

Transforming Growth Factor β_2 Inhibits Cerebrovascular Changes and Brain Edema Formation in the Tumor Necrosis Factor α -Independent Early Phase of Experimental Pneumococcal Meningitis

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Summary

Macrophages and granulocytes seem to play a key role in the pathogenesis of bacterial meningitis. Transforming growth factor β (TGF- β) leads to macrophage deactivation, as well as to inhibition of cytokine production and of endothelial granulocyte adhesion. We have investigated the influence of TGF- β on regional cerebral blood flow (rCBF), intracranial pressure (ICP), and brain edema formation during the early phase of experimental meningitis. Rats which were inoculated intracisternally with live pneumococci or with pneumococcal cell wall hydrolyzed by the M1 muramidase (PCW-M) developed an increase of rCBF and ICP within 4 h postintracisternal challenge. A single intraperitoneal injection of TGF- β_2 but not of TGF- β_2 vehicle-control prevented the changes of rCBF. Furthermore, TGF- β_2 significantly reduced the increase of ICP in rats inoculated with PCW-M. Likewise, the elevation of brain water content after intracisternal injection of pneumococci or PCW-M was blocked by pretreatment of rats with TGF- β_2 . TGF- β_1 exhibited similar inhibitory effects in PCW-M-injected rats. The beneficial effects of TGF- β_2 on the initial phase after pneumococcal inoculation seem to be tumor necrosis factor α - (TNF- α) independent since (a) intracisternal or intraperitoneal injection of neutralizing anti-TNF- α antibodies did not significantly influence rCBF, ICP, and brain water content in PCW-M-induced meningitis; and (b) TNF- α was only occasionally detected at low levels in cerebrospinal fluid at 4 h after PCW-M application.

In bacterial meningitis, the major determinants of cerebral lesions seem to be alterations in cerebral blood flow, brain edema, and elevated intracranial pressure (ICP) (1). The mechanisms leading to these changes involve both bacterial components and host factors such as cytokines, arachidonic acid metabolites, platelet activating factor, complement factors, granulocytes, and reactive oxygen intermediates (1, 2). Elevated levels of TNF- α and IL-1 β have been detected in cerebrospinal fluid (CSF) of patients with bacterial meningitis, and in animal models of bacterial meningitis (3, 4). In addition, a mAb to TNF- α reduced inflammation and was also protective against brain edema in a rabbit model of pneumococcal meningitis (5). Therefore, it has been suggested that TNF- α may play a key role in the development of tissue damage in bacterial meningitis.

There is increasing evidence that TGF- β suppresses the immune response by inhibiting the expression of MHC class

II antigens, the growth of lymphocytes, and the formation of antibodies in vivo (6, 7). Furthermore, TGF- β may modulate the inflammatory response through its interference with the production of cytokines, the activation of fibroblasts and endothelial cells, and with the deposition of extracellular matrix (for review see reference 8). In the present report we demonstrate that TGF- β suppresses disease-related cerebrovascular changes and brain edema formation in the early TNF- α -independent phase of experimental pneumococcal meningitis.

Materials and Methods

Animal Model of Pneumococcal Meningitis. A well-characterized meningitis model, that was previously described in detail, was used (2). Male Wistar rats weighing 250–350 g were anesthetized intraperitoneally with thiobutabarbital (100 mg/kg), tracheotomized, and artificially ventilated (model 683 small animal ventilator; Harvard Apparatus Co. Inc., South Natick, MA). A catheter

connected to a Statham P23 pressure transducer (Viggo-Spectramed, Oxnard, CA) was inserted into the cisterna magna. A craniotomy with a diameter of 5 mm was made in the right parietal bone for the placement of the laser-Doppler probe (model BPM 403; TSI Inc., St. Paul, MN). The dura was left intact in all preparations. Regional cerebral blood flow (rCBF), ICP, mean arterial blood pressure (Statham P23 pressure transducer), and endexpiratory CO₂ (infrared CO₂ analyzer, model 2200; Heyer, Bad Ems, Germany) were continuously monitored on a multichannel paper strip recorder (model BD 101; Kipp & Zonen, Delft, The Netherlands) and by a personal computer system after analog digital conversion for signal processing. In our previous experiments using live pneumococci, the increase in rCBF reached a plateau between 4- and 6-h postinfection with only slight fluctuations in the mean values (2). Therefore, the current investigations were limited to the observation period of 4-h postintracisternal injection.

Brain Water Content Determination. At 4-h postintracisternal inoculation, the rats were killed by exsanguination. The brain was removed, weighed in a glass dish, and dried in a stove for 16 h at 130°C to a stable weight. The brain water content was calculated by (wet wt - dry wt)/wet wt × 100.

Intracisternal Inoculum. Highly purified pneumococcal cell wall solubilized by the M1 muramidase (PCW-M) was used in this study. This preparation contains cell wall in the form of disaccharide peptides and their oligomers, with and without attached choline teichoic acid. Live pneumococci strain 6b was prepared as previously described (2).

Induction of Meningitis. When a stable baseline of rCBF and ICP for 30 min was achieved, 75 μl CSF was removed through the intracisternal catheter, as described previously (2). The rats were challenged intracisternally with 75 μl 10⁷ CFU/ml live pneumococci, or 200 μg PCW-M. Both preparations had a pH of 7.4. PBS was injected into the control animals.

Effect of TGF-β₂. Recombinant human TGF-β₂ (10 μg) (6) was injected intraperitoneally in a volume of 0.2 ml PBS containing 0.1% BSA. TGF-β₂ or the vehicle of TGF-β₂ (veh_{TGF-β}) was injected 1 h before intracisternal pneumococcal challenge. TGF-β was tested at 10 μg, since this dose has been found recently to be most effective (6, 7). In an additional series (three rats), we tested the effect of 10 μg TGF-β₁ on rCBF and ICP in PCW-M-injected rats.

Effect of a Polyclonal TNF-α Ab We used a neutralizing polyclonal rabbit anti-mouse TNF-α antiserum (anti-TNF-α; Genzyme Corp., Boston, MA) crossreacting with rat TNF-α. We showed that this Ab completely neutralized TNF-α activity of LPS-stimulated rat macrophage supernatants with at least 10⁵ neutralizing U/ml. Anti-TNF were either injected intracisternally (20 μl) or intraperitoneally (100 μl) together with PCW-M (three animals each).

Assay for TNF-α. TNF-α was quantitated in a bioassay using the TNF-sensitive L-M cells (3).

Statistical Analysis. rCBF and ICP were compared against time from the point of intracisternal injection every 30 min for 4 h. The different groups of animals were compared for rCBF, ICP, and brain water content using one-way analysis of variance and Student-Newman-Keuls multiple comparisons. A *p* value of <0.05 was considered significant. Data are given as mean ± SD.

Results and Discussion

Irrespective of whether rats were inoculated with live pneumococci or with PCW-M, and irrespective of TGF-β₂ treatment P_aO₂, P_aCO₂, pH, mean arterial blood pressure, and

hematocrit levels remained within the normal ranges in all groups during the whole experiment (data not shown). Histological examinations at 4 h after injection revealed slight meningeal inflammatory infiltrates located mainly in the leptomeningeal space of the posterior fossa and the base of the cerebrum. Furthermore, at 4 h after infection, meningeal polymorphonuclear inflammation was detected in only 7/15 rats pretreated with veh_{TGF-β}, and in 2/9 rats pretreated with TGF-β₂. Intracerebral inoculation of PBS did not lead to meningeal inflammation in rats pretreated with veh_{TGF-β} or pretreated with TGF-β₂ (six rats each). Previously, we have shown that at 6-h postinfection, histological evidence of meningeal inflammation was uniformly detectable (2).

rCBF and ICP did not change in rats pretreated with TGF-β₂ or veh_{TGF-β} and then injected intracisternally with PBS (see legend to Fig. 1). In rats pretreated with veh_{TGF-β} and inoculated intracisternally with live pneumococci or PCW-M, rCBF continuously increased during the 4-h period postintracisternal injection (Fig. 1). Furthermore, an elevation of ICP was noted at 4 h after intracisternal challenge of PCW-M (Fig. 1) or live pneumococci (data not shown). TGF-β₂ inhibited the increase of both rCBF and ICP. The suppressive effect of TGF-β₂ on rCBF was observed at all time points recorded after 1 h of intracisternal infection with pneumococci or PCW-M (Fig. 1). TGF-β₁ inhibited the increase of rCBF and ICP in PCW-M-injected rats in a similar fashion (e.g., at 4-h postintracisternal injection rCBF was 121.5 ± 14.7%, and ICP was 4.4 ± 0.8 mmHg).

Brain water content was significantly elevated in rats pretreated with veh_{TGF-β} and inoculated intracisternally with live pneumococci (79.27 ± 0.23%) or PCW-M (79.35 ± 0.18%), as compared to rats pretreated with veh_{TGF-β} and inoculated intracisternally with PBS (78.87 ± 0.19%), or rats pretreated with TGF-β₂ and inoculated intracisternally with PBS (78.90 ± 0.24%). TGF-β₂ pretreatment prevented the increase of brain water content in rats challenged intracisternally with live pneumococci (78.90 ± 0.12%) or PCW-M (78.98 ± 0.23%) (*p* < 0.05).

Unlike TGF-β₂, anti-TNF-α antibodies given intracisternally or intraperitoneally did not significantly reduce the increase of rCBF in animals inoculated with PCW-M (Fig. 2). ICP increased from a baseline of 3.9 ± 0.4 mmHg to 5.8 ± 2.2 mmHg in intracisternal anti-TNF-α-treated rats, and from a baseline of 3.4 ± 2.4 mmHg to 5.7 ± 1.8 in intraperitoneal anti-TNF-α-treated rats (NS compared with 9.8 ± 2.9 mmHg in PCW-M-injected, untreated rats). Brain water content was elevated both in intracisternal anti-TNF-α-treated (79.40 ± 0.16%) and intraperitoneal anti-TNF-α-treated rats (79.29 ± 0.01%) (NS compared to 79.33 ± 0.18% in PCW-M-injected, untreated rats). Effectiveness of anti-TNF-α treatment is documented by the observation of decreased CSF cell counts at later time points after infection, e.g., at 6 h (1,620 ± 1,417 cells/μl, *n* = 5, with treatment, 4,725 ± 2,636 cells/μl, *n* = 4, without treatment, *p* < 0.05). These data confirm recent studies in rabbits infected with pneumococci in which, 6 h after infection, anti-TNF-α antibodies reduced pleocytosis (5).

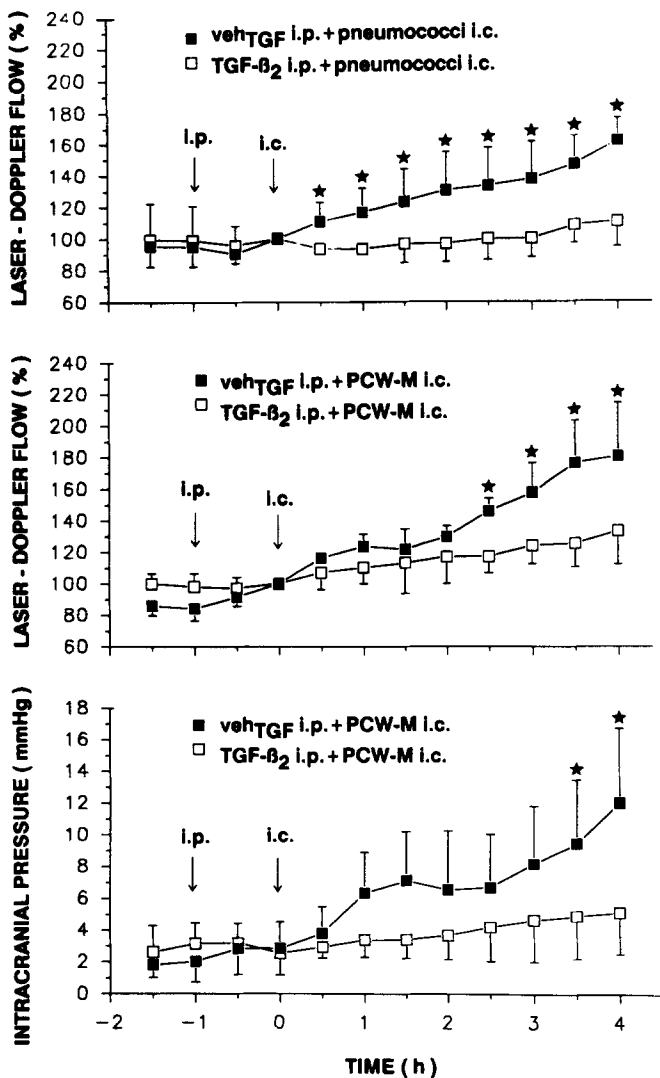


Figure 1. Effect of TGF- β_2 on rCBF in rats challenged with live pneumococci or pneumococcal cell wall components (PCW-M). rCBF was expressed as percentage of the baseline values (=100%). Within 4 h postintracisternal (*i.c.*) injection of PBS rCBF did not change in rats pretreated intraperitoneally (*i.p.*) with TGF- β_2 ($n = 7$) or veh_{TGF- β} ($n = 7$) (rCBF $103.8 \pm 16.3\%$ and $110.0 \pm 10.8\%$, respectively). However, in rats pretreated with veh_{TGF- β} and inoculated intracisternally with live pneumococci, rCBF increased to $161.0 \pm 14.3\%$ within 4-h postintracisternal injection ($n = 8$). The increase in rCBF was inhibited by TGF- β_2 pretreatment ($110.2 \pm 16.0\%$ at 4-h postintracisternal injection; $n = 7$). At 4-h postintracisternal inoculation, rats pretreated with veh_{TGF- β} and inoculated intracisternally with PCW-M ($n = 5$) showed an increase of rCBF and ICP to $182.4 \pm 49.4\%$ and 12.0 ± 4.7 mmHg, respectively. TGF- β_2 greatly attenuated both the increase in rCBF and ICP after intracisternal injection of PCW-M ($n = 5$). At 4 h after intracisternal challenge, rCBF and ICP increased to $133.2 \pm 21.2\%$ and 5.1 ± 2.6 mmHg, respectively. Data are expressed as means \pm SD. (*) $p < 0.05$, compared to rats pretreated with TGF- β_2 and inoculated intracisternally with live pneumococci or PCW-M.

The pathogenesis of the increase of rCBF, ICP, and edema formation in bacterial meningitis are only poorly understood and may be the result of a complex interplay of host factors and bacterial components. Dexamethasone, indomethacin, and

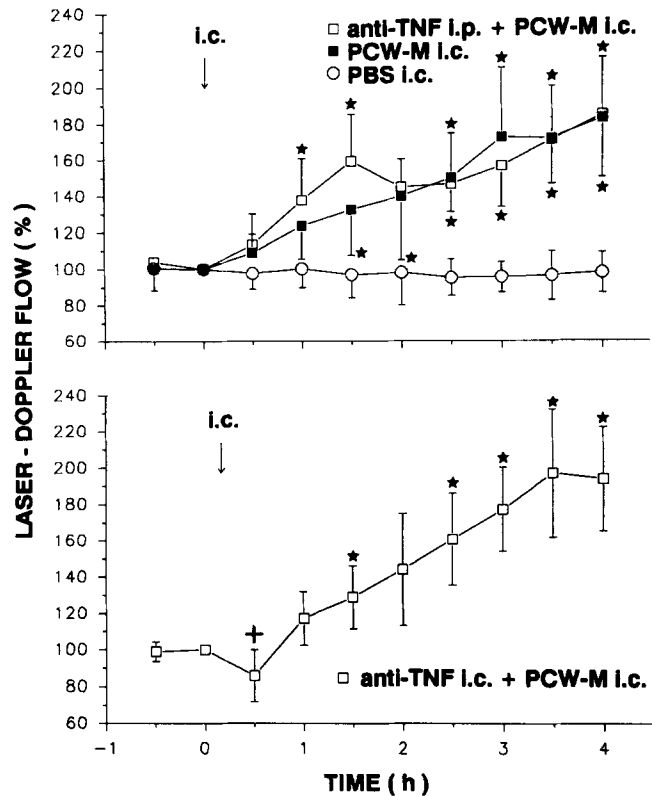


Figure 2. Effect of a polyclonal antibody to TNF- α (anti-TNF- α) on rCBF. rCBF was expressed as percentage of the baseline values (=100%). Within 4 h after intracisternal (*i.c.*) injection, rCBF increased to $193.6 \pm 28.5\%$ in intracisternal anti-TNF- α -treated ($n = 3$) and to $184.9 \pm 30.6\%$ in intraperitoneal (*i.p.*) anti-TNF- α -treated rats ($n = 3$). Data are expressed as means \pm SD. (*) $p < 0.05$, compared to PBS-injected rats; (+) $p < 0.05$, compared to rats inoculated intracisternally with PCW-M.

superoxide dismutase have been shown to attenuate the development of microvascular changes during the early phase of experimental meningitis (2). These data point to an involvement of molecules from the arachidonic acid metabolism and of oxygen-derived free radicals. The potential role of cytokines in the meningeal inflammatory process is outlined by the induction of pleocytosis in CSF and brain edema upon injection of IL-1 β , TNF- α , and macrophage inflammatory protein (5, 9). The effect of TGF- β to minimize the initial pathological events in experimental meningitis may either be due to inhibition of production of "inflammatory mediators" or may reflect interference with the effect of inflammatory mediators on target cells. TGF- β inhibits the production of TNF- α , IL-1 receptor expression and oxygen-free radicals by macrophages (8, 10-12). By the induction of the production of the IL-1 β receptor antagonist protein by monocytes, TGF- β blocks IL-1 β -mediated events (13). In addition, TGF- β blunted induction of nitric oxide release from macrophages by inflammatory agents (14). Nitric oxide is a candidate as a mediator of hyperemia as seen in our model. TGF- β has also been shown to prevent vascular endothelial cells from exhibiting an adhesive phenotype for neutrophils (15). The

transendothelial neutrophil passage is promoted by IL-1 β and TNF- α through their stimulatory effects on endothelial cells (16). Thus, TGF- β may prevent the initial alteration of the blood brain barrier in the early phase of bacterial meningitis by modulating the expression of adhesion molecules on endothelial cells, and by prevention of the action of cytokines on the endothelial cell layer. Extensive studies have characterized the production of TNF- α in bacterial meningitis including infections with pneumococci (3). However, in the present study, only low concentrations (10–60 U/ml) of TNF- α were identified in 4 of 12 CSF samples of animals at 4 h after intracisternal pneumococcal infection, and in none of 6 CSF taken 4 h after intracisternal inoculation of PCW-

M. Furthermore, an anti-TNF- α Ab injected intraperitoneally or intracisternally failed to alter the increase of rCBF, ICP, and brain water content as observed in PCW-M-challenged rats. These in vivo findings do not support a central role of TNF- α in the induction of microvascular changes and brain edema formation in the very initial phase of pneumococcal meningitis in our rat model. Thus, the protective role of TGF- β is independent of TNF- α production or biological functions. Provided that TGF- β is produced in vivo in bacterial infection, it may comprise a host factor interfering with immune pathological events altering the integrity of the endothelial barrier.

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