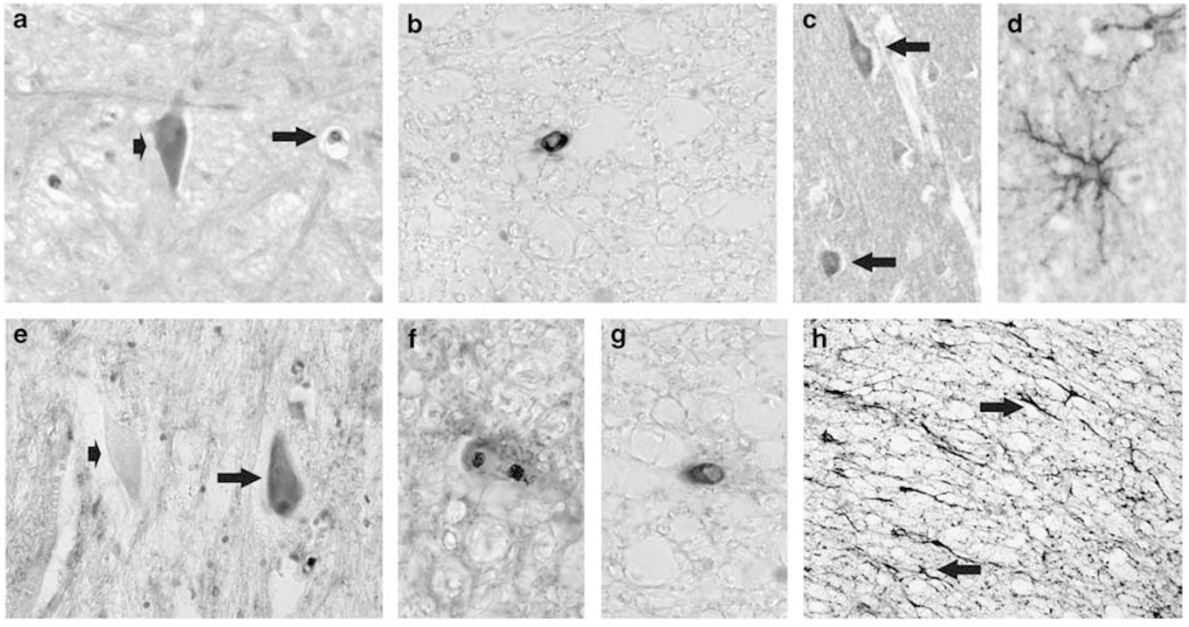

Figure 1 TGF-β1 and TGF-β2 expression in the normal human brain and spinal cord. (**a**) In the normal brain, TGF-β1 immunohistochemistry demonstrates neuronal staining (arrowhead) as well as intravascular monocytes (arrow) in both white and grey matter. (**b**) Occasional blood vessel walls also demonstrate TGF-β1 staining in sections of control brains. (**c** and **d**) TGF-β2 immunoreactivity in the brain was confined to occasional neurons (arrows) (**c**) and ramified microglia (**d**). (**e**–**g**) In the spinal cord, some grey matter motoneurons reveal a strong cytoplasmic TGF-β1 immunoreactivity (arrow). Nearby, a non-stained neuron can be found (arrowhead, **e**). Furthermore, intravascular monocytes (**f**) and blood vessels (**g**) are TGF-β1 positive. (**h**) In the normal spinal cord white matter, GFAP staining reveals astrocytic cell bodies (arrows) in between a homogenous network of processes. (**a**–**c** and **e**–**h**) magnification × 320 and (**d**) magnification × 640. TGF, transforming growth factor.

Table 2 Amount of immunopositive cells at the lesion site at different survival times following human SCI

	Control	*2 days*	*4–8 days*	*10–11 days*	*24 days*	*4 months*	*10 months*
	Lesion epicentre						
CD68	0/+	+	+++	++++	++++	++	+
CD68/TGFβ1	0/+	+	+++	++++	+++	++	+
CD68/TGFβ2	0	0	0	0	++++	++	+
	Intermediate zone						
CD68	0/+	+	++	+++	+++	+	+
CD68/TGFβ1	0/+	+	++	++	++	+	+
CD68/TGFβ2	0	0	0	0	+++	+	+
GFAP	++	+	+	+	+	0/+	0/+
GFAP/TGFβ1	0	+	+	+	+	0/+	0/+
GFAP/TGFβ2	0	0	0	0	0	0/+	0/+
Tubulin	++	++	+/++	+	+	+	+
Tubulin/TGFβ1	0/+	++	+/++	+	+	0/+	0/+

Abbreviations: SCI, spinal cord injury; TGF, transforming growth factor.
The figures reflect the number of immunopositive cells using an arbitrary rating scale from 0 (no cells) to ++++ (maximum amount of cells) in sections from control spinal cords and the lesion site (epicentre and intermediate zone) at various survival times after SCI stained for the different antigens.

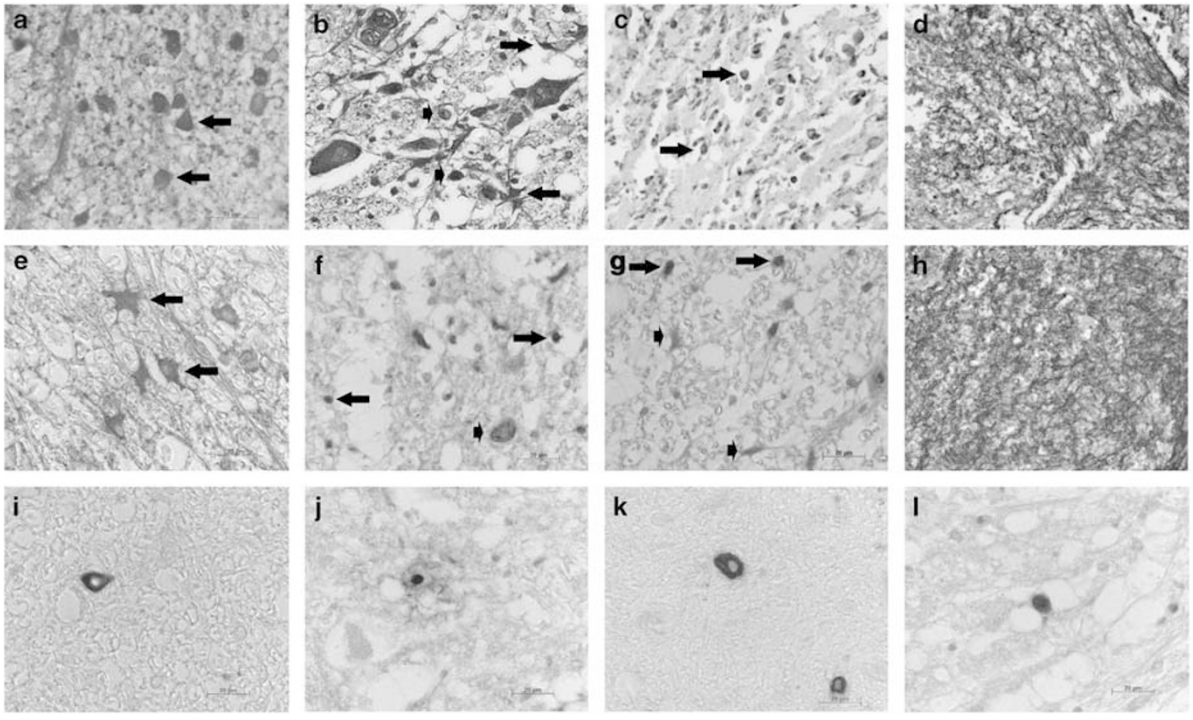

Figure 2 TGF-β1 and TGF-β2 expression in the lesioned human spinal cord. (**a**) Two days after traumatic spinal cord injury (P1), TGF-β1 staining on sections from the lesion epicentre shows round to oval cells with the morphology of monocytes/macrophages (arrows). (**b**) Furthermore, in adjacent areas in the intermediate zone, multiple cells which morphologically include neurons, astrocytes (arrows) and invading macrophages (small arrows) are TGF-β1 positive. (**c**) Twenty-four days after trauma (P7), a dramatic upregulation of TGF-β2 is detectable at the lesion epicentre, mostly in cells with a rounded, foamy morphology (arrows). (**d**) In a section from the intermediate zone of a patient who died 24 days after injury (P7), GFAP immunohistochemistry reveals areas with a dense irregular scar of astrocytic processes in both the grey and white (this picture) matter. (**e**) Four months after injury (P8), activated, hypertrophied astrocytes (arrows) display strong TGF-β1 immunoreactivity in the intermediate zone. (**f**) In a section from the same 4 months case, TGF-β1 remains upregulated in neurons (small arrow) and macrophages (arrows). Compared to earlier time points the number of positive cells is reduced. (**g**) At a post-injury survival time of 4 months (P8), TGF-β2 immunohistochemistry demonstrates some rounded macrophages (arrows) and stellate astrocytes (arrowheads) in the intermediate zone. (**h**) Four months after trauma (P8), the intermediate zone is filled with a highly GFAP-immunopositive scar; cell bodies can no longer be seen. (**i**) One year after SCI (P10), TGF-β1 staining is again restricted to blood vessel walls. (**j**) In a section close to the lesion site of a patient who died 1 year after trauma (P10), the spinal cord parenchyma is devoid of TGF-β2 immunoreactivity and only a single intravascular monocyte can be detected. (**k**) Four months after SCI with an injury at cervical level C3 (P8), TGF-β1 staining in the thoracic corticospinal tract is restricted to occasional blood vessels. (**l**) In an adjacent section of the same case, TGF-β2 immunoreactivity can be detected in a single intravascular monocyte. (**a**–**d** and **f**–**h**) magnification × 320, **e** and **i**–**l** magnification × 640 and see scale bars. SCI, spinal cord injury; TGF, transforming growth factor.

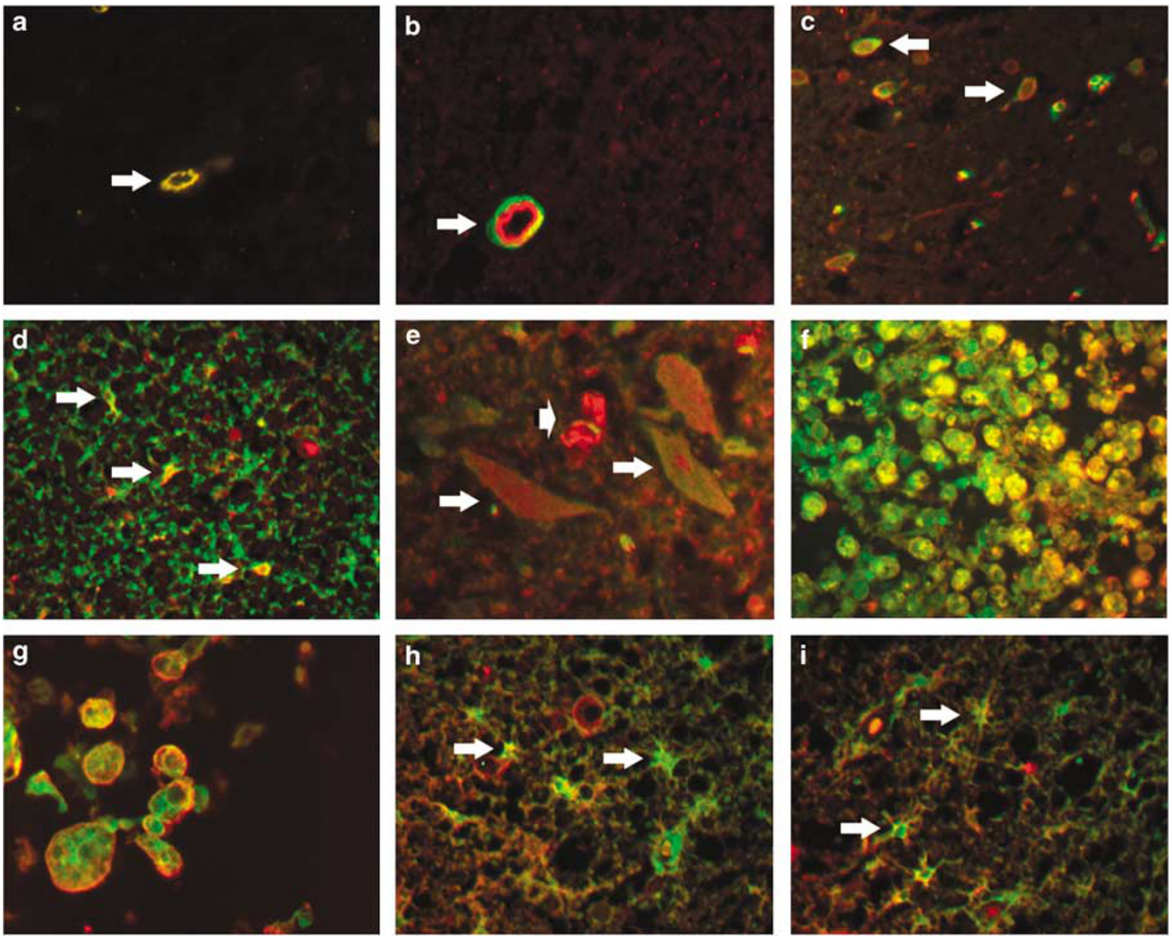

Figure 3 Double immunofluorescence of TGF-β1 and TGF-β2 immunohistochemistry to demonstrate cellular/structural identity. (**a**) In the spinal cord white matter of a control case, double staining for TGF-β1 (red) and CD31 (green) reveals the endothelium of a blood vessel with colocalized TGF-β1 immunoreactivity (arrow). (**b**) In a section from the same control case, double immunofluorescence for TGF-β1 (red) and smooth muscle actin (green) demonstrates the absence of TGF-β1 in the smooth muscle cell layer of a blood vessel in the white matter (arrow). (**c**) In a section from a patient who died 2 days after traumatic SCI (P1), double staining for TGF-β1 (red) and CD68 (green) demonstrates macrophages/microglia with TGF-β1 immunoreactivity (arrows). (**d**) In a section from a patient who died 4 days after traumatic SCI (P2), double staining for TGF-β1 (red) and GFAP (green) reveals TGF-β1-positive activated astrocytes in the intermediate zone (arrows). (**e**) In a section from a patient who died 4 days after traumatic SCI (P2), double staining for TGF-β1 (red) and β III tubulin (green) reveals TGF-β1-immunopositive neurons (yellowish combined signal, arrows). Furthermore, a TGF-β1-immunopositive blood vessel can be found (arrowhead). (**f**) In a section from a patient who died 24 days after SCI (P7), double staining for TGF-β2 (red) and CD68 (green) demonstrates multiple TGF-β2-positive macrophages/microglia at the lesion site. (**g**) In the same section (P7), higher magnification reveals the morphology of foamy macrophages. (**h**) Four months after SCI (P8), TGF-β1 (red) and GFAP (green) immunohistochemistry reveals large TGF-β1-immunopositive activated astrocytes at the site of injury (arrows). (**i**) Four months after SCI (P8), TGF-β2 (red) and GFAP (green) immunohistochemistry shows activated astrocytes with TGF-β2 immunoreactivity at the site of injury (arrows). (**a–f** and **h** and **i**) magnification $\times 400$ and (**g**) magnification $\times 640$. SCI, spinal cord injury; TGF, transforming growth factor.

pattern was comparable in all cases with survival times up to 24 days after SCI.

In contrast to the rapid increase in TGF-β1 immunoreactivity, elevated TGF-β2 immunoreactivity was only detected at 24 days after traumatic injury. At this survival time, many cells demonstrated immunoreactivity for TGF-β2, both at the lesion epicentre and in the intermediate zone (Figure 2c). The large, rounded morphology of these cells and double staining for CD68 confirmed the macrophage/microglial identity of the TGF-β2-positive cells (Figures 3f and g). The upregulation of TGF-β2 in the intermediate zone coincided with the appearance of a dense GFAP-positive reactive gliosis/astrocytic scar formation (Figure 2d).

By 4 and 10 months after SCI, microglia/macrophages remained TGF-β1 positive at the lesion epicentre and in the intermediate zone, but the number of immunoreactive cells was strongly reduced compared to earlier time points (Figure 2f). Few microglia/macrophages were TGF-β2 immunoreactive at this time. In the intermediate zone, some reactive astrocytes also demonstrated TGF-β1 and TGF-β2 immunoreactivity (mainly at the border of the lesion site, Figures 2e, g, 3h and i) with only occasional cells being detectable by 10 months. Most neurons retained moderate TGF-β1 immunoreactivity up to 10 months post injury, but no nuclear staining could be detected (Figure 2e). In all patients with a survival time of 4 months and longer, the

intermediate zone of the lesion site was filled with an irregular, GFAP-positive astrocytic scar (Figure 2h). In cases with survival times ranging from 1 to 30 years, no TGF-β1 or TGF-β2 immunoreactivity could be detected at the lesion epicentre. In the intermediate zone, TGF-β1 immunoreactivity was restricted to occasional motoneurons and blood vessel walls (Figure 2i) while TGF-β2 immunoreactivity was found in occasional, intravascular monocytes (Figure 2j).

Distribution of TGF-β1 and TGF-β2 in white matter tracts undergoing Wallerian degeneration

The distribution of TGF-β1 and TGF-β2 immunoreactivity in nerve fibre tracts undergoing Wallerian degeneration after traumatic SCI. Particular attention was paid to degenerating descending corticospinal (motor) and ascending dorsal column (sensory) tracts. To exclude any contribution from the inflammatory milieu at the lesion site, only sections prepared from blocks 2 or more segments away from the site of injury were investigated. There was no indication of either TGF-β1 or TGF-β2 expression by reactive astrocytes in the fibre tracts undergoing Wallerian degeneration, even in those tracts which showed a massive anisotropic astrocytic scar formation (cases with survival times of 1 year or greater). Only occasional blood vessels and intravascular monocytes were found to be immunoreactive for TGF-β1 or for TGF-β2 (Figures 2k and l).

Discussion

Inflammation in the CNS, in response to injury, is of major importance but remains only partially understood. Both beneficial and detrimental components of the inflammatory cascade have been identified (for example, Hausmann[1]). In contrast to studies using experimental animals, there is relatively little correlative information about cytokine expression in human tissues following SCI or insult-induced Wallerian degeneration.[11] TGF-β1 and TGF-β2 have previously been shown to be upregulated following experimental spinal cord lesions and to play important roles in the early inflammatory response, in particular being involved in the glial scar formation at and around the lesion site. Since correlative data in human traumatic SCI are lacking, the present immunohistochemical investigation was undertaken to identify the spatio-temporal expression pattern of TGF-β1 and TGF-β2 in both normal and lesioned human spinal cord.

Expression of TGF-β1 and TGF-β2 in the normal CNS

In the normal, unlesioned human brain TGF-β1 and TGF-β2 immunoreactivity could only be detected in scattered cellular profiles. TGF-β1 immunoreactivity was detected in some scattered neurons as well in the walls of occasional blood vessels, localized in the endothelium, of brain and spinal cord sections. TGF-β2 could also be detected in some neurons (as well as microglia) of the brain, but was only present in occasional intravascular monocytes in the spinal cord of the same cases. This suggests the presence of distinct populations of microglia in the normal human brain and spinal cord, as well as a distinctive pattern of TGF-β2-positive neurons in the normal human brain. Prior investigations in human CNS tissue have reported a range of TGF-β1 distributions including peri-neuronal[12] or microglial patterns. Other reports have failed to detect any TGF-β1 immunoreactivity in the CNS.[9] In contrast, immunohistochemistry for TGF-β2 in normal human CNS has resulted in the detection of neuronal,[7,9,12] microglial[7,9] and astrocytic distribution patterns.[9] This distribution pattern is similar to that obtained using experimental animals, where TGF-β1 and TGF-β2 could be found in neurons and astrocytes of the adult rodent spinal cord.[8,13] The function of both cytokines in the normal CNS is presently not clear. *In vitro* and *in vivo* studies have demonstrated a neuroprotective and growth promoting effect of TGF-β on a number of neuronal populations[14,15] as well as both growth-inhibitory and growth-promoting effects on astrocytes (depending on their phenotype when exposed to the TGF-β[16]).

The detection of TGF-β1 immunoreactivity that was associated with the endothelium of blood vessel walls probably reflects attachment of the cytokine to extracellular matrix proteins and might play a role in the retention, delivery and clearance of the cytokine.[17,18]

Expression of TGF-β1 and TGF-β2 at the lesion site after traumatic SCI

The present data show a rapid and dramatic induction of TGF-β1 immunoreactivity, both extracellularly and intracellularly (that is, in macrophages, astrocytes and neurons) supporting the suggestion that it plays a role in the early inflammatory reaction cascade following traumatic injury to the human spinal cord. This expression pattern is in line with experimental studies revealing an early and maintained increase of TGF-β at the mRNA and protein levels following experimental crush and contusion injuries of the adult rat spinal cord.[8,10,19] Furthermore, the cell types demonstrating TGF-β1 immunoreactivity following human SCI (that is, macrophages, some astrocytes and neurons) also show a striking similarity to what has already been identified at the mRNA levels in experimental animals.[8,10,19] In the post-mortem human SCI samples TGF-β1 expression remained elevated for up to 10 months; however, the longest time point of TGF-β1 elevation demonstrated in experimental animals was 54 days after injury.[8] Therefore, the early and prolonged upregulation of TGF-β1 after experimental SCI appears to correlate with the events that take place following humans SCI. Experimental SCI studies have already suggested roles in both pro- and anti-inflammatory effects as well as involvement in scar formation.

The lesion-induced increase of TGF-β1 may exert its pro-inflammatory effects via direct (acting as a potent chemoattractant) and indirect means (inducing adhesion molecules as well as the degradation of the endothelial basement membrane).[4] Furthermore, TGF-β1 stimulates monocytes as well as astrocytes to express a variety of pro-inflammatory cytokines and induces Fcγ receptors on the mononuclear cell surface.[4] The anti-inflammatory effects of TGF-β1 are exerted via a number of mechanisms including the modulation of cytokine and chemokine expression, the inhibition of

hydrogen peroxide production and the inhibition of the inducible nitric oxide synthase. Furthermore, the anti-inflammatory effect is brought about via indirect means; rendering cells unresponsive to pro-inflammatory cytokines.[4] This bi-directional role of TGF-β1 in inflammation results from more pro-inflammatory effects on resting/non-activated cells in the initial phase and more anti-inflammatory effects on activated cells in the later inflammatory cascade.

In both experimental animals and humans the prolonged upregulation of TGF-β1 after SCI is in contrast to other cytokines such as interleukin-1β and tumour necrosis factor-α which demonstrate an early but short-lived induction of 1–3 days after trauma.[11,19] The reason for this difference might be that on top of an important function in the early inflammatory cascade, as shared with interleukin-1β and tumour necrosis factor-α, TGF-β1 is also involved in scar formation as previously shown in several experimental studies.[8,10,19]

Lagord *et al*.[8] proposed that TGF-β1 is mainly involved in the inflammatory reactions while TGF-β2 is involved in scar formation following crush injuries to the rat spinal cord. In their study, astroglial scarring and concomitantly TGF-β2 upregulation was detected from around 5 to 28 days after injury. In the present investigation in post-mortem human tissue, the expression pattern of TGF-β2 also correlated well with the formation of the astrocytic scar at the lesion site. The first indication of astrocytic scar formation, 24 days after injury, coincided with the upregulation of TGF-β2 in macrophages at the lesion site. Raised levels of TGF-β2 could be detected for up to a 1-year after injury, by which time, scar formation should have been completed. This expression pattern of TGF-β2 after human SCI is delayed and prolonged compared to animal data but correlates to the delayed formation of astroglial scarring in humans,[20] starting approximately 24 days after injury (at which time scarring was nearly complete in the rats[8]). The reason for delayed scar formation following human SCI is presently not clear but the present results suggest that the lack of an early TGF-β2 upregulation might be involved in this difference.

The confirmation of the spatio-temporal upregulation of TGF-β1 and TGF-β2 in human SCI highlights their potential as possible targets for therapeutic interventions aimed at promoting CNS axon regeneration. In experimental studies, the treatment of SCI injured animals with neutralizing antibodies to TGF-β1 and TGF-β2 has demonstrated a reduction in scar formation and a concomitant decrease of associated growth-inhibitory proteins such as chondroitin sulphate proteoglycans (CSPG).[21] However, other groups have reported that this approach did not lead to enhanced regeneration of lesioned nerve fibre tracts[22] and suggested that the neutralization of TGF-β alone may not be sufficient for promoting functional repair.

Lack of involvement of TGF-β1 and TGF-β2 in nerve fibre tracts undergoing Wallerian degeneration

A transient upregulation of TGF-β1 and TGF-β2 in oligodendrocytes of white matter tracts undergoing Wallerian degeneration has been reported following experimental SCI and points to a possible involvement of both cytokines in the formation of the astrocytic scar in the degenerated fibre tracts. In the present investigation, possible changes of TGF-β1 and TGF-β2 expressions were investigated in both ascending (sensory) and descending (motor) human spinal cord white matter tracts undergoing Wallerian degeneration. At no time point after SCI, could elevated levels of TGF-β1 or TGF-β2 be seen in degenerating dorsal columns (ascending, sensory) or corticospinal (descending, motor) fibre tracts. This was despite the detection of a dense astroglial scar in cases with survival times of 1 year or greater (see also Buss *et al*.[23]). It may be concluded that neither TGF-β1 nor TGF-β2 are involved in oligodendrocytic responses, nor in the clearance of axonal and myelin debris or in the long-term astrocytic scarring during Wallerian degeneration of human white matter tracts.[9] This strongly suggests that the process of scar formation at the primary site of injury and in degenerating white matter tracts follow different molecular programs. As with other cytokines, TGF-β isoforms most likely exert multiple functions in traumatic CNS injury and further studies are needed to elucidate the exact mechanism of action on the wide range of CNS cell populations at different time points after injury.

Conclusion

The present study demonstrates the spatio-temporal pattern of TGF-β1 and TGF-β2 upregulation after traumatic human SCI. The data also demonstrated that, although the timing of these events in experimental animal models of SCI and in post-mortem human SCI cases may not be identical, the general spatio-temporal expression patterns are similar, thus confirming the clinical relevance of the experimental model for this aspect of the lesion-induced inflammatory cascade. TGF-β1 was rapidly upregulated after human SCI and may be involved in the inflammatory reaction as well as in scar formation at the lesion site. In contrast, TGF-β2 demonstrated a delayed upregulation and may be involved in the formation or maintenance of the astrocytic scar around the lesion site. The confirmation of the spatio-temporal upregulation of TGF-β1 and TGF-β2 in human SCI highlights their potential as possible targets for therapeutic interventions aimed at promoting CNS axon regeneration.

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