The Neuropathological and Behavioral Consequences of Intraspinal Microglial/Macrophage Activation

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Abstract. Activated microglia and macrophages (CNS macrophages) have been implicated in the secondary or “bystander” pathology (e.g. axon injury, demyelination) that accompanies traumatic or autoimmune injury to the brain and spinal cord. These cells also can provide neurotrophic support and promote axonal regeneration. Studying the divergent functional potential of CNS macrophages in trauma models is especially difficult due to the various degenerative mechanisms that are initiated prior to or concomitant with microglial/macrophage activation (e.g. hemorrhage, edema, excitotoxicity, lipid peroxidation). To study the potential impact of activated CNS macrophages on the spinal cord parenchyma, we have characterized an in vivo model of non-traumatic spinal cord neuroinflammation. Specifically, focal activation of CNS macrophages was achieved using stereotactic microinjections of zymosan. Although microinjection does not cause direct mechanical trauma, localized activation of macrophages with zymosan acts as an “inflammatory scalpel” causing tissue injury at and nearby the injection site. The present data reveal that activation of CNS macrophages in vivo can result in permanent axonal injury and demyelination. Moreover, the pathology can be graded and localized to specific white matter tracts to produce quantifiable behavioral deficits. Further development of this model will help to clarify the biological potential of microglia and macrophages and the molecular signals that control their function within the spinal cord.

Key Words: Axonal injury; Demyelination; Functional recovery; Funiculotomy; Neuroinflammation; Spinal cord injury.

INTRODUCTION

Inflammation is an inevitable but poorly understood consequence of traumatic central nervous system (CNS) injury. The influx of hematogenous macrophages and activation of resident microglia (collectively referred to as CNS macrophages) can contribute to delayed neuronal and glial cell death, a process referred to as secondary degeneration (2–4). However, these same cells can promote axonal regeneration (5, 6). Unfortunately, interpreting the biological effects of neuroinflammation in models of traumatic CNS injury is confounded by an assortment of injurious enzymatic and biochemical cascades that are initiated prior to or in parallel with inflammation. Specifically, hemorrhage, edema, extracellular accumulation of neuroexcitotoxins, and ionic imbalances make it difficult to determine the predominant effect of CNS inflammation on tissues that survive the initial traumatic event. However, a recent study by Fitch et al (7) confirmed the deleterious potential of CNS macrophages in the corpus callosum by showing that activation of these cells created a cavity devoid of astrocytes or axons. By adapting that model to the spinal cord, we now describe an in vivo method for studying the biological consequences of intraspinal macrophage activation without the confounding effects of physical trauma. Specifically, using minimally invasive stereotaxic microinjections of zymosan (a yeast cell wall preparation and potent activator of microglia/macrophages), we demonstrate that focal activation of CNS macrophages can cause permanent axonal injury and demyelination. Zymosan microinjections in radiation bone-marrow chimeric rats reveal that monocytes/hematogenous macrophages are the principal effectors of pathology in this model. Furthermore, although zymosan-mediated pathology can produce clinically silent lesions, the injection protocol can be modified such that larger injection volumes localized to discrete spinal tracts can serve as an “inflammatory scalpel” to produce significant deficits in overground locomotion. The present data illustrate the destructive potential of CNS macrophages and demonstrate the utility of this approach as a non-traumatic method of performing funiculotomies as well as a novel method for studying the functional potential of CNS macrophages in vivo.

MATERIALS AND METHODS

Animals and Microinjections

Sterile glass micropipettes were pulled to an external tip diameter of 30–50 μm and were filled with sterile phosphate-buffered saline (PBS) (0.1 M) or zymosan (Sigma, St. Louis, MO; 12.5 mg/ml) dissolved in sterile PBS. Anesthetized animals (ketamine: 50 mg/kg and xylazine: 80 mg/kg) received a single level laminectomy at the level of T8/T9 then were suspended in a stereotactic frame by the adjacent vertebral processes. Pipettes were inserted through the opened dura 1–1.2...
mm lateral to the spinal cord midline and 500–700 μm deep for lateral funiculi injections, or 300 μm from the midline and 1.7 mm deep for ventral funiculi injections. Injections (50 nl) were carried out over 5 min using calibrated pressure ejection. Adult female Sprague-Dawley rats (Harlan, Indianapolis, IN) were randomized into groups designated for anatomical/behavioral analysis of injections placed into the lateral funiculus (n = 24), ventral funiculus (n = 19), or spinal gray matter (n = 10). Although most animals survived for 3 days after injection, a subset of animals injected into the lateral funiculi survived for 7 (n = 2), 14 (n = 3) or 21 days (n = 2). A separate group of animals received up to 600 nl bilaterally into the ventral funiculi and survived for either 1, 3, or 6 wk post-injection (n = 4–7/time point).

Tissue Processing

At designated post-injection times, animals were anesthetized as before and then perfused intracardially with 100 ml of cold PBS (pH 7.4), followed by 300 ml of 4% paraformaldehyde. After marking the injection site on the dorsal spinal surface, spinal cords were removed, post-fixed for 30 min, then rinsed and stored overnight in 0.2 M phosphate buffer (PB). Tissues were cryoprotected on the following day by immersing them in 30% sucrose for 48 hours (h). Spinal cords were sequentially blocked in the transverse plane over a distance of 2 cm, using the injection site as a central reference point. Blocked tissues were embedded in cryomolds containing OCT and then frozen on dry ice. Frozen tissue blocks were sectioned at 12 μm on a cryostat (Microm, Kalamazoo, MI) and collected sequentially on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA). From a subset of tissues, a 1-mm transverse tissue block at the injection site was removed then immersion fixed in 1% glutaraldehyde/4% paraformaldehyde in 0.1 M PB. These tissue blocks were rinsed in 0.1 M PB for 30 min then post-fixed in 2% OsO4, dehydrated in ascending alcohols, and embedded in TAAB resin. Transverse semi-thin sections (1 μm) were cut from each block then were stained with alkaline toluidine blue and coverslipped.

Immunohistochemistry and Image Analysis

Standard histochemical and immunohistochemical protocols were used to characterize the cellular response to intraspinal zymosan injections (Table) (8). For morphometric comparisons of microglial/macrophage activation and regions of axonal or astroglial damage, the injection site and 2 evenly spaced (~150 μm apart) rostral and caudal tissue sections were digitized into individual channels of an image analysis system (MCID M5+, Imaging Research, St. Catherines, Ontario). The injection site was determined by identifying the section with largest area of round OX42+ microglia/macrophages. Regions of interest were manually outlined and the area quantified using the MCID system. Unpaired t-tests were used to compare area data between regions for a given antibody. Linear regression analyses were conducted to evaluate the relationship between microglia/macroglial activation and regions containing dystrophic axons (or regions devoid of axonal labeling). Statistical significance was set at p < 0.05.

Preparation of Chimeric Rats

To definitively characterize the composition of the macrophage reaction elicited by zymosan injections, we used a well-characterized radiation bone-marrow chimeric rat model (9, 10). A total of 4 chimeric rats received zymosan injections. In chimeric animals, antibodies raised against unique cell surface molecules expressed on bone marrow-derived cells (BMCs) were used to distinguish infiltrating BMCs from resident microglial-derived macrophages. Brown-Norway (BN) and (BN x Lewis)F1 rats were obtained from Harlan breeders at about 6 wk of age. BN and Lewis were used for bone marrow recipients, the F1, hybrid animals were the marrow donors. Bone marrow donors were euthanized and their femurs, tibiae, and humeri removed and placed on ice. From each bone, the epiphysial plate was snapped off exposing the marrow cavity in the shaft of the tubular bone. Using ice-cold Dulbecco’s phosphate buffered saline (DPBS), the marrow core was forced out of the bone shaft by fluid pressure exerted via a syringe fit with a 20-gauge needle. The extruded marrow was collected

**Immunohistochemical Reagents**

<table>
<thead>
<tr>
<th>Antibody Clones</th>
<th>Working dilution</th>
<th>Specificity</th>
<th>Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>I1-69</td>
<td>1:4</td>
<td>MHC RT-1A† (Lewis MHC class I) CD11b (complement type 3 receptor) on macrophages and activated microglia</td>
<td>*Serotec (Raleigh, NC)</td>
</tr>
<tr>
<td>OX 42 (anti-CD11b)</td>
<td>1:4000</td>
<td>200 kD phosphorylated neurofilament protein (axons)</td>
<td>DSHB†</td>
</tr>
<tr>
<td>RT97</td>
<td>1:2000</td>
<td>100 kD glycoprotein expressed on lysosomal membrane of activated microglia and macrophages; similar to CD68 in humans and macrosialin in mice</td>
<td>Serotec</td>
</tr>
<tr>
<td>ED 1</td>
<td>1:8000</td>
<td>Glial fibrillary acidic protein (astrocytes)</td>
<td>Sigma (St. Louis, MO)</td>
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* The I1-69 antibody was a generous gift from Dr. Hiromitsu Kimura.
† Developmental Studies Hybridoma Bank - University of Iowa (Iowa City, IA).
INTRASPINAL MICROGLIAL/MACROPHAGE ACTIVATION


Fig. 1. Intraspinal micropipette insertion is non-traumatic. Microinjections of PBS (single arrow in A and B) failed to elicit macrophage activation (A, ED1) or cause axon damage (B, neurofilament), whereas identical injections of zymosan (double arrows) produced localized inflammation and axon damage. Scale bar = 500 μm.

Behavioral Analysis

Hind limb locomotor function was evaluated daily for up to 1 wk in a total of 36 animals then at weekly intervals for 6 wk (n = 17) using a standardized open-field locomotor rating scale based on operational definitions of hind limb movement, paw placement, and coordination (11). Within a group, individual hind limb scores were averaged. Because behavioral deficits were usually not apparent for more than 3–5 days, data are not presented as function of time. Instead, data are expressed as a percent of baseline for each animal (i.e. pre-injection score of 21). For each group, pre- and post-injection behavioral scores were compared using paired t-tests. Statistical significance was set at p < 0.05.

RESULTS

Intraspinal Microinjection Is Non-Traumatic

To minimize mechanical injury to the spinal parenchyma (thereby limiting trauma-induced inflammation), glass micropipettes were pulled to an outer tip diameter of 30–50 μm. Light microscopy revealed no evidence of microglial activation or tissue injury (i.e. axons or myelin damage) due to micropipette placement (Fig. 1). Behavioral deficits were never observed (n = 5 animals) following PBS injections into the lateral or ventral thoracic white matter (not shown).

Intraspinal Activation of Microglia and Macrophages With Zymosan Causes Chronic Pathology

Nanoinjections of zymosan (50 nl) into lateral white matter of thoracic spinal cord caused marked activation of microglia and infiltration of blood-monocytes (Fig. 2A, B). Although it is logical to suspect blood-brain barrier disruption at the injection site, we did not observe erythrocytes or hemosiderin-positive macrophages at any time post-injection. Thus, bleeding and vascular injury were presumably minimal or absent. Uptregulation of ED1 immunoreactivity and the appearance of luxol fast blue in these cells (indicating myelin phagocytosis; not shown) exemplifies their increased phagocytic function (Fig. 2C).

Zones of activated microglia/macrophages clearly delineated and predicted regions of axon and astroglial loss (Figs. 2C–E, 4). In fact, phagocytic macrophages and regions of axon damage could be superimposed over a distance of 1 millimeter from the injection site (Fig. 3). However, reactive microglia were still evident beyond (200–600 μm) the injection site and regions of axon loss (Figs. 3, 4). These cells were morphologically distinct from those associated with regions of axon loss.

By 1 wk post-injection, marked inflammation, axon loss, and myelin vesiculation/demyelination remained a prominent feature of the injection site (Fig. 2F). However, by 2 wk, the injection site consisted almost entirely of inflammatory cells, connective tissue, and microvasculature (Fig. 2G). By 6 wk, inflammatory cells were
Fig. 2. Analysis of cellular responses at sites of zymosan injection. Zymosan microinjections elicit a pronounced macrophage response as identified by OX42 (A, B) and ED1 antibodies (C). Macrophages demarcate regions of axon loss (D, neurofilament staining) and areas devoid of GFAP immunoreactivity (E). Semi-thin (1 μm) sections (toluidine blue stain) through the injection site at 7 (F), 14 (G), and 42 days (H) post-injection reveal 3 distinct phases of the inflammatory reaction (see text for details). Astroglial reactivity around the site of injection (assessed by GFAP staining) is minimal at 7 days (I) but becomes increasingly more prominent at 14 (J) and 21 days post-injection (K). Note the increased thickness of the glial boundary by 21 days post-injection (K, double arrows). Box in (A) indicates region shown in sequential sections (B–E). Scale bars: A = 100 μm; B–K = 25 μm.

fewer in number (Fig. 2H) and despite the presence of endothelia and connective tissue within the injection site, neither axons nor myelin profiles were evident. An astroglial scar surrounding the injection site was minimal at 1 wk (Fig. 2I) but was more prominent between 2 and 3 wk post-injection (Fig. 2J, K, respectively). GFAP immunoreactivity was not evident within the injection site.
Fig. 3. Activated microglia/macrophages delineate regions of axon injury within and rostral/caudal to the injection site. Adjacent longitudinal sections reveal a disparity between the rostro-caudal extent of microglial/macrophage activation (A) and axon loss (absence of neurofilament staining demarcated by arrows in D). At the site of injection, phagocytic macrophages (B) co-localize with regions of axon damage (E). Note the close apposition of the large phagocytic macrophages (unlabeled cells surrounding the asterisk in E) and the prominent axonal retraction bulbs/dystrophic axons (arrows in E) at the lesion edge. Beyond the region of axon loss (box F in D), microglial activation is still evident (box C in A; high power in C). Within these rostral/caudal regions, some neurons express abnormal accumulations of phosphorylated neurofilament (arrow in F) indicative of axonal injury that presumably occurred at the injection site. Scale bars: A, D = 290 μm; B, E, F = 36 μm; C = 72 μm.
Intraspinal Activation of CNS Macrophages is Distinct in Gray and White Matter

Multiple biological variables between gray and white matter could influence inflammatory-mediated injury of the CNS. Specifically, the density of microvasculature and microglia as well as the expression of chemokines/cytokines and their signaling molecules (e.g. NFκB) are more pronounced in gray matter after SCI (12–15). Thus, we reasoned that comparative analysis of zymosan-induced inflammatory injury in spinal cord gray and white matter would be distinct. Two-dimensional quantitative morphometric analysis revealed a significant correlation between microglial/macrophage activation and tissue regions that either were devoid of axons or contained dystrophic axons (Fig. 4). We also noted that despite equivalent injection volumes into gray and white matter, morphometric measures of CNS macrophage activation were larger in the gray matter (Fig. 4B–D).

Hematogenous Macrophages are the Predominant Cell Type Associated with Axon and Myelin Pathology

To determine whether the pathology that we observed was associated with resident microglia or infiltrating monocytes, zymosan injections were performed in radiation bone-marrow chimeric rats. Using these animals, routine immunohistochemical stains can differentiate between macrophage sub-populations (9, 16). By 3 days post-injection, strong OX42 immunoreactivity (labels resident microglia and recruited macrophages) was detected at the injection site and in the surrounding white matter. However, within the lesion center where axon pathology, astroglial loss, and demyelination were greatest (Figs. 2–4), the majority of cells were I169+ revealing their hematogenous origin (i.e. derived from blood monocytes) (Fig. 5).

Neurological Consequences of In Vivo Macrophage Activation

Despite the axon pathology and demyelination described above, only 17% of animals (n = 6 of 35) revealed any abnormalities in open-field locomotion as long as 21 days post-injection. When present, deficits were manifested as occasional toe drags and/or the tail being down as the animal stepped. These deficits were usually restricted to the first 3 days post-injection.

Because the macrophage-mediated pathology caused by stereotaxic injection of zymosan can be localized to anatomically distinct regions of the spinal cord, we determined whether the inflammatory reaction could be
**DISCUSSION**

Previous studies examining the neurological consequences of surgical ablation of the ventral or lateral funiculi in cats (17–19) and rats (20) revealed significant inter-animal variability in the lesion. Ideally, to evaluate the role played by specific axonal systems on motor and/or sensory function, one would like to create selective spinal cord lesions wherein the placement and extent of the lesion can be controlled and varied in magnitude. Stereotaxic microinjection lesions can provide this precision. Indeed, well-circumscribed demyelinating lesions can be created using injections of ethidium bromide and other gliotoxins into the spinal cord (21, 22). Unfortunately, these approaches also have limitations. Specifically, unless animals are X-irradiated, ethidium bromide lesions spontaneously remyelinate and irradiation can adversely affect locomotor behavior and produce noticeable skin lesions (23). Thus, the methodological constraints of ethidium bromide would make interpretation of behavioral data difficult and the skin changes would preclude blinded trials. In the present report, we demonstrate that focal activation of macrophages via stereotaxic injection of zymosan serves as a mild, non-traumatic “inflammatory scalpel” capable of producing precise funiculotomies characterized by permanent axon loss and demyelination at the injection site. These lesions can be titrated (by varying the volume of injectate) to produce dose-dependent behavioral deficits. Thus, this model offers several advantages over more conventional approaches, including the induction of clear-cut behavioral deficits without the confounding effects of spontaneous remyelination. Moreover, because the inflammatory injury occurs in the absence of physical trauma, the cellular and molecular signals that control macrophage-mediated pathology in the CNS may be examined.

**Comparing Neuroinflammation in Brain and Spinal Cord**

Previously, Fitch et al showed that microinjections of zymosan into rat corpus callosum resulted in rapid activation of CNS macrophages with subsequent cavitation and glial scarring (7). The present data corroborate those findings with 2 notable exceptions. Cavities formed by zymosan-activated macrophages in the callosum decreased in size by 2 wk post-injection and became partially repopulated by reactive astrocytes. Following intraspinal injection of zymosan, we did not detect a change in lesion size over time nor did we observe significant repopulation of the lesion by astrocytes. These distinct cellular responses in brain and spinal cord may be due to unique mechanisms controlling inflammation in these CNS compartments.

It is has been shown that similar mechanical injuries in cortex and spinal cord elicit distinct patterns of neutrophil...
Fig. 6. Stereotaxic microinjections of zymosan into ventral spinal white matter create discrete lesions with quantifiable behavioral deficits. Although nanoliter quantities of zymosan rarely cause motor deficits in lateral or ventral funiculi, microliter quantities into ventral white matter induces tissue pathology (A) that is accompanied by variable degrees of locomotor dysfunction (B; n = 7, 4, 6 for 1, 0.6, and 0.4 μl injection, respectively). Note that by 6 wk post-injection (A, C), pathology is largely restricted to the injection site and is surrounded by a rim of hypertrophied glia (arrows in A; also see Figs. 2, 3) and morphologically intact myelinated axons (arrows in C). Morphometric analysis of lesion size (tissue area devoid of GFAP labeling) as a function of time illustrates lesion stability over a period of 3 wk post-injection (D). **p < 0.01 or *p < 0.05 vs pre-injection control function. Scale bars: A = 160 μm; C = 19 μm.

and macrophage recruitment (24). Specifically, when equivalent trauma is caused in brain and spinal cord, subsequent breakdown of the blood-brain barrier and parenchymal inflammation is markedly enhanced in spinal cord (24). These differences may be attributed to unique patterns of cytokine/chemokine signaling between these CNS compartments. For example, cytokine injections (e.g. TNF-α or IL1-β) into the cerebral cortex fail to elicit vascular damage or leukocyte recruitment, while marked inflammation is noted after similar injections into spinal cord (25, 26). The differential cellular responses in brain and spinal cord to immune-mediated injury also may be explained by regionally specific chemoanatomy and cellular composition. For example, intraocular zymosan injections promote macrophage activation that is associated with increased regeneration of injured retinal ganglion cell axons (27). The reparative potential of macrophages elicited by zymosan in that model is distinct from our present results and those reported by Fitch et al (7). However, the microenvironment of the eye can effectively attenuate the detrimental potential of activated inflammatory cells (28).

This is believed to be due in part to elevated levels of anti-inflammatory cytokines (e.g. TGFβ) (29). Similar principles of CNS site-specific regulation of immune activation may underlie the differential susceptibility of the brainstem and cerebral cortex to neuroinflammatory reactions in multiple sclerosis. Phillips et al have demonstrated enhanced lymphocyte infiltration and microglial activation within the brainstem compared with the hippocampus after immune challenge (i.e. IFNγ injections) (30). Thus, generalizing neuroimmune phenomena in the brain to the spinal cord (or vice versa) may obviate the development of effective therapies and limit our ability to reveal mechanisms of neuroimmune-mediated injury/repair.

Intraspinal Microinjections as a Model for Revealing the Divergent Functional Potential of CNS Macrophages

Although the microenvironmental cues capable of activating macrophages are undoubtedly greater in the traumatically injured CNS than following intraspinal zymosan injection, controlled delivery of pro-inflammatory
agents may be a useful tool for extrapolating the functional potential of macrophages subsets activated by spinal cord injury (SCI). For example, phagocytosis of zymosan or the myelin/neuronal debris liberated by zymosan or trauma could trigger shared intracellular signaling pathways. Such a redundancy in macrophage signaling can be inferred from studies showing NF-κB-dependent transcription of inflammatory cytokines and nitric oxide (NO) in macrophages exposed to zymosan or activated after SCI (15, 31, 32). Although specific triggering events are required to elicit microglia/macrophage functions, these signals are poorly understood in CNS trauma models. Intraparenchymal injections of zymosan or lipopolysaccharide (LPS) have proven useful in revealing some of the functional consequences of CNS macrophage activation.

In the absence of macrophages or microglia, zymosan is not toxic to neurons or glia (7). However, conditioned medium from zymosan-stimulated microglia/macrophages significantly reduced neuronal survival indicating the soluble nature of zymosan-induced neurotoxins. Glutamate, NO, proinflammatory cytokines, and matrix metalloproteinases (MMPs) are produced by zymosan-activated macrophages and have been localized to CNS macrophages in models of trauma or neuroinflammatory disease (33–36). Regardless of the triggering event (zymosan or trauma), excess accumulation of these molecules (e.g. MMPs or glutamate) at inflammatory foci could result in axon damage and demyelination (37–39). Zymosan-conditioned medium from CNS macrophage cultures also induces astrocyte migration and synthesis of extracellular matrix molecules associated with the inhibition of axonal regeneration (e.g. tenascin and chondroitin sulfate proteoglycans) (7, 40). These data reveal that some CNS macrophages also may impair neuritic sprouting and axonal regeneration thereby thwarting endogenous repair efforts by the injured CNS (3, 41).

Although we have described the pathological potential of intraparenchymal zymosan injections, others have shown that zymosan-activated macrophages can promote neural repair/regeneration (27). A similar dichotomy of macrophage-mediated repair and degeneration has been described in models of CNS trauma (2, 3, 5, 42). Thus, there may be considerable overlap in the molecular and biochemical cues that control the functional diversity in CNS macrophages. Through β2-integrins (CD11b/CD18), CNS trauma and zymosan can stimulate macrophage phagocytosis and the release of oxygen free radicals (43–45). Like zymosan and CNS trauma, intracerebral injections of LPS trigger NF-κB activation and potently induce neutrophin, cytokine, and NO production. Each of these byproducts of immune activation can affect neuronal survival and regeneration and are produced by CNS macrophages after injury (33, 46, 47). Moreover, the kinetics and composition of cytokine mRNA transcription following intracerebral LPS injection overlap with those cascades elicited by brain or spinal trauma (48–51) further substantiating the use of LPS as a generic macrophage triggering molecule in the CNS.

Further studies are required to fully evaluate the range of receptors and signaling pathways that are involved in a controlled setting like the zymosan model (or LPS) and models of greater inherent biological complexity (e.g. trauma, stroke, neurodegeneration). Indeed, macrophages (and microglia) express receptors that are essential for the rapid recognition of highly conserved molecular patterns found on microorganisms (52, 53). Zymosan and LPS (see below) bind to these pattern recognition receptors (PRR). For instance, zymosan binds to the Toll-like receptor TLR2 (54). More recently, macrophages were found to express the PRR dectin-1, which is specific for β-D-glucan carbohydrate polymers that are abundant on zymosan (55). It is not known whether microglia express dectin-1 or whether natural ligands exist for this or other PRRs in the CNS. Similarly, despite a convergence of macrophage function following trauma or LPS-mediated activation, LPS may trigger functions through the TLR4 receptor that are not elicited by trauma (54). To date, there is no evidence of a natural ligand for TLR4 in the CNS.

Together, these data suggest that zymosan, LPS and trauma elicit comparable effector functions in CNS macrophages, possibly through the activation of shared intracellular signaling pathways. By revealing distinct neurotoxic or neurotrophic functions in a simplified microenvironment, i.e. one without the cellular and biochemical cascades initiated by trauma, we may begin to reveal macrophage-specific contributions to delayed degenerative and repair processes after brain or spinal trauma.

Summary and Clinical Significance

There are several features of the present model that distinguish it from other minimally invasive techniques designed to evoke CNS-immune crosstalk. The limited solubility of zymosan minimizes diffusion allowing one to create localized and consistent cavitating lesions. These lesions can be increased in size by increasing injection volumes. Also, the injected zymosan represents a finite challenge, i.e. it is eventually cleared by recruited phagocytes. Consequently, inflammatory secretory cascades should terminate along with the possibility of lesion expansion. Given that the tissue injury caused by the inflammatory reaction is limited, subsequent inflammatory responses are likely to be minimal or absent. In fact, the increased astrogliosis around the lesion and the fact that neither astrocytes, Schwann cells, nor axons repopulate the lesion core up to 6 wk post-injection suggests the lesion is relatively stable. In contrast, LPS, TNFα, or IFNγ are soluble/diffusible mediators that once injected...
cause widespread microglial activation with or without notable pathology in brain or spinal cord (56–58). Comparative studies that evaluate the molecular and cellular responses to zymosan and non-cavitating inflammatory agents should help to unravel the complexities of the functional heterogeneity of microglia and macrophage function in the CNS. The need for this information has become increasingly more vital given that the safety of intraspinal macrophage transplantation to treat human spinal cord injury is currently being tested in clinical trials (59). A more detailed examination of the ligand-receptor interactions that lead to CNS macrophage activation should reveal molecular pathways specific to neuroinflammatory-mediated injury or repair. This information is essential for developing targeted (i.e. gene or cell-specific) immune therapies to treat virtually any neurological disease associated with activated macrophages.

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