Published in final edited form as:

Curr Pharm Des. 2008 ; 14(16): 1620–1624.

Cytokine Transport Across the Injured Blood-Spinal Cord Barrier

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Abstract

Spinal cord injury (SCI) induces dynamic changes of the blood-spinal cord barrier and even the more distant blood-brain barrier. Besides an immediate increase of paracellular permeability resulting from the direct impact of the injury, the transport systems for selective cytokines undergo regulatory changes. Since many of the transported molecules play essential roles in neuroregeneration, we propose that this altered peripheral tissue / CNS interaction benefits remodeling of the spinal cord and functional recovery after SCI. This review examines the transport of cytokines and neurotrophic factors into the spinal cord, emphasizing the upregulation of two cytokines – tumor necrosis factor α (TNF) and leukemia inhibitory factor (LIF) - during the course of SCI. The increased transport of TNF and LIF after SCI remains saturable and does not coincide with generalized BBB disruption, highlighting a pivotal regulatory role for the blood-spinal cord barrier.

Keywords

Spinal cord injury; blood-brain barrier; cytokines; neurotrophins; TNF; LIF

1. INTRODUCTION

The blood-spinal cord barrier (BSCB) resembles the blood-brain barrier (BBB) in many ways. Their structural characteristics are essentially the same in most areas [1,2]. Whereas the BBB is adjacent to circumventricular organs in several midline regions, the BSCB is bordered by the filum terminali caudally and the nerve root entry zones laterally. By contrast to the blood supply from the carotid and vertebrobasilar systems in the brain, the spinal cord is innervated by branches from the vertebral arteries and intercostal arteries. Electron microscopy of this barrier shows endothelial cells in the spinal cord connected by tight junctions and surrounded by a thick basement membrane, as in the brain. The vascular marker lanthanum, with an atomic number of 57 in the periodic table, does not penetrate the endothelial cell membrane of the BBB at the spinal cord. Other markers, such as Evans blue albumin, iodine, and horseradish peroxidase (HRP), are also unable to cross the endothelial cells of the spinal cord in uninjured animals, showing the similarity of the spinal cord endothelial cell membrane with that of the cerebral endothelium [2].

After spinal cord injury (SCI), part of the spinal cord pathology results from changes in BSCB permeability. The importance of the BSCB is shown by MRI studies in rats in which

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restoration of barrier function after SCI at 17 days correlates with improvement in motor function [3]. Another study showed that the BSCB impermeability to HRP is restored 14 d after SCI [4]. Before this time, disruption of the BSCB after SCI plays a major role in cell and tissue injury in the spinal cord. Certainly, the extent and duration of barrier disruption are dependent on the type and severity of the injury.

Along with many others in the field of spinal cord regeneration research, we recently summarized our understanding of neural plasticity after SCI, strategies to create a regenerating environment, and approaches to target neurite growth inhibitors and extracellular matrix components, also in the journal *Current Pharmaceutical Design* [5–7]. We now know that the damaged spinal cord not only allows permeation of certain toxic substances from blood to the spinal cord temporarily, but also shows regulatory changes of transport systems at the BSCB. The transported cytokines may either serve to provide an adequate inflammatory response to facilitate neuroregeneration, or they may trigger cellular death pathways. There is evidence for both possibilities, and studies are ongoing to test the complex interactions at the BSCB and within the spinal cord.

2. TUMOR NECROSIS FACTOR- α (TNF) IN SCI

2a. TNF in CNS Inflammation

TNF expression is induced in inflammation, trauma, ischemia, and autoimmune diseases. Its increase precedes the increase of most other cytokines in conditions such as SCI [8]. TNF is transported into brain and spinal cord by a saturable transport system separate from that of interleukin 1 and all other cytokines tested [9,10]. The transport is receptor mediated, and is completely abolished in TNFR1 (p55) and TNFR2 (p75) double knockout mice [11]. Cellular studies on cerebromicrovessel endothelial cells from the receptor knockout mice and in cellular models with overexpression of TNFR1 or TNFR2 receptors show that TNFR2 plays a major role in surface binding of TNF, whereas TNFR1 mediates more efficient endocytosis [12–14].

TNF has dual actions in the CNS [15]. On the one hand, after SCI it is involved in inflammation, myelin destruction, apoptotic neuronal cell death, and astrocyte toxicity. On the other hand, TNF can stimulate neurite outgrowth, induce secretion of growth factors, and help in tissue remodeling. In this sense, TNF is expected to facilitate regeneration of the spinal cord [16–20]. Thus, upregulation of its transport after SCI could compensate for the increased need of the injured spinal cord while the saturability of the transport system limits excessive entry.

2b. Upregulation of TNF Transport after SCI

Our results of the effects of SCI on BSCB permeability to TNF evolved along several types of mouse SCI models, each addressing different aspects of injury and imitating different human injury situations. The first study involved complete transection of the lumbar spinal cord. The increased permeability in the spinal cord to TNF 24 h later could not be explained by disruption of the BSCB because of the lack of increased entry of albumin or even sucrose in most regions [21]. Moreover, the increased entry of the radioactively labeled TNF was self-inhibited by a dose of 1 μ g/mouse of unlabeled TNF. This showed that rather than being abolished by disruption of the BSCB, the transport system for TNF is enhanced in SCI mice in regions proximal to the lesion.

The second study of TNF transport after SCI involved compression of the lumbar spinal cord at L1#x2013;L2 for 5 sec. Although both albumin and TNF entry into the lumbar spinal cord increased acutely at 5 min, returning to basal levels by 1 h, the second peak of TNF entry occurred at 48 h with a corresponding increase in albumin and persisted until the end

of the study at 120 h [22]. Moreover, the increased permeability to TNF was not confined to the lumbar spinal cord but also was evident in the brain and distal spinal cord segments. As in the first study, the increased entry of TNF was abolished by addition of excess unlabeled TNF. Thus, the increased TNF transport system could not be explained by diffusion or leakage and remained saturable after SCI.

The third study involved SCI in the thoracic region by both of the methods used in the first two studies: transection and compression [23]. After transection in this higher area, entry of albumin was limited to the area of injury and only occurred immediately afterwards. TNF entered then but also again at 5 days, evident in the lumbar area. After compression, non-specific disruption of the thoracic segment was evident immediately and persisted for a 2 day period unaccompanied by an increase in the lumbar or cervical areas. By contrast, there was an increased entry of TNF in the thoracic area after compression that lasted throughout the 5 day observation period; TNF entry in the lumbar areas was significant at 4 days. This study showed that the upregulation of TNF entry after SCI is specific for time, region, and type of spinal cord lesion.

The fourth study of TNF permeation across the BSCB after SCI involved hemisection of the right lumbar spinal cord, resembling the Brown-Sequard clinical syndrome. Entry of albumin and TNF occurred immediately and only in the lumbar area [24]. By contrast, specific increase in the entry of TNF occurred in all spinal cord areas 1 week later, as well as in the brain, and this coincided with sensorimotor and gait improvement. Again, the transport system for TNF remained saturable, showing its upregulation. As an additional control, interleukin (IL)-1 β followed the pattern of albumin, not TNF. The results support the involvement of the BSCB in the recovery of SCI.

As above, the increase of TNF transport occurs later than the disruption of the BSCB immediately after SCI, at a time when general paracellular permeability is decreasing to its baseline. The dissociation of TNF and albumin uptake by the injured spinal cord suggests that the changes are not solely explained by higher paracellular permeability after injury [22,25]. The increase of TNF permeation shows specificity to the type of injury, site, and time after injury [26]. Interestingly, the increase in the entry of TNF is not only confined to the injury site (e.g., thoracic spinal cord), but it involves more distal areas, suggesting the presence of a blood-borne or other generalized regulator(s).

There are two more pieces of evidence supporting the specificity of the upregulation of TNF transport. First, the second phase of increase is not seen for interleukin (IL)-1 β , insulin-like growth factor-I, or brain-derived growth factor. Second, excess unlabeled TNF can suppress the enhanced uptake of radioactively labeled TNF, showing competition for entry *via* the known transport system for TNF. The enhanced entry of TNF at 1 week after SCI coincided with sensorimotor and gait improvement of the mouse. Thus, the permeating TNF at least does not inhibit functional recovery and perhaps facilitates it [27].

In RNA preparations from the injured spinal cord, quantitative RT-PCR analysis further showed that the increase of p55 receptor expression is more robust, being seen between 12 h and 1 wk after SCI, whereas the increase of p75 receptor expression occurs later and involves fewer regions. This indicates that upregulation of TNF transport is related to transcriptional activation of both p55 and p75 receptors [12]. Thus, the differential upregulation of p55 and p75 receptors indicates that permeation of TNF across the injured BSCB remains a regulated process.

2c. TNF Trafficking

In a fifth SCI study, the spinal cord was compressed for 5 sec LI in single and double TNF receptor knockout mice. We had previously established that deletion of either of the two TNF receptors resulted in significantly decreased entry of TNF into the spinal cord; deletion of both receptors completely abolished transport [26]. After SCI, deletion of either of the TNF receptors decreased the selective upregulation seen at 3 days in controls, the decrease being greater in the p55 (TNFR1) knockouts [12]. This is consistent with findings of the sequential increase of p55 (TNFR1) and p75 (TNFR2) mRNA preceding the time of maximal increase of TNF transport in the wildtype mice [12].

In primary mouse brain microvessel endothelial cells from the double receptor knockouts, transcytosis was also reduced even though most of the TNF crossed in intact form [12]. TNF was also exocytosed in intact form in an RBE4 model of the BBB involving both biotinylated TNF amplified by streptavidin-Quantum dots for confocal microscopy and radioactively iodinated TNF detected by electron microscopic autoradiography [13]. The results show that intact TNF can cross the BBB by vesicular trafficking.

The ability of both TNFR1 and TNFR2 to mediate TNF transcytosis is further illustrated by receptor overexpression and transport assays. TNFR1-mediated uptake of TNF was faster than TNFR2-mediated uptake of TNF. TNFR2, however, exhibited greater capacity, leading to a higher percentage release of TNF into the exocytosis medium. Rather than being degraded, most of the TNF inside the cell remained intact for 1 h. Both receptors exerted protective roles against degradation, but there was no cooperativity between them [14]. Apparently, much more needs to be done to better understand the trafficking process and the signals guiding the exocytosis events.

3. THREE RELATED MODELS OF INJURED BSCB

3a. EAE

As occurs in SCI, in acute experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, the penetration of the BBB/BSCB by TNF is enhanced. This increased transport occurs in all areas of the spinal cord as well as the brain, remains saturable, and is not explained by damage of the BBB/BSCB [27]. Dose-dependent changes in body weight and EAE scores after subcutaneous daily injection of TNF have provided some clues to the functional implications of the enhanced TNF transport. It appears that low doses of TNF may facilitate recovery, whereas high doses increase the severity of sickness [27].

As some of the CNS effects of TNF are not directly induced by TNF receptors on the target cells but are conveyed by secondary mediators, we used the cDNA microarray approach to profile the cerebral endothelial response to TNF treatment in-vitro. One of the most prominent candidates induced by TNF is the IL 15 system. As IL 15 plays important roles in EAE, studies are ongoing to determine the interrelationship between the TNF and IL 15 systems in disease progression of EAE and in generalized neuroinflammation [28].

3b. mTBI

In minimal traumatic brain injury (mTBI) to the right temporal region induced by weight drop extracranially, TNF permeation was selectively increased only in the right hippocampus 24 h after mTBI, returning to normal by 1 week [29]. This was not accompanied by increased uptake of the vascular marker albumin or IL-1 β . In these CD1 mice, there also was a change in exploratory behavior but not locomotor activity. It is

possible that altered BBB transport of TNF provides a partial explanation of reduced psychomotor performance after concussion injury.

3c. tMCAO

In a mouse model of transient middle cerebral artery occlusion (tMCAO) by filament, TNF entry into the brain was increased 5 days later, unaccompanied by an increase in albumin permeation [30]. Similar to observations in the SCI studies, this upregulation of TNF transport after tMCAO remained saturable. In cerebral microvessels obtained from either the ischemic or contralateral hemisphere, mRNA expression of both the p55 and p75 receptors for TNF showed more than a 20-fold increase 5–7 days later. Nonetheless, the mice started to recover by day 3. One may speculate that this delayed transcriptional activation of microvascular TNF receptors is induced by neuroimmune modulation of circulatory factors or by tissue remodeling factors derived from the recovering brain. Regardless, the TNF transport system participates in rewiring of the CNS after stroke.

Thus, all models of CNS injury tested show a selective upregulation of TNF transport that not only remains saturable, but functions at a higher capacity. They point to an essential role of TNF and the BBB in neuroplasticity.

4. LEUKEMIA INHIBITORY FACTOR (LIF) IN SCI

4a. LIF Transport in Normal Endothelia and Involvement of the Receptors

The neurotrophic cytokine LIF can enter the brain from blood at a rate much faster than that of the vascular marker albumin [31]. Although ciliary neurotrophic factor (CNTF) and LIF bind to the same receptor complex and saturably enter the brain, they do not use the same transport system [31,32]. The entry of LIF into spinal cord also is saturable in these normal mice.

In another study in normal mice, entry of LIF into spinal cord was decreased by inclusion of a polyclonal antibody directed against the extracellular domain of the specific gp190 receptor for LIF (LIFR) but not by a control antibody against the EGF receptor [33]. *In vitro* in RBE4 cells, both excess LIF and the LIF antibody significantly decreased the permeability coefficient.

The in-vivo studies certainly point to a mediatory role of the gp190 specific receptor for LIF on its transport across the BBB. TNF treatment causes a dose- and time-dependent decrease of gp190 protein expression by post-transcriptional regulation, mainly at the level of protein degradation by ubiquitin-proteasome pathways [34]. Concurrently, there is an increase of gp130 mRNA and protein. The reason for such dissociation of the direction of regulatory changes is not clear, but the involvement of NFκB transcription factor has been proved by use of specific inhibitors. TNF treatment causes a reduction of LIF-induced STAT3 activation and decreases LIF endocytosis by RBE4 cells [34,35]. The results suggest that LIFR (gp190) plays a greater role than gp130 in both LIF transport and signal transduction. The results also show that TNF “preconditions” the endothelia to decrease the amplitude of response to LIF, both by signaling within the cells and transporting LIF to CNS parenchyma. Here we see a lack of synergy of two proinflammatory cytokines, by contrast to what has been shown by the cooperativity of the adipokines leptin and urocortin for BBB transport [36–40].

4b. Upregulation of LIF Transport

BSCB permeability to ¹²⁵I-LIF was determined in groups of mice one week after compression SCI at the level of upper lumbar spinal cord (LI), or after sham surgery. The

lumbar spinal cord/serum ratio of ^{125}I -LIF 10 min after intravenous injection of the radiotracer was significantly higher in the SCI mice than the controls [41]. This increase was suppressed in the presence of excess unlabeled LIF or the blocking antibody against LIFR, neither of which affected the entry of albumin. The enhanced transport of LIF correlated with increased expression of LIFR, but not its non-selective gp130 receptor, as shown by immunofluorescent staining and western blot [41]. Thus, like TNF but apparently unlike BDNF and IGF1, the receptors for LIF are crucial for the regulatory function of the BSCB in SCI.

Besides SCI, the endotoxin from Gram-negative bacteria wall lipopolysaccharide (LPS) also induced upregulation of LIF transport. Mice were treated with an intraperitoneal dose of LPS (5 mg/kg), and the permeability of BBB and BSCB to ^{125}I -LIF was determined 48 h later. The LPS treated mice showed a significantly higher influx rate from blood to the CNS in comparison with the saline-treated controls. LPS treatment was accompanied by increased gp130 mRNA and protein expression, though the increase of LIFR protein was not significant. Part of the effect of LPS was mediated by TNFR1 and TNFR2, both of which showed upregulation at the level of mRNA and protein expression, since the double TNF receptor knockout mice failed to show the increase of the transporting receptors seen in the wildtype mice [42]. The increased unidirectional influx rate of LIF far exceeded the minimal change of sodium fluorescein, a paracellular permeability marker that does not cross the BBB and can even be pumped out of the brain by efflux drug transporters. Overall, LPS appears to be a positive regulator to enhance LIF permeation across the BBB/BSCB. This contrasts with the inhibiting effect of TNF on LIF endocytosis seen in the cultured cerebral endothelial RBE4 cell line described above. This suggests that part of the LPS effects may be mediated by direct activation of Toll-like receptors, and other mediators in addition to TNF.

5. SPECIFICITY

The upregulated transport of TNF and LIF after SCI, as indicated previously in this review, is not paralleled by a corresponding increase in the permeation of the vascular marker albumin. In addition, by the same multiple-time regression analysis [43] used for TNF and LIF, SCI does not increase the permeation of IL-1 β , BDNF, IGF1, or the MSH analog ebitaride [21,24,44]. Moreover, inhibition of the early phase of BSCB disruption by SCI with a bradykinin antagonist fails to affect the selective upregulation of TNF transport [45].

The entry of pituitary adenylate cyclase-activating polypeptide (PACAP)-38 was examined after SCI by transection of the cord between L2 and L3. Unlike TNF and LIF, the permeability of PACAP38 decreased in various region of the spinal cord for up to 3 days [46]. An increase occurred in all regions except the proximal lumbar spinal cord at 7 days, but not before. Saturation was tested in unlesioned mice, but not in those subjected to SCI; saturation was observed in the cervical and thoracic spinal cord, but not in the lumbar areas.

6. ENTRY OF OTHER CYTOKINES/NEUTROPHINS INTO THE UNINJURED SPINAL CORD

The studies discussed previously in this review are essentially the only ones examining the transport of peptides/polypeptides from blood to spinal cord after SCI. In unlesioned animals, several cytokines/neurotrophins have been shown to enter the spinal cord by saturable transport systems. These include IL-1 α [47], interferon- γ (saturable in cervical but not thoracic or lumbosacral areas) [10], granulocyte-macrophage colony-stimulating factor (GM-CSF) [48], and neuregulin-1- β 1 [49].

The entry of some other cytokines/neurotrophins into the spinal cord from blood does not show the self-inhibition of a saturable transport system. These include nerve growth factor (NGF), neurotrophin 4/5 (NT5), and β NGF [50]. However, the effects of SCI on the permeation of the spinal cord by these substances have not been determined.

7. CONCLUSION

SCI results in early generalized opening of the BBB/BSCB followed by later transport of a few cytokines/neurotrophic agents into the spinal cord. Most striking is the later selective transport of TNF and LIF. Their entry not only is increased, but it also remains self-inhibitable, showing saturable upregulation. This emphasizes the regulatory role of the BBB/BSCB in SCI.

Acknowledgments

Current grant support for WP and AJK is provided by NIH NS45751, NS46528, and DK54880.

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