RESEARCH ARTICLE

Lesion-associated Expression of Transforming Growth Factor-Beta-2 in the Rat Nervous System: Evidence for Down-regulating the Phagocytic Activity of Microglia and Macrophages

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The mechanisms that control the phagocytic activities of microglia and macrophages during disorders of the nervous system are largely unknown. In the present investigation, we assessed the functional role of transforming growth factor (TGF)β2 in vitro and studied TGFβ-2mRNA and protein expression in two CNS lesion paradigms in vivo characterized by fundamental differences in microglia/macrophage behaviour: optic nerve crush exhibiting slow, and focal cerebral ischemia exhibiting rapid phagocytic transformation. Furthermore, we used sciatic nerve crush injury as a PNS lesion paradigm comparable to brain ischemia in its rapid phagocyte response. In normal and degenerating optic nerves, astrocytes strongly and continuously expressed TGF-β2 immunoreactivity. In contrast, TGF-β2 was downregulated in Schwann cells of degenerating sciatic nerves, and was not expressed by reactive astrocytes in the vicinity of focal ischemic brain lesions during the acute phagocytic phase. In line with its differential lesion-associated expression pattern, exogenous TGF-β2 suppressed spontaneous myelin phagocytosis by microglia/macrophages in a mouse ex vivo assay of CNS and PNS Wallerian degeneration. In conclusion, we have identified TGF-β2 as a nervous system intrinsic cytokine that could account for the differential regulation of phagocytic activities of microglia and macrophages during injury.

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INTRODUCTION

Microglia play an essential role in the pathophysiology of neurodegenerative, ischemic and inflammatory autoimmune disorders of the central nervous system (CNS) by acting as effector cells in tissue damage and as scavenger cells in tissue remodeling (1, 21, 42, 44). In the normal CNS, microglia are in a quiescent state and exhibit a ramified morphology. Upon activation microglia upregulate immunological effector molecules such as MHC class II, and depending on the underlying pathology, can undergo phagocytic transformation (11, 21). In vitro, brain-isolated microglia spontaneously transform into amoeboid, phagocytosing cells (14,16), a process that can be reversed by growing microglia on an astrocytic cell layer (39, 45). In contrast to culture systems, little is known about the mechanisms that control microglial phagocytic activity in vivo. This is of functional importance since insufficient phagocytic activation and concomitant delayed clearance of growth-inhibitory myelin remnants have been identified as one major cause for regeneration failure after fibre tract injury in the CNS (6,12, 29, 30, 43).

Transforming growth factor-βs (TGF-β) encompass a family of multifunctional cytokines which exert profound immunomodulatory effects, chemotaxis, stimulate angiogenesis and scar formation, and act as tumor suppressors and regulators of cell differentiation and death (2, 3, 25). Three isoforms, TGF-β1, -β2, and -β3 have so far been identified in mammals. Each isoform is encoded by a distinct gene and expressed in a tissue-specific fashion. In the adult nervous system of rodents, TGF-β2 and -β3 are constitutively expressed in subpopulations of neurons, in astroglia and Schwann cells (35, 46), while TGF-β1 is virtually absent, but rapidly upregulated after injury (18). Although it is apparent from studies in transgenic mice that TGF-β isoforms exert distinct effects (34, 38), their specific functions during disorders of the nervous system are incompletely understood (10).

In the present investigation, we show that TGF-β2 and -β1 are differentially regulated in CNS and peripheral nerve injury, and we could identify TGF-β2 as a principal cytokine controlling phagocytic activity of microglia and macrophages. We first analyzed the spatiotemporal expression of TGF-β2/1 in two CNS lesion paradigms characterized by fundamental differences in microglial behaviour: *i)* optic nerve crush, in which microglia are activated, but only slowly transform into phagocytes (28, 33, 43), and *ii)* photochemically induced focal cerebral infarcts, in which microglia account for the majority of phagocytes during early stages of infarct development (19, 36). As a model of focal cerebral ischemia, rat photothrombosis, originally described by Watson and colleagues (47), was chosen because location and size of the ischemic lesions are highly reproducible, and cellular responses develop in a predictable sequence (15, 36, 37). In this model, a photosensitive dye, rose bengal, is injected intravenously, and subsequentially activated locally by a light beam through the intact skull which leads to free-radical formation and consecutive thrombosis of small vessels of the illuminated cerebral cortex (9). Based on the different expression patterns, we further analyzed the functional effects of TGF-β2 on myelin clearance using an in vitro assay of Wallerian degeneration (22).

MATERIALS AND METHODS

Animal experiments. All experiments were approved by the Bezirksregierung

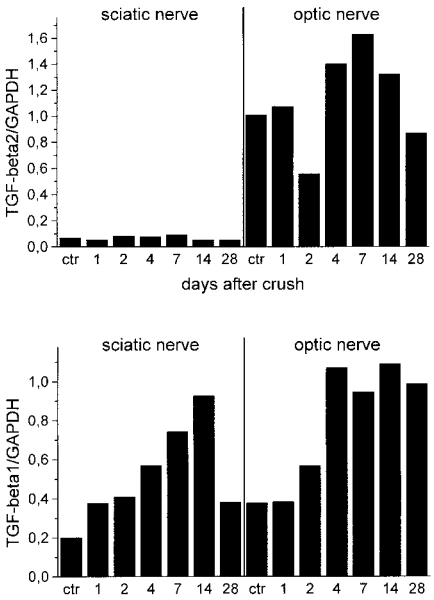

Figure 1. *TGF-β2 and TGF-β1-mRNA expression in normal sciatic (SN) and optic nerves (ON), and in the distal nerve segments during the course of Wallerian degeneration (WD)* (values of 20 pooled nerve segments at each time point). Semiquantitative evaluation of TGF-β2 and-1 mRNA levels after normalization against the "house keeping" gene GAPDH. Note the virtual absence of TGF-β2 mRNA in SN and the high constitutive expression in ON without significant modulation during nerve degeneration. In contrast, TGF-β1 mRNA is present in both SN and ON, and increases during WD in both tissues.

Braunschweig, Nordrhein, or Unterfranken respectively, and performed in accordance with institutional guidelines.

Nerve crush and transection. Nerves of 8- to 10-week-old Wistar rats were crushed on one side in deep anesthesia at the sciatic notch (sciatic nerve crush) or behind the eye ball (optic nerve crush), respectively, for one minute using a jeweller's forceps. Nerve specimens were later removed at the time points given below. For morphological studies (immunocytochemistry) sciatic nerve samples (n = 4 per time point) were collected at days 0, 2, 4, 8, and 16 after crush, optic nerve specimens at days 0, 4, 7, 14, and 28 after injury. Rats were perfused in deep anesthesia with 4% paraformaldehyde in 0.1M sodium phosphate buffer, pH 7.4. Sciatic or optic nerve specimens were removed, postfixed in the same fixative overnight at 4°C, rinsed with buffer, infiltrated with 20% sucrose and then frozen. Some nerve samples were also embedded in paraffin. In addition, sciatic nerves of 8- to 10-week-old C57BL/6 mice were transected, fixed with 4% paraformaldehyde and frozen for immunocytochemistry.

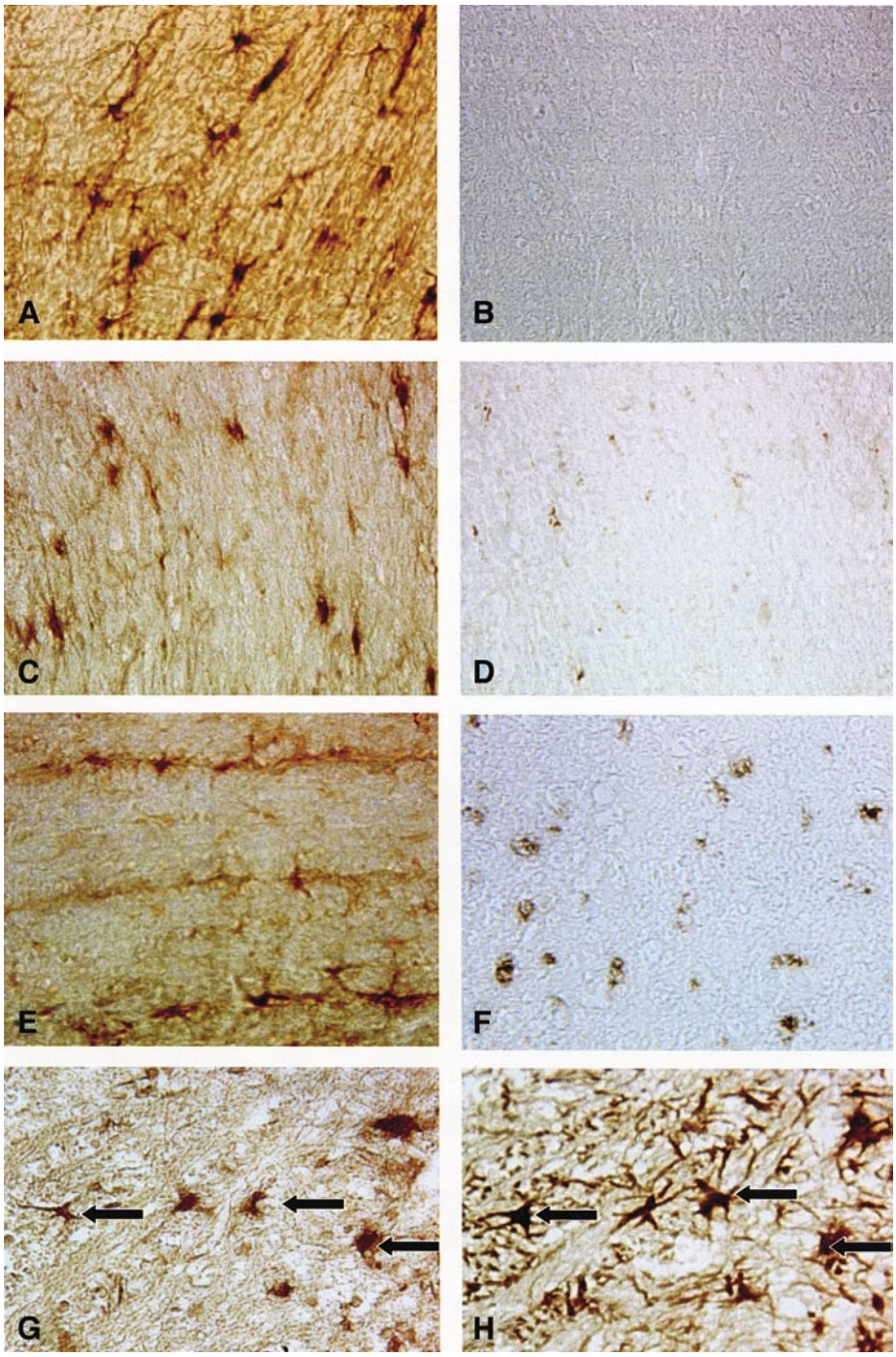

Figure 2. *Expression of TGF-β2 protein in normal optic nerve (ON) (**A, B**) and after ON crush (**C, E**).* In normal ON, astrocytes identified by their typical stellate morphology constitutively express TGF-β2 (**A**). TGF-β2 immunoreactivity was abolished by preabsorption of antibodies with TGF-β2 protein (**B**). TGF-β2 immunoreactivity persists in astrocytes during optic nerve degeneration as exemplified at days 4 (**C**) and 14 (**E**). ED1 immunoreactivity indicative of phagocytic activity of microglia only slowly increases (**D, F**), and is much less compared to the dense ED1 positive macrophage infiltrates seen in sciatic nerve crush at day 14 (Figure 3). (**G, H**) are serial paraffin sections stained for TGF-β2 (**G**) and GFAP (**H**), a marker for astrocytes. Note colocalisation as marked by arrows.
Magnification (**A-H**) ×250.

For reverse-transcriptase-polymerase chain reaction (RT-PCR) analysis, Wistar rats along with normal controls were sacrificed by an overdose of ether on days 1, 2, 4, 7, 14 , and 28 after sciatic or optic nerve crush, respectively. For RNA preparation, 20 nerve segments were pooled at each time point. Sciatic nerve specimens distal

to the crush site were rapidly removed, snap frozen in liquid nitrogen, and stored at -80°C until RNA preparation. The intracranial proportions of the crushed optic nerves (excluding the crush site) were rapidly removed, and stored in RNA-later™ solution (Ambion Inc., Austin, Tex) at 4°C until preparation of RNA.

Focal cerebral infarcts. Focal cortical infarctions were induced in adult Wistar rats by photothrombosis of cortical microvessels under anesthesia with enflurane in a O_2/N_2 (1:2) atmosphere as described in detail elsewhere (15, 47). Briefly, the fiber optic bundle of a cold light source was centered stereotactically 4 mm posterior and 4 mm lateral from Bregma on the exposed skull. Via a femoral vein catheter, 0.4 ml of a sterile-filtered Rose Bengal solution (10 g/l) was given and the brain was illuminated for 20 minutes. Afterwards, the skin was sutured. This procedure resulted in cone-shaped pure cortical infarctions which were readily visible at 4 hours after induction of ischemia. For morphological studies rats (n = 3 at each time point) were sacrificed at 3 days, 6 days, 14 days and 28 days after induction of cortical photothrombosis and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Whole brains were removed from the skull, postfixed in the same fixative overnight at 4°C, and cryoprotected by overnight infiltration with 20% sucrose in phosphate buffer at 4°C. For RT-PCR analysis, ischemic lesions and contralateral cortex homotopic to the infarct were prepared at 4 hours, 16 hours, 24 hours, 2 days, 3 days and 7 days (n=4 per time point) and snap frozen. From each site approximately 30 to 50 mg tissue (wet weight) was obtained.

Immunocytochemistry. Serial free-floating 40-µm cryostat sections of rat sciatic and optic nerves as well as brain infarcts were cut and washed 3 times in Tris-buffered saline containing 0.05% Triton X-100 (TBS-T) pH 7.5. Endogenous peroxidase was blocked by 30 minute incubation in 0.3% H_2O_2 in TBS-T. After 3 washes in TBS-T, sections were incubated with rabbit polyclonal antibodies against rat TGF-β2 1:5000 (sc-90; SantaCruz Biotech, Santa Cruz, Calif), which shows no crossreactivity with TGF-β1 and -β3,

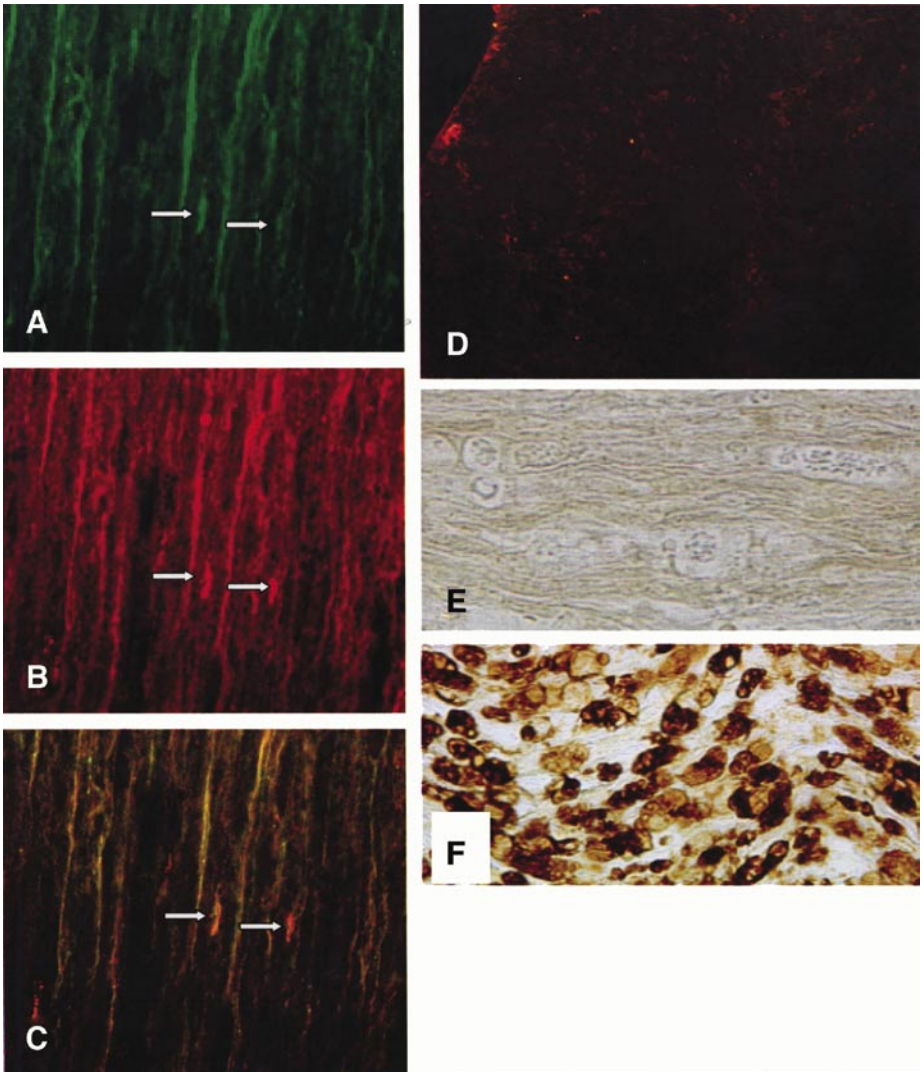

Figure 3. *TGF-β2 expression in peripheral nerves*: In normal mouse sciatic nerves S100-positive Schwann cells marked by arrows in (**A**) strongly express TGF-β2 in (**B**) as revealed by the yellow/orange overlay picture in (**C**). During Wallerian degeneration TGF-β2-immunoreactivity disappears in mouse (**D**) as well as rat (**E**) sciatic nerves which are heavily infiltrated by ED-1 positive macrophages (**F**). Magnification (**A-D**) ×400; (**E, F**) ×550.

mouse monoclonal antibody ED1 1: 2000, a marker for the phagocytic state of microglia/macrophages (11, 41, 43), and mouse anti-GFAP 1:2000, a marker for astrocytes, in 2% normal goat serum in TBS-T for 16 hours at 4°C. Following 3 washes in TBS-T, bound antibody was detected using biotinylated goat anti-rabbit or goat anti-mouse IgG (Vector Laboratories, Burlingame, Calif), respectively, and the ABC Elite kit (Vector) with diaminobenzidine as substrate. Control experiments in which TGF-β2 antibodies were either omitted, or preadsorbed with a 10-fold excess of recombinant TGF-β2 (blocking peptide sc-90P, SantaCruz) revealed complete loss of specific immunostaining. Sections were mounted onto gelatine-coated slides, air-dried, dehydrated with ascending series of ethanol, cleared, and coverslipped with Entellan™ (Merck, Darmstadt, Germany). In addition, 5-µm serial paraffin sections of rat optic nerves were stained for TGF-β2 and GFAP to identify TGF-β2-positive cells.

Cryostat sections of normal and transected sciatic nerves of adult C57BL/6 mice were stained by immunofluorescence with primary antibodies directed against Schwann cells (S-100 antigen) (polyclonal rabbit anti-S-100, Dako, code No. Z 0311, dilution 1:200), TGF-β2 (polyclonal goat anti-TGF-β2, R&D Sytems, catalog number: AF-302-NA, dilution 1:10) and the monoclonal rat anti-mouse macrophage marker F4/80 (Serotec, dilution 1:50). Secondary antibody for S-100 staining was a Cy2-conjugated goat anti-rabbit IgG, diluted 1:100 (Jackson Immuno

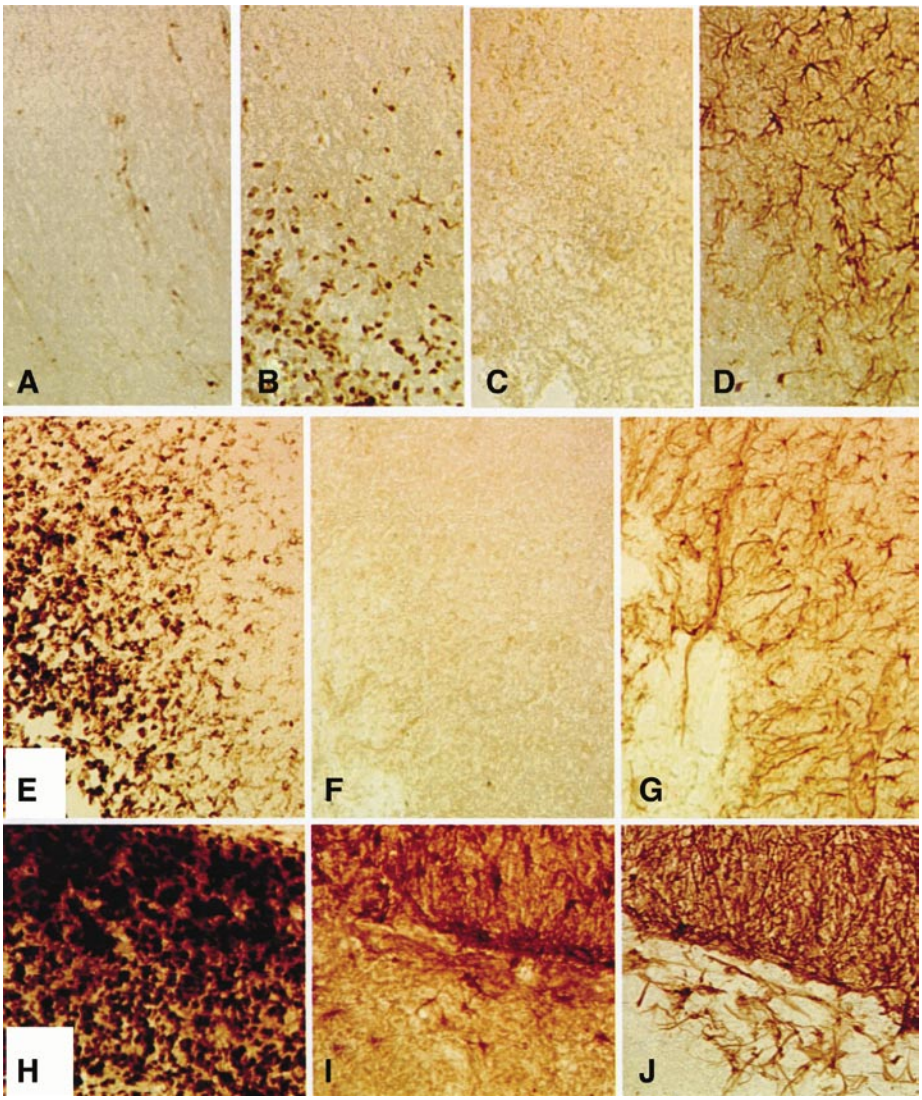

Figure 4. *Delayed induction of TGF-β2 in focal cerebral ischemia.* Border zone of cerebral infarcts stained for microglia/macrophages with antibody ED1 (**B, E, H**), TGF-β2 (**C, F, I**), and GFAP, a marker for astrocytes (**D, G, J**) at days 3 (**B-D**), 6 (**E-G**), and 28 (**H-J**) after photothrombosis. (**B-D**), (**E-G**), and (**H-J**) represent serial free floating sections. (**A**) shows a degenerating optic nerve stained with ED-1 ab for comparison. Note that a large number of ED1 phagocytes representing transformed microglia (Schroeter et al, 1997) is already present early during infarct development (**B**) and by far outnumbers ED1-positive structures in ON injury (**A**). Cortical astrocytes respond by upregulation of GFAP expression (**D**), but lack TGF-β2 immunoreactivity (**C**). At day 6 the number of phagocytic microglia had further increased (**E**), and reactive astrocytes (**G**) are still TGF-β2-negative (**F**). Only at late stages of tissue remodelling GFAP-positive astrocytes (**J**) strongly express TGF-β2 immunoreactivity (**I**). Astrocytes now overlap with large infiltrates of ED1-positive phagocytes (**H**).
Magnification (**A-J**) ×160.

Research, code number: 111-225-003). Secondary antibody for TGF-β2 staining was a biotinylated anti-goat Ig diluted 1: 50 (Amersham Pharmacia, RPN 1025), followed by strepatvidin conjugated Cy3 diluted 1:100 (Jackson Immuno Research, code number: 016-160-084). Secondary antibody for F4/80 staining was a Cy2-conjugated goat anti-rat IgG, diluted 1:100 (Jackson Immuno Research, code number: 112-225-003).

Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was isolated from pooled rat optic and sciatic nerve tissue samples, and ischemic and nonischemic contralateral cortex, respectively, using the TRIzol reagent (GIBCO BRL, Gaithersburg, Md) according to the manufacturer's instructions. RNA was quantitated spectrophotometrically. One µg of RNA was reverse transcribed using oligo$(dT)_{20}$ primers and SuperscriptII-Reverse Transcriptase (GIBCO BRL) essentially to the manufacturer's protocol. cDNA equivalent to 20 ng of total RNA was subjected to subsequent PCR analysis as detailed elsewhere (17) using primer pairs specific for rat TGF-β1 (Clontech), TGF-β2 (20), and glyceraldehyde-phosphate dehydrogenase (GAPDH) (Gillen et al, 1998). For each primer combination, optimal cycling conditions allowing linear amplification of cDNA were determined in preliminary experiments as described (13). Cycle numbers were: TGF-β1 = 24, TGF-β2 = 32, GAPDH = 20. Annealing was performed at 60°C (TGF-β1), 58°C (TGF-β2), and 58°C (GAPDH). Controls included RNA subjected to RT-PCR without addition of reverse transcriptase and PCR performed in the absence of cDNA which always yielded negative results. For each PCR product representative samples were sequenced on an ABI PRISM 310 genetic analyzer (Perkin Elmer, Oak Brook, Ill). Densitometric quantification of PCR products was done as described (17).

In vitro assay of Wallerian degeneration. Sciatic or optic nerves from adult C57BL/6 mice of either sex were dissected and the nerve sheaths were removed. Sciatic or optic nerve segments of 3 to 4 mm length were cultured in the presence or absence of macrophages for 10 days. Peritoneal macrophages were harvested from the same animals three days after intraperitoneal injection of thioglycollate by peritoneal lavage as described in detail previously (22). Medium containing 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.8 mM glutamine and 10 µM cytosine arabinoside was supplemented with recombinant TGF-β2 (R&D Systems, catalog number: 302-B2) in a concentration of 0.2 ng/ml. The medium was changed every second day. In control experiments, recombinant TGF-β2 was omitted. Three independent sets of experiments were performed.

At the end of the experiments, the nerve segments were washed in PBS, immediately fixed in 3% glutaraldehyde and postfixed in 1% osmium tetroxide. Specimens were embedded in Araldite. Semithin sections (1 µm) were stained with toluidine blue. The total area of each semithin section was determined by a 4-fold magnification (Olympus BX51) and by computer aided software ("analySIS" Soft Imaging System Muenster, Germany). The number of microglia/

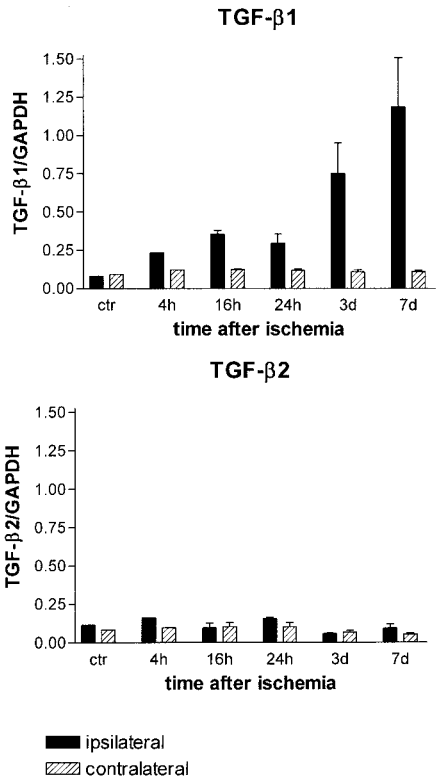

Figure 5. *TGF-β2 and TGF- β1-mRNA expression after focal cerebral ischemia* as assessed by RT-PCR in the ischemic cortex (black bars) and contralateral nonischemic hemisphere (hatched bars). Semiquantitative evaluation of TGF-β2 and-1 levels after normalization against the "house keeping" gene GAPDH. Values are given as mean ± S.E.M. from 5 animals per time point. Note the virtual absence of TGF-β2 transcripts in the normal cerebral cortex and after cerebral ischemia within the first week. Contrastingly, TGF-β1 mRNA levels steadily increased in the ischemic, but not contralateral hemisphere.

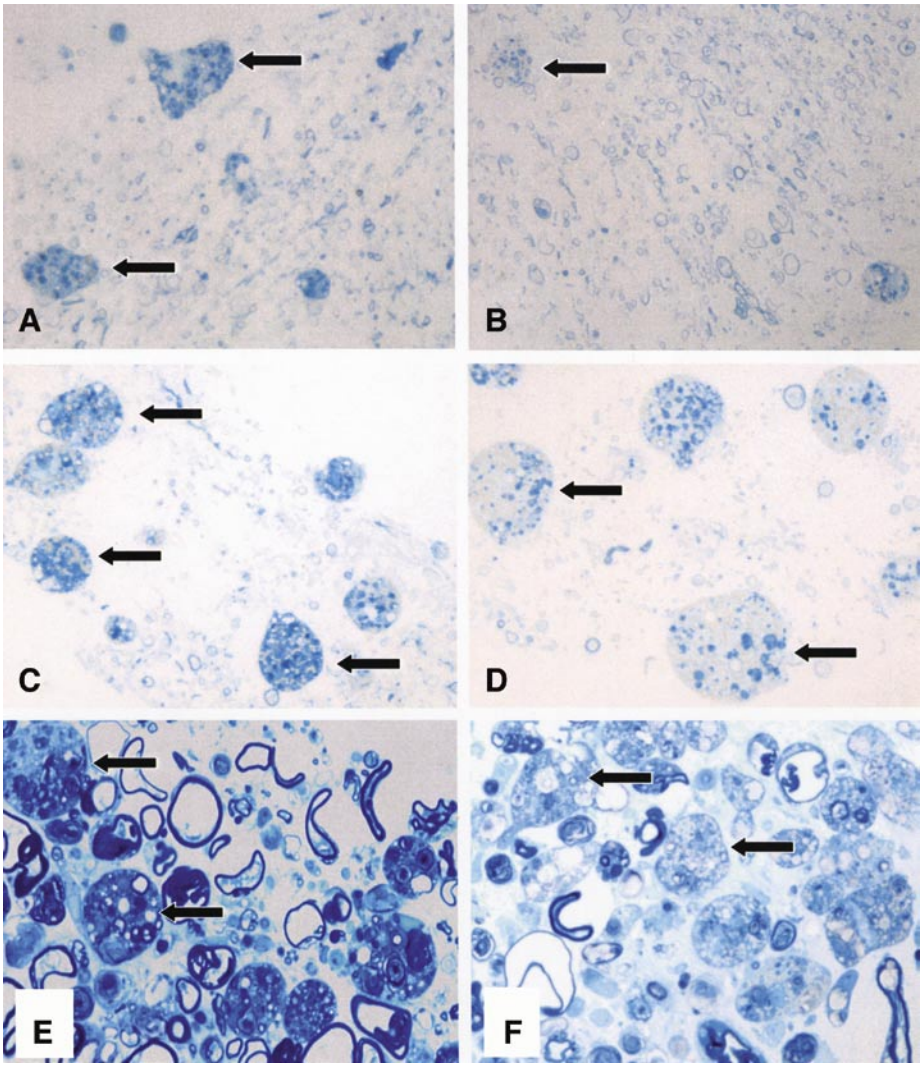

Figure 6. *In vitro assay of phagocytic activity of microglia and macrophages in degenerating ON and SN segments after incubation with TGF-β2:* (**A, B**) represent semithin sections from cultures optic nerve fragments which show spontaneous myelin phagocytosis by microglia in the absence (arrows in A) or presence of TGF-β2 (arrow in **B**). Note that myelin uptake is almost completely abolished by TGF-β2. When peritoneal macrophages were added to degenerating optic (**C, D**), or sciatic nerve (**E, F**) segments, treatment of cultures with TGF-β2 (**D, F**) similarly reduced myelin phagocytosis by macrophages compared to controls (**C, E**).

macrophages within each section was counted at a 100-fold magnification. From these data the number of macrophages per mm^2 was calculated. For determination of the myelin load in microglia/macrophages, at least 10 microglia/macrophages per semithin section were evaluated at a 100-fold magnification (Olympus BX51) and by computer aided software ("analySIS"). For measurements the program allows to create a mesh consisting of squares with 5 × 5 µm. Cross-points of the mesh which covered the cytoplasm of phagocytic cells were counted and afterwards those hitting phagocytozed myelin in the same cell were counted. The myelin load (hits on myelin) was then calculated as percent of microglia/macrophage cytoplasm (the latter = 100%). Pooled numbers of each experimental group are expressed as mean ± SD.

RESULTS

TGF-β2 persists during Wallerian degeneration in the central but is downregulated in the peripheral nervous system. After optic nerve crush microglia only slowly transform into phagocytes (29, 43). RT-PCR analysis of normal optic nerves showed high constitutive TGF-β2 transcript levels that were maintained in nerve segments undergoing Wallerian degeneration after crush (Figure 1A). By contrast, RT-PCR analysis of both normal and degenerating sciatic nerves revealed only minute TGF-β2 mRNA amounts (Figure 1A). TGF-β1 mRNA transcript levels were low in normal optic and sciatic nerves, and increased to a similar extent after nerve crush (Figure 1B). In accordance with a previous study (46), astrocytes strongly expressed TGF-β2 immunoreactivity in normal optic nerve (Figure 2A). During Wallerian degeneration of optic nerve astrocytic TGF-β2 expression persisted (Figure 2C, E, G, H). We used an antibody against TGF-β2 with no crossreactivity to the other TGF subtypes (Unsicker et al, 1991). Preabsorption of anti-TGF-β2 antibodies with recombinant TGF-β2 protein completely abolished immunoreactivity in astrocytes (Figure 2B). Staining of adjacent free floating sections with antibodies against ED1, a lysosomal marker, confirmed the limited phagocytic

	ON	ON + TGF-β2	ON + M	ON + M+ TGF-β2	SN + M	SN + M + TGF-β2
Myelin load (%)	27.7 ± 16.2	18.4 ± 10.6*	26.7 ± 13.4	13.7 ± 9.8*	37.3 ± 13.9	19.7 ± 11.5*
Macrophages or microglia/mm^2	37.4 ± 28.3	40.1 ± 31.7	566.6 ± 302.3	734.4 ± 427.1	349.4 ± 133.2	357 ± 189.9

Table 1. Myelin load and macrophage numbers in sciatic and optic nerves. SN = sciatic nerve; ON = optic nerve; M = macrophages. *: p<0.0001 against cultures without TGF-β2.

transformation of microglia in degenerating optic nerves (Figure 2D, F).

In the PNS, Schwann cells expressed TGF-β2 immunoreactivity in normal sciatic nerves (Figure 3A-C). TGF-β2 expression declined in injured sciatic nerves (Figure 3D, E) coincident with the appearance of phagocytic macrophages (Figure 3F).

Delayed TGF-β2 induction in focal cerebral infarcts. In the normal brain TGF-β2 is expressed by a subpopulation of cortical neurons and astrocytes in the white matter, but not cerebral cortex. Microglia rapidly transform into phagocytes in the vicinity of ischemic brain lesions (36). Accordingly, microglia showed intense ED1 immunoreactivity indicating robust phagocytic activation at day 3 after ischemia (Figure 4B) in contrast to microglia in the lesioned optic nerve (Figures 1D, 4A). Activated GFAP-positive cortical astrocytes at the infarct site remained TGF-β2 negative at day 3 (Figure 4C, D). At day 6, the number of phagocytes had further increased and besides typical ameboid microglia, transitional forms of ED1 positive microglia exhibiting cellular processes were abundant in the infarct border zone (Figure 4E). TGF-β2 immunoreactivity was still lacking (Figure 4F). Only after completed phagocytic transformation of microglia and macrophage infiltration at days 14 and 28 after infarction (Figure 4H), GFAP-positive astrocytes at the infarct border became TGF-β2 positive (Figure 4I, J). The absence of TGF-β2 in the vicinity of cortical ischemic lesions during the first week could be confirmed on the mRNA-level by RT-PCR. Only minute amounts of TGF-β2-mRNA could be detected in ischemic brain lesions which were not different from normal control cortex and the contralateral nonischemic hemisphere of ischemic rats (Figure 5). In contrast, TGF-β1 mRNA levels increased within 48 hours in ischemic brain lesions as reported before (24, 48).

In the white matter tracts undergoing secondary Wallerian degeneration due to destruction of the corresponding cortical neurons by focal ischemia, astrocytes maintained their TGF-β2 expression similar to optic nerves after crush, and accordingly showed a similar delayed phagocytic transformation of microglia.

Regulation of myelin phagocytosis by TGF-β2. Based on our observations that the expression of TGF-β2 was inversely related to morphological signs of phagocytic activity in microglia after traumatic and ischemic CNS lesions, we directly addressed the functional role of TGF-β2 in debris removal in an in vitro assay of Wallerian degeneration of the PNS and CNS (22). To investigate the phagocytic capacity of resident microglia cells, optic nerves were cultured in the absence of cocultured peritoneal macrophages for 10 days. In these cultures, only few resident phagocytic microglial cells were observed (Figure 6A). These cells extensively ingested the myelin fragments. Treatment with TGF-β2 significantly reduced their myelin-phagocytic capacity (Figure 6B; Table 1). When optic nerve fragments were cultured in the presence of peritoneal macrophages, the number of invading phagocytes significantly increased (Figure 6C; Table 1). Their myelin content (26.7 ± 13.4) was similar to that of resident microglia. TGF-β2 treatment significantly reduced their cytoplasmic myelin load (13.7 ± 9.8) indicating an impaired myelin phagocytic capacity (Table 1).

In addition, degenerating sciatic nerve fragments were cocultured with peritoneal macrophages for 10 days to determine their basic myelin phagocytosis rate. The cocultured macrophages invaded the degenerating nerve segments and extensively ingested the collapsed and condensed myelin fragments. The relative myelin load of the macrophage cytoplasm was 37.3 ± 13.9 (Figure 6E; Table 1). TGF-β2 treatment significantly suppressed myelin phagocytosis by macrophages leading to a lower cytoplasmic myelin load in the macrophages (19.7 ± 11.5) (Figure 6F; Table 1). TGF-β2 had no effect on macrophage infiltration into optic or sciatic nerves (Table 1).

DISCUSSION

In the present study, we have identified TGF-β2 as a lesion-associated cytokine in the CNS and PNS that could account for the suppression of phagocytic activities of microglia and macrophages. Our conclusion is based on the following observations: *i)* TGF-β2 was expressed at high levels in optic nerve injury exhibiting delayed myelin clearance, *ii)* TGF-β2 was virtually absent in the acute phase of ischemic brain lesions showing rapid phagocytic transformation of microglia, and during peripheral nerve injury characterized by fast debris removal by infiltrating macrophages, and *iii)* TGF-β2 functionally blocked myelin phagocytosis of microglia and macrophages in an in vitro assay of Wallerian degeneration.

Insufficient microglia activation is considered one major cause for the lack of spontaneous nerve regeneration in the CNS (28, 29, 33, 43). While in peripheral nerve injury, macrophages rapidly infiltrate degenerating nerves and remove growth inhibitory myelin debris (4, 29, 41), they are virtually excluded from degenerating optic nerve or central fibre tracts (12). Local microglia could compensate for the lack of entry of inflammatory cells by phagocytic transformation (11). However, although microglia recognize nerve damage and respond by upregulation of CD4, major histocompatibility complex class II molecules and increased expression of the proinflammatory cytokine interleukin-18, they only undergo slow phagocytic transformation as indicated by the long persistence of myelin debris and limited expression of the lysosomal marker ED1 (28, 33). Similarly, peritoneal macrophages preincubated with optic nerve segments exerted reduced phagocytic capacities (23). Our combined in vivo and in vitro data suggest that TGF-β2 could be the responsible suppressor of phagocytic activities. We could show

that TGF-β2 almost completely blocked the spontaneous myelin phagocytosis of microglia in vitro. Similarly, myelin phagocytosis by peritoneal macrophages added to degenerating peripheral nerve segments was reduced by 50% by adding TGF-β2 to the culture medium. Importantly, the number of macrophages was not different indicating normal macrophage recruitment into degenerating nerves in our in vitro system. However, macrophages seem to remove PNS myelin much more effectively than CNS myelin as shown by their higher myelin load. This may be due to either a phagocytosis suppressive CNS milieu or a more effective myelin recognition in the PNS. In contrast to TGF-β2, the TGF-β1 isoform which is upregulated in all kinds of injury in the nervous system, did not affect phagocytic capacities of microglia and macrophages in previous investigations (7, 40), thus pointing to distinct functions of TGF-β isoforms in the nervous system.

Vice versa, lesions with prompt phagocytic transformation of microglia such as ischemic brain infarctions were devoid of TGF-β2 expression. Cortical astrocytes in the vicinity of ischemic brain lesions quickly responded by increased expression of GFAP, but these reactive astrocytes were initially TGF-β2 negative. At a later stage of tissue reorganization when debris was mostly removed and microglia phagocytically transformed already, reactive astrocytes surrounding and demarcating the infarct area strongly upregulated TGF-β2. The functional role of delayed TGF-β2 expression in focal ischemia remains speculative at present. Astrocytic TGF-β2 could be involved in scar formation (27) and in the limitation of the ongoing inflammatory response since targeted depletion of GFAP-positive astrocytes led to a significant increase in leukocyte infiltration and neuronal degeneration after stab injury (5). In CNS autoimmunity, treatment of animals with experimental autoimmune encephalomyelitis with TGF-β2 ameliorated clinical disease (31) while mice with transgenic astrocytes developed more severe clinical signs and increased inflammation in this model for human multiple sclerosis (26).

Little is known on molecular factors that control the cellular expression of TGF-β2. While TGF-β1 can be induced by a variety of defined growth factors and cytokines (8), undefined factors within supernatants of activated T-cells were shown to modify glial TGF-β2 expression (32). Unraveling the signaling pathways of TGF-β2 maintenance and induction in the nervous system is a major challenge in respect to its pleiotropic functions which involves scar formation, immunosuppression and control of phagocytosis as shown in the present study, and may have profound therapeutic implications.

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