Cytokine-induced Acute Inflammation in the Brain and Spinal Cord

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Abstract. Different compartments in the central nervous system mount distinct inflammatory responses. The meninges and choroid plexus respond to pro-inflammatory stimuli in a manner reminiscent of a peripheral inflammatory response, whereas the brain parenchyma is refractory. Trauma-induced lesions in brain and spinal cord are associated with leukocyte infiltration, blood-brain barrier (BBB) breakdown, and secondary tissue destruction. Unexpectedly, these phenomena are generally more pronounced in the parenchyma of the spinal cord than in the parenchyma of the brain. To investigate whether these differences between brain and spinal cord can be attributed, at least in part, to differing sensitivities to proinflammatory cytokines, we stereotactically injected recombinant rat (r) TNFα or rIFN-α into the striatum or the spinal cord of Wistar rats. In the brain, the injection of rTNFα failed to evoke BBB breakdown or leukocyte recruitment, whereas in the spinal cord injection of TNFα resulted in marked BBB breakdown and leukocyte recruitment. Similarly, the injection of rIFN-α into the brain parenchyma failed to induce BBB breakdown and gave rise to only minimal neutrophil recruitment, whereas the injection of rIFN-α into the spinal cord induced significant BBB breakdown and recruitment of neutrophils and lymphocytes. Thus, using a minimally invasive injection technique, equivalent in both circumstances, we have shown that there are marked differences in the inflammatory response between the brain parenchyma and spinal cord parenchyma. This observation has important implications for the treatment of spinal cord injuries.

Keywords: Blood-brain barrier; Cytokines; Interleukin-1β; Meningitis; Spinal cord injury; Tumor necrosis factor-α.

INTRODUCTION

In recent years it has been acknowledged that Alzheimer disease (1), Parkinson disease (2), prion diseases (3), and AIDS-related dementia (4) all have an inflammatory component in addition to the classic neuroinflammatory pathologies, namely multiple sclerosis and stroke. This has highlighted the need to investigate the effect the host response to an inflammatory challenge has on the integrity of the CNS. Study of inflammation in the CNS is complicated by the fact that the different compartments in the CNS—for instance the meninges, choroid plexus, and brain parenchyma—mount different inflammatory responses (5, 6). Injection of IL-1β or TNFα into the vitreous humor of the eye results in the development of an acute inflammatory response associated with hemorrhage, edema, and delays in conduction of visually evoked potentials from the eye (7, 8). Similarly, injections of IL-1β or TNFα into the ventricles induce significant neutrophil emigration into the cerebrospinal fluid and increased vascular permeability to serum proteins (9–11). However, we have shown that the direct injection of either TNFα or IL-1β into the brain parenchyma does not induce leukocyte recruitment or tissue edema acutely in adult animals (6). The factors that govern the atypical nature of the inflammatory response in the brain parenchyma compared with the response in other compartments in the CNS and to peripheral tissues remain unclear. The effect cannot be simply attributed to the lack of appropriate adhesion molecules on CNS endothelium, since important adhesion molecules are either constitutively expressed on the vasculature or are upregulated upon challenge (12). The varied inflammatory responses in different parts of the CNS may account for the way in which individual pathologies manifest themselves in human disease. Neuroinflammatory diseases, such as multiple sclerosis, also seem to affect the spinal cord more often than other parts of the CNS and traumatic lesions to the spinal cord appear to be associated with a greater inflammatory response (13). However, the inflammatory response in the spinal cord has not been studied to the same extent as the response elsewhere in the CNS.

Neuronal cell death is a prominent sequela following traumatic injury to the spinal cord and to the brain and is accompanied by a mixed, neutrophil-rich, inflammatory cell infiltrate (14, 15). Importantly, cell death continues for a period after the initial injury and may, in the spinal cord, involve longitudinal spreading of lesions in patients, leading to further loss of function (15, 16). Interestingly, the available evidence suggests that a stronger inflammatory reaction is evoked in the spinal cord following an experimentally induced lesion than is evoked in the brain (15, 17-20). The neurological outcome from spinal cord injury and traumatic brain injury results from both the primary mechanical trauma and a subsequent cascade of cellular and molecular events that are termed the “secondary injury” (21, 22). Assessing the relative contributions of these 2 components is difficult in the existing models of spinal cord and brain injury. Equally
difficult is the assessment of the contribution secondary injury makes to the outcome of traumatic brain injury as compared with spinal cord injury. This is a consequence of the technical difficulties associated with making comparable lesions in the brain and spinal cord. It is also the case that the anatomical arrangement in the brain versus the spinal cord is different: the position of the grey and white matter relative to the meninges is reversed. The mechanisms associated with secondary injury have been attributed to a number of factors, such as ischemia resulting from disrupted and blocked blood vessels, glutamate excitotoxicity (23), and free radical damage (22).

The precise functions and effects of the recruited inflammatory cells and their secreted factors are largely unclear. Therapeutically, anti-inflammatory treatment with glucocorticosteroids is given as soon as possible after acute spinal cord injury and is the regime of choice (24).

There is now a wealth of evidence suggesting that soluble IL-1β and TNFα are involved in CNS inflammation. In multiple sclerosis, the presence of TNFα and IL-1β in the cerebrospinal fluid correlates with disease activity (25), and, in animals sensitized to develop experimental allergic encephalomyelitis (EAE), intra-peritoneal injection of IL-1α promoted disease, whereas the injection of soluble IL-1 receptor antagonist delayed the onset and shortened the duration of disease (26). A role for TNFα is also implicated in EAE: for example, Rolipram, a type-IV phosphodiesterase inhibitor that suppresses the production of TNFα, is an effective treatment (27). In infectious meningitis and cerebral malaria the presence of TNFα has been correlated with the outcome of the infection (28). Within the spinal cord, as in situ hybridization demonstrates, transcripts for the proinflammatory cytokines TNFα and IL-1β as well as the chemokines MIP-1α and MIP-1β are upregulated within the first hour following experimental traumatic injury (29). Recently, the administration of exogenous TNFα or IL-1β to an experimental spinal cord lesion in the mouse after one day resulted in an increased recruitment and activation of macrophages and microglial cells in the lesion area (30). On a previous occasion, the direct injection of TNFα into naive spinal cord has been shown to give rise to cuffs of mononuclear cells around vessels and to induce mild meningitis (31).

Thus in the present study we investigate the inflammatory effects of recombinant rat (r) IL-1β or rTNFα in the brain parenchyma and in the spinal cord, using a stereotactic technique that is comparable in brain and spinal cord. In the absence of compounding and uncontrollable effects of a concomitant traumatic lesion, we use this approach to evaluate the effects of these inflammatory cytokines on vessel permeability and on leukocyte recruitment in these 2 CNS compartments at different times after challenge.

MATERIALS AND METHODS

Animals

Three-month-old Wistar rats (Charles River, U.K.) were used throughout. In each experiment, at least 4 animals were used per group to examine the response to cytokines over the time course.

Reagents

Rat recombinant IL-1β and rat recombinant TNFα were obtained from the National Institute for Biological Standards and Controls (NIBSC, Potters Bar, U.K.). All other reagents were obtained from Merck (Poole, U.K.) and were of AnaLAR grade unless otherwise stated.

Intra-cranial Stereotaxic Injections

The rats were anesthetized with Avertin (1 ml/100 g) and placed in a stereotactic frame. An incision was made in the scalp to expose bone and a 2-mm diameter burr hole was drilled through the skull to allow the tip of a finely drawn calibrated glass capillary tube to be inserted (external diameter at the tip = 50 μm). 1 μl injections of a range of doses of either IL-1β or TNFα were placed stereotaxically into the striatum, which was chosen as a large region of brain parenchyma distant from the meninges. The coordinates for the injection site were bregma, +1.2; lateral, +3.0 mm; and depth 4.5 mm. The animals recovered from the anesthetic, before being killed at either 4 hours or 24 hours. The proinflammatory cytokines were dissolved in a solution of 0.1 percent bovine serum albumin in phosphate-buffered saline. This vehicle was tested for endotoxin and was found to contain less than 1 pg/μl (5 x 10^-4 EU/μl), which, in view of previous studies (5), was considered negligible in the context of these experiments. Control injections of the bovine serum albumin in phosphate-buffered saline solution were also carried out.

Stereotaxic Spinal Cord Injections

The skin overlying the vertebral column was incised, the muscles detached from the vertebrae and a partial laminectomy performed at thoracic level 8 (T8). By clamping the vertebrae 7 and 9, the animal was then suspended in a stereotactic frame. A finely drawn calibrated glass capillary tube was stereotaxically inserted through a small opening in the dura into the gray matter of the spinal cord (+0.4 laterally and to a depth of 1.4 mm). This site was chosen for its distance from the meninges and for its close proximity to the white matter of the lateral funiculus. The injection of 1 μl of cytokine was performed over a 5-minute period. The capillary tube was then slowly withdrawn, the muscles were reapproached with sutures, and the skin was closed with wound clips. In all cases recovery was uneventful, with no overt clinical signs.

Perfusion and Tissue Preparation

After the various survival times, the rats were deeply anesthetized with sodium pentobarbital. Transcardiac perfusions were carried out with periodate-lysine paraformaldehyde containing 0.05% glutaraldehyde (PLP) (32). After dissection, brains and spinal cords were postfixed for several hours in the fixative and then immersed in 30% sucrose buffer for 24 hours.
to cryoprotect. The tissue was then embedded in Tissue Tek (Miles Inc, Elkhart, Ind.) and frozen in isopentane at −40°C.

Assessment of Blood-brain Barrier Permeability

Thirty minutes before being killed, the animals were injected intravenously with type-II horseradish peroxidase (HRP) (Sigma Chemical Co., St Louis, Mo., 10 U/kg, as a tracer of increased BBB permeability. The animals were then perfusion-fixed. Coronal, free-floating, sections were cut for HRP localization by a modified Hanke-Yates method (33). HRP has been used extensively as a tracer of altered vessel permeability, and does not increase endothelial transport during normal conditions (34, 35).

Identification of Leukocytes

Cresyl violet-stained, 50-μm-thick sections were examined for signs of neuronal damage and for the presence of leukocytes (phagocytic cells could also be identified in the Hanke-Yates-treated sections, as they are peroxidase positive). Leukocytes referred to as ‘margined’ in the text, were those cells that appeared to be adherent to the luminal side of the vascular endothelium. Cells described as ‘cuffed’ appeared to be on the abluminal side of vessels, and leukocytes referred to in the text as being ‘recruited’, were those cells that had crossed the vascular endothelium, the basement membrane, and were clearly present in the tissue. Immunohistochemistry was used to confirm the presence and distribution of specific cell populations. Frozen, 10-μm-thick serial sections were cut from the PLP fixed tissue and mounted on gelatin-coated glass slides. Antigens were detected using a 3-step indirect method (36). Polymorphonuclear neutrophils were identified using the anti-neutrophil serum HB199 (37) and macrophages were identified using the monoclonal antibodies ED1 (38), which stains most macrophage populations including recruited monocytes, but not quiescent microglia, and ED2, which is a marker for perivascular macrophages in the CNS (39). T-cells were identified using the mouse monoclonal OX19 (40) and B-cells were identified with the mouse monoclonal OX33 (41).

Quantification of Leukocyte Numbers

Neutrophils, identified by their nuclear morphology, were counted in 50-μm-thick cresyl violet-stained sections from regions immediately adjacent to the injection site. Three non-overlapping fields, containing the highest density of recruited cells within the parenchyma, were chosen and the number of neutrophils was calculated as an average number per mm² for each animal. Mononuclear cells were counted in a similar fashion. All these counts were verified immunohistochemically.

Analysis

The data are presented as the mean ± SD and where statistical analysis has been employed, Student’s t-test was applied.

RESULTS

Controls

Injection of the vehicle for rIL-1β and rTNFα into the brain did not result in any overt breakdown of the blood-brain barrier or leukocyte recruitment after 4 hours or 24 hours (Fig. 1a, b). The spinal cord, however, was more reactive to the vehicle challenge. After 4 hours we observed margination of neutrophils in the penetrating vessels, and by 24 hours there were clearly a small number of cells in the spinal cord parenchyma. However, the number in the parenchyma (120 neutrophils/mm²) was very small compared with the number recruited following the injection of either IL-1β or TNFα (see below) into the cord. Some HRP extravasation accompanied the appearance of the neutrophils in the grey matter of the spinal cord parenchyma at 24 hours (Fig. 1b).

Effects of Recombinant Rat IL-1β on Leukocyte Recruitment in the Brain and Spinal Cord

In the animals killed 4 hours after the intra-striatal injection of 1 ng rIL-1β (a previous study having shown 1 ng to be the optimal dose) there was a pronounced meningitis. The leukocyte recruitment to the meninges was predominantly neutrophilic, identified by their nuclear morphology when stained with cresyl violet (Fig. 2a) and by their immunoreactivity with HB199 (Fig. 2b). However, in HB199-stained brain sections adjacent to the injection site, only minimal margination, capping, or recruitment of leukocytes (19 neutrophils per mm² and 17 mononuclear cells per mm²) was observed 4 hours after the injection of rIL-1β (Fig. 3a). By 24 hours the number of cells had risen slightly to 145 neutrophils per mm² and 78 mononuclear cells per mm² (Fig. 3b).

In comparison to the result obtained for the brain, the pattern of leukocyte recruitment in the spinal cord in response to rIL-1β was markedly different (Fig. 2c). After 4 hours, significantly more neutrophils (Fig. 3a) and mononuclear cells had been recruited to the spinal cord (1,173 neutrophils per mm² and 220 mononuclear cells per mm²). Employing an immunohistochemical technique, T-cells and B-cells were also found to have margined within vessels within the grey matter (Fig. 2h, i). This was not a feature of rIL-1β-induced inflammation in the brain parenchyma. By 24 hours the infiltrate was predominantly comprised of neutrophils (4,930 cells/mm²), but a large number of recruited mononuclear cells were also present (848 cells/mm²). At both 4 hours and 24 hours the white matter in the spinal cord appeared to be more resistant to leukocyte recruitment when compared with the cord grey matter. However, there were always more neutrophils present in the white matter of spinal cord than were present in the brain parenchyma.

Effects of Recombinant Rat TNFα on Leukocyte Recruitment in the Brain and Spinal Cord

After 4 hours the intra-striatal injection of 300 ng rTNFα into the brain produced little or no neutrophil recruitment to the brain (3 cells/mm²) (Fig. 4a). However, some monocyte margination and monocyte recruitment
immature perivascular macrophages, which is accompanied by the de novo synthesis of ED-2 reactive antigen.

In spinal cord grey matter, the inflammatory response was brisker and the cell infiltrate 'mixed' when compared with the pattern of recruitment in the brain. Both mononuclear cells (218 cells/mm²) and neutrophils (115 cells/mm²) were recruited to the brain parenchyma by 4 hours (Fig. 2d). Although the number of mononuclear cells in the spinal cord had doubled at 4 hours, by 24 hours the proportion of neutrophils (1,366 cells/mm²) compared with mononuclear cells (1,688 cells/mm²) was much the same (Fig. 4b). The white matter of the spinal cord, however, was more resistant to the proinflammatory effects of TNFα and more restricted in terms of the population of cells recruited. Relatively few neutrophils (57 cells/mm²) had been recruited to the spinal cord by 24 hours compared with the number of recruited mononuclear cells (336 cells/mm²).

**Leukocyte Traffic in the Penetrating Vessels of the Spinal Cord White Matter**

Perhaps one of the most striking observations concerning the recruitment of leukocytes to the spinal cord following the injection of rIL-1β was the marked diapedesis of neutrophils into the abluminal space surrounding the ventro-medial vessels and other large vessels penetrating the white matter. This was most apparent in the Hanka-Yates-stained sections, where peroxidase-positive cells were seen to 'outline' the vessels (Fig. 2e). On the cresyl violet-stained sections it was clear that the peroxidase-positive cells were mainly neutrophils (Fig. 2f).

**Effects of Recombinant Rat IL-1β on Blood-brain Barrier Leakage in the Brain and Spinal Cord**

Following the injection of rIL-1β, an increase in the permeability of the BBB to serum proteins was observed around the larger penetrating vessels in the superficial layers of the cortex and in the meninges (Fig. 1c) after 4 hours. However, no increase in BBB permeability was observed around the injection site in the center of the striatum some 3.5 mm from the penetrating vessels in the overlying cortex. Furthermore, in those animals killed 24 hours after an injection of 1 ng rIL-1β, although some neutrophils were present in the brain parenchyma, there was again no evidence of BBB breakdown to HRP (Fig. 1d).

In marked contrast, the injection of rIL-1β into the spinal cord had a wholly different effect. At 4 hours,
chyma. (b) Neutrophils, identified immunohistochemically with HB199, present in the meninges 4 hours after the injection of IL-1 into the brain (magnification: ×1,200). (c) A cresyl violet-stained section of a region close to the injection site 4 hours after the injection of rIL-1β into the spinal cord (magnification: ×560). Arrows indicate the presence of neutrophils. (d) A cresyl violet-stained section of a region close to the injection site 4 hours after the injection of TNFα into the spinal cord (magnification: ×560). Note the conspicuous recruitment of mononuclear cells (arrows). (e) Peroxidase-positive cells in a Hanser-Yates-stained section following the injection of IL-1β at 4 hours. The arrow indicates the abuminar 'cuffed' cells associated with the ventromedial vessels. (f) A cresyl violet-stained section of the ventromedial vessels in an animal injected with IL-1β into the spinal cord (magnification: ×560). Large arrow indicates the large numbers of neutrophils present in the abuminar space. (g) Macrophages, identified immunohistochemically with ED1, present in the grey matter of the spinal cord 24 hours after the injection of TNFα (magnification: ×560). (h) B-cells, identified immunohistochemically with OX33, present in vessels in the spinal cord 4 hours after the injection of IL-1β. (i) T-cells, identified immunohistochemically with OX22, present in vessels in the spinal cord 4 hours after the injection of IL-1β (magnification: ×1,200). (j) ED-2-positive perivascular macrophages, in association with large penetrating vessels in the brain, following the injection of TNFα into the brain after 24 hours (magnification: ×1,200).
unlike brain, there was significant BBB breakdown. Extravasation of HRP was most marked in the grey matter and meninges, but there was also some HRP extravasation in the white matter (Fig. 1c). HRP extravasation was observed up to 6 mm both rostral and caudal to the injection site. 24 hours after the injection of rIL-1β, the BBB was still permeable to HRP. The extent of HRP extravasation was reduced compared with 4 hours after the injection of rIL-1β, but still extended 3 mm rostral and caudal to the injection site (Fig. 1d).

Effects of Recombinant Rat TNFα on Blood-brain Barrier Leakage in the Brain and Spinal Cord

In the brain, the injection of 300 ng rTNFα failed to evoke BBB breakdown after 4 hours or after 24 hours at and around the site of injection (Fig. 1e, f). After 24 hours, however, there was some evidence of mild meningitis. By contrast, in the spinal cord, injection of the same amount of rTNFα resulted in marked BBB breakdown after 4 hours (Fig. 1e), which was reminiscent of a peripheral response to the proinflammatory effects of rTNFα. Breakdown of the BBB extended for up to 3 mm rostral and caudal to the injection site. By 24 hours the area of extravasation was less pronounced, but still extended for up to 2 millimeters on either side of the injection site (Fig. 1f).

DISCUSSION

General

We have shown that, in response to comparable inflammatory challenges, there are marked differences in the inflammatory response between the brain parenchyma and the spinal cord parenchyma. In addition, there are clear differences in the response between the grey matter and the white matter in the spinal cord.

In a previous study, we found that the inflammatory response to CNS injury is much stronger in the spinal cord than in the cerebral cortex using identical lesioning techniques, and that at least part of the exacerbation of the primary lesion is due to infiltration of inflammatory cells from the periphery (42). The present study was undertaken to determine whether a tissue-specific response to individual cytokines could account, at least in part, for the observed differences. Thus, we injected IL-1β and TNFα, 2 well-established proinflammatory cytokines, into the brain and spinal cord by employing a minimally invasive stereotaxic technique. Thus, any changes in vessel permeability following the injection of cytokine can be attributed to the action of the cytokines on their receptors rather than to trauma. No increases in vessel permeability or recruitment of leukocyte to the parenchyma were associated with the injection of vehicle into the brain or into the spinal cord at 4 hours. A small amount of plasma extravasation was observed in the spinal cords of the control animals at 24 hours. However, this modest response to the injection of vehicle into the spinal cord, whereas there was no response in brain, also highlights the increased susceptibility of the spinal cord to microtrauma.

Responses of the Brain to Inflammatory Cytokines

Clearly, the brain parenchyma of an adult rat is remarkably resistant to inflammation induced by IL-1β, in contrast to the effects induced when this cytokine is injected into the spinal cord. Other investigations have focused on the effects of IL-1β on the blood-CSF barrier
Intra-cerebral injection of TNFα provoked little response in this study. Some leukocyte recruitment was observed, which was primarily monocytic, and no significant increase in vascular permeability was observed at any time after challenge at the doses employed. Following TNFα injections (10³ U) into the eye, others have noted a predominantly monocytic response (44) and a subsequent increase in vascular permeability (45). Wright and Merchant have performed intra-cerebral injections of human rTNFα: 6 × 10⁶ U provoked leukocyte adherence and cuffing from 4 hours to 48 hours (46). There was an area of leukocyte recruitment, predominantly neutrophils, in the parenchyma in the region surrounding the injection site at 48 hours. It seems likely, however, that more trauma was inflicted by their injection protocol, since even their vehicle-injected animals exhibited some vascular cuffing, comprising neutrophils and macrophages, from 4 hours to 48 hours.

In vitro experiments have regularly reported changes in permeability of brain endothelial cell monolayers in response to inflammatory cytokines. In one study using cerebrovascular-derived endothelial cells, IL-1, TNFα, and IL-6 all induced a decrease in the trans-endothelial cell resistance in vitro (47). Our findings do not support the view that there is a generalized response of the blood-brain barrier to proinflammatory cytokine in vivo. Thus, for in vitro experiments to be of relevance, the properties of an in vitro endothelial cell barrier must reflect the characteristics of the in vivo barrier it is meant to be mimicking.

Response of the Spinal Cord to the Inflammatory Cytokines

To our knowledge, only one other study has investigated the direct injection of proinflammatory cytokines into the spinal cord. Simmons and Willenborg (31) directly injected TNFα into the lumbar sacral spinal cord of rats producing meningitis and cuffs of mononuclear cells within the spinal cord. Our results support their finding: TNFα injected into the spinal cord generated an inflammatory response in the grey matter very reminiscent of a peripheral inflammatory response. Neutrophils and monocytes were rapidly recruited. In the white matter of the spinal cord, however, the response was attenuated and restricted in terms of the population of leukocytes recruited. Those signals, present in the grey matter of the spinal cord, which result in the recruitment of neutrophils, do not seem to be present in the brain or in the white matter of the spinal cord where a mononuclear cell response is predominant.

Following a standardized contusion injury to the spinal cord there is a rapid increase in the production of protein and mRNA for IL-1β (48). Before the present study, however, it was unclear what effect the synthesis of IL-1β in the spinal cord might have on the inflammatory...
response, given the unusual nature of the inflammatory response to IL-1β in the brain parenchyma. However, IL-1β injected into the spinal cord induced a marked increase in vessel permeability and recruitment of leukocytes. The recruited population, in contrast to the effect of TNFs, was predominantly made up of neutrophils. The number of neutrophils recruited to the grey matter of the spinal cord was strikingly larger than the number recruited to brain. Given the close proximity of the spinal cord white matter to the site of cytokine administration and the extent of the lesion induced, there were surprisingly few neutrophils within the fiber tracts. However, most of the larger penetrating vessels within the white matter were packed with neutrophils, which were also present abuminally as perivascular cuffs. Thus the neutrophils appeared to be excluded from entering the white matter itself—a similar result to that obtained with TNFα. However, given the response in the grey matter of the cord, there is clearly a role for IL-1β in the inflammatory response following trauma.

Mechanisms for the Disparity in Cellular Response Between Brain and Spinal Cord

It is clear from neutrophil depletion experiments that the observed increase in IL-1β-induced vessel permeability in the CNS is dependent on an interaction between endothelial cells and neutrophils, and is not due to the action of IL-1β on endothelial cells alone (6). Indeed, although IL-1 receptors are clearly present on brain endothelial cells in vivo (49), an intrastriatal injection of IL-1β did not result in an increase in vessel permeability immediately adjacent to the injection site. An injection of IL-1β does, however, trigger an increase in immunoreactive ICAM-1 on endothelial cells (50). This, and the fact that other adhesion molecules are also upregulated on brain endothelial cells (12, 51), would seem to rule out an absence of the appropriate adhesion molecules as a potential explanation for the disparity in the cellular response between CNS compartments. In trauma models too, ICAM-1 and PECAM-1 were upregulated on blood vessels in the vicinity of the lesion in both CNS compartments to a similar extent. However, upregulation of PECAM-1 did occur more quickly in the spinal (42).

Tissue invasion by inflammatory cells requires not only the presence of appropriate adhesion molecules, but also the production of chemokines to attract the cells to their target. IL-1β and TNFα are not chemotactants per se, but are known to be inducers of chemokines. One possible explanation for the observed differences in the inflammatory response in different CNS compartments may be the differential induction of chemokines. IL-1β induces interleukin-8 (IL-8) in humans and cytokine-induced neutrophil chemoattractant (CINC) in rodents, both of which are potent and specific neutrophil chemoattractants (52). We have previously demonstrated that intracerebral injection of recombinant murine macrophage inflammatory protein 2 (MIP-2) and IL-8 will overcome the resistance of adult mouse brain to neutrophil recruitment and cause BBB breakdown (53). A recent study examining the mRNA expression of MIP-2, a C-X-C chemokine involved in neutrophil recruitment, revealed no expression of this particular chemokine following spinal cord lesion despite early expression of MIP-1α mRNA 45 minutes after trauma. Other members of the C-X-C chemokine family have not been studied in the spinal cord, but are known to be expressed in the brain following ischemic lesions (54). The factors responsible for differential recruitment of neutrophils in brain and spinal cord require further investigation.

Anatomical considerations are also likely to be of importance and of these the vascular arrangement is of particular interest. Leukocytes are preferentially recruited via the venous vessels and only occasionally from the arterial side. In the rat spinal cord, the number of veins entering from the anterior median sulcus are almost twice as numerous as the arteries (55) and these constitutively express relevant adhesion molecules (ICAM-1, VCAM, PECAM-1). The vasculature of the rat spinal cord differs from the spinal cord in that there is a roughly equal proportion of veins and arteries.

Tissue Destruction by Leukocytes in the CNS

What evidence is there that the inflammatory response per se is harmful? A principal function of macrophages is the removal of tissue debris by phagocytosis. In the CNS, this process is much slower than elsewhere, and macrophages in areas of Wallerian degeneration can persist for several months (56). Macrophages secrete a large array of cytokines and proteases which can influence a variety of other cell types, but which may also cause tissue destruction directly (37). The prolonged presence of activated macrophages in CNS tissue could therefore result in a number of secondary effects, either beneficial or deleterious to functional neural recovery. Activation of microglia may also lead to the production of growth factors essential for neuronal survival and tissue repair. As an example, IL-6 will induce NGF production by astrocytes in culture (57). In contrast, the prolonged release by macrophages of proinflammatory cytokines or proteases may contribute to subsequent exacerbation of the initial tissue damage. The direct injection into the CNS of matrix metalloproteinases, which are synthesized by macrophages in multiple sclerosis and stroke lesions, results in demyelination and neuronal cell death (37). The dementia associated with AIDS has been linked to the neurotoxic substances secreted by HIV-infected activated microglia (38). Furthermore, in the developing CNS the secretion of NGF by microglia may be neurotoxic (59).
In a delayed-type hypersensitivity response in the CNS, which is directed against a non-CNS antigen, both demyelination and neuronal loss are features of the macrophage and T-lymphocyte-dominated lesions (60). In the spinal cord following a compression injury, the depletion of leukocytes or the administration of an anti-P-selectin monoclonal antibody reduced the accumulation of neutrophils in the damaged spinal cord and reduced motor disturbances (61). Thus, on balance, the existing evidence would seem to favor the argument that the presence of leukocytes within the brain and spinal cord parenchyma is very likely to be a destructive element.

Summary and Clinical Significance

The gray matter of the spinal cord, unlike brain, is susceptible to the proinflammatory effects of IL-1β and TNFα. This exaggerated inflammatory activity could have important implications for the treatment of spinal cord injuries, and may explain why certain human pathologies (such as multiple sclerosis) and experimental pathologies (such as experimental autoimmune encephalomyelitis) typically seem to affect the spinal cord more than the brain.

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