Innate and adaptive immune responses can be beneficial for CNS repair

Michal Schwartz, Gila Moalem, Raya Leibowitz-Amit and Irun R. Cohen

The limitation of immune responsiveness in the mammalian CNS has been attributed to the intricate nature of neuronal networks, which would appear to be more susceptible than other tissues to the threat of permanent disorganization when exposed to massive inflammation. This line of logic led to the conclusion that all forms of CNS inflammation would do more harm than good, and hence, the less immune intervention the better. However, mounting evidence indicates that some forms of immune-system intervention can help to protect or restore CNS integrity. We have shown that the innate immune system, represented by activated macrophages, can facilitate the processes of regeneration in the severed spinal cord. More recently, we found that autoimmune T cells that are specific for a component of myelin can protect CNS neurons from the catastrophic secondary degeneration, which extends traumatic lesions to adjacent CNS areas that did not suffer direct damage. The challenge, therefore, is to learn how to modify immune interactions in the traumatized CNS in order to promote its post-injury maintenance and repair.


Acknowledgments
The authors’ research was supported by the Human Frontier Science Program Organization (LT-174/96). Dr R. Cohen currently holds a fellowship from the Medical Research Council of Canada and U-K.H. is supported by the German Research Foundation (SFB 507).

THE PHENOMENON of ‘immune privilege’ in the mammalian CNS is thought to derive from an evolutionary adaptation that restricts immune responses within the CNS (Ref. 1). Several mechanisms contribute to the status of the CNS as a site of immune activity of unique and possibly autonomous character. The most prominent element involved in these mechanisms is the blood-brain barrier, an anatomical and physiological barrier that keeps the CNS free from intruders. An additional mechanism is an immunological barrier, manifested by: (1) the reduced expression of major histocompatibility complex class-I and class-II antigens on certain cells in the CNS (Refs 3, 4), and (2) an immune-suppressive micro-environment that contains, for example, astrocytes that suppress or anergize invading T cells5, and locally produces factors that suppress and regulate the production of immune responses in the CNS (Refs 7, 8). These two barriers, which limit both the entry of immune cells into the CNS and their activity there, are thought to protect against remodeling of the dynamic

Michal Schwartz and Raya Leibowitz-Amit are at the Dept of Neurobiology, Gila Moalem is at the Depts of Neurobiology and Immunology, and Irun R. Cohen is at the Dept of Immunology, The Weizmann Institute of Science, 76100 Rehovot, Israel.
and complex neural network of the brain. A unique immunological feature of the CNS, which possibly evolved as partial compensation for the above limitation, is the abundant presence of resident macrophages, the microglia, which occur in resting form and account for approximately 12% of the total brain-cell population.

In tissues other than the CNS, immune responses have a pivotal role in maintenance and repair, which raises the following questions: does the CNS, like other tissues, depend on immune responses for its maintenance and repair, particularly after injury? And, if so, is the restricted activity of CNS-resident immune cells sufficient for this purpose? The data presented in this article suggest that the injured CNS does indeed require immune intervention in order to limit damage and to activate healing, but that under normal circumstances the CNS is relatively inaccessible to such intervention because of the restrictions associated with its immune-privileged status.

The involvement of immune activity in CNS repair and maintenance can be seen, for example, in the case of white-matter injury, that is, injury to axons. Such injury initiates a process of degeneration at the injury site, which is accompanied by an inability of the nerve fibers to regrow and reconnect, and usually culminates in the death of the corresponding cell bodies. In addition, un-damaged fibers in the vicinity of the injured axons become affected by the lateral spread of damage and consequently undergo secondary degeneration, unless they are treated adequately. This lateral, secondary degeneration appears to be mediated by agents such as glutamate, free radicals or additional mediators of toxicity, some of which might be associated exclusively with CNS axonal injury rather than a direct injury to cell bodies. Attempts to minimize the spread of degeneration following axonal injury, using the rat optic nerve or spinal cord as a model, have yielded some insights into the mechanisms involved in degeneration and have led to the identification of specific molecules with neuroprotective properties. Thus, for example, the observed reduction in secondary degeneration that followed immediate treatment of an injury with a single dose of methylprednisolone was attributed to the reduction of local inflammation that this compound produces. Accordingly, inflammatory cells were considered to be harmful to damaged axons. The beneficial effect of methylprednisolone, either as a result of its anti-inflammatory activity or of its other actions, might be as a neuroprotective agent and, accordingly, it might be exerted only at an early posttraumatic stage. For regrowth, however, inflammatory cells appear to be important. In vitro studies have shown that the phagocytic activity of macrophages is enhanced on their exposure to PNS nerve segments and then applying equal numbers of macrophages to the site of injury in the optic nerve or spinal-cord models. By means of anterograde and retrograde labeling of transected optic nerves of adult rats, we have demonstrated morphologically that PNS-activated macrophages are more beneficial for axonal regrowth than are CNS-activated or non-activated macrophages. Regrowth of axons is correlated with the speedy clearance of myelin from the treated axons and with the abundant distribution of PNS-activated macrophages along the distal part of the damaged axons (in contrast to the limited macrophage distribution in transected, untreated axons, or in transected axons exposed to CNS-activated macrophages or to non-activated macrophages). The beneficial effect of the macrophages on regrowth is not diminished by treatment with dexamethasone. Some functional recovery has been demonstrated in studies of adult-rat spinal cord, where local application of PNS-activated macrophages to the completely transected spinal cord leads to the partial recovery of otherwise paraplegic rats. Recovery is manifested by acquisition of locomotor activity, which is tested in an open-field by measuring the generation of mototelic potential responses in the hind-limb muscles, and by morphological alterations that meet specific criteria. The above data support three conclusions. First, the CNS is not intrinsically refractory to the processes of healing and regrowth. Second, the ability of activated macrophages to promote CNS healing and regrowth is, in principle, not unlike that promoted by the innate inflammatory response in other organs. Third, the failure of the CNS to regrow can be attributed, at least in part, to a relative inability of the damaged CNS to recruit and activate a restorative inflammatory response to tissue damage. Thus, it appears that the immune-privileged status of the CNS, at least in some circumstances, might be disadvantageous and even detrimental. The remainder of this article contains a review of our recent findings that autoimmune T cells, like activated macrophages, can benefit the damaged CNS.
Passive transfer of CNS-antigen-specific autoimmune T cells limits secondary degeneration

Macrophages do not bear receptors for antigens and they lack immune memory, thus representing the non-adaptive, innate arm of the immune response. T cells, in contrast, respond to specific antigens and ‘remember’ past experience, and so represent part of the adaptive arm of the immune response. When activated, the T cells can kill their target cells or produce signal molecules that activate or suppress the growth, movement or differentiation of other cells. Thus, T cells are involved in protecting the individual against foreign invaders as well as in maintaining body function. The blood–brain barrier of the CNS is normally impermeable to resting T cells, but is permeable to activated T cells. Activated T cells, however, do not accumulate in the healthy CNS unless they recognize and are able to react to their specific antigen there.

In order to determine whether increasing T-cell accumulation is beneficial or harmful to the injured CNS, we used an experimental model of a partial lesion of the rat optic nerve, which allows the assessment of nerve maintenance after traumatic axonal injury. We found that axonal injury was followed by a transient accumulation of endogenous T cells in the injured PNS than in the injured CNS (Ref. 45). Moreover, the CNS shows a marked propensity for elimination of T cells via apoptosis, whereas this mechanism is less effective in the PNS and is almost absent in other tissues such as muscle and skin. These findings suggest that the T-cell response in the traumatized CNS is both restricted and tightly regulated. Is this limitation in T-cell response disadvantageous to the CNS?

Comparative studies of the T-cell response at sites of optic-nerve and sciatic-nerve injury, using T-cell immunocytochemistry, have revealed a significantly greater accumulation of endogenous T cells in the injured PNS than in the injured CNS (Ref. 45). Moreover, the CNS shows a marked propensity for elimination of T cells via apoptosis, whereas this mechanism is less effective in the PNS and is almost absent in other tissues such as muscle and skin. These findings suggest that the T-cell response in the traumatized CNS is both restricted and tightly regulated. This limitation in T-cell response disadvantageous to the CNS?

Interestingly, the protection of neurons from secondary degeneration was not related to the intrinsic pathogenicity of the anti-MBP T cells. The disease induced by T cells specific to a cryptic epitope of MBP, p51–70, was significantly milder, if seen at all, than that induced by the anti-MBP T cells. Nevertheless, this weakly pathogenic anti-p51–70 T-cell line was as effective in reducing secondary degeneration as the highly pathogenic anti-MBP T-cell line. Thus, the induction of clinical autoimmune disease was not a prerequisite for the protection against secondary degeneration mediated by the anti-MBP T-cell lines.

We do not yet know how the anti-MBP T cells arrest the progression of secondary degeneration, although it is known that T cells can synthesize cytokines and...
and to growth-factor secretion, resulting in neuroprotection.

To growth-factor secretion, resulting in neuroprotection. Adoptive transfer of autoimmune T cells (left) led to a reduction in energy requirements, caused by transient inhibition of nerve conduction ('neuronal rest'), resulting in axonal regeneration. Macrophage implantation (right) led to the removal of neuronal growth inhibitors by myelin clearance and to growth-factor secretion. A model of partial white-matter injury was used for studying regeneration and a model of complete transection in mice. These experiments with respect to their cytokine and neurotrophic factors. Alternatively, or in addition, it is known clinically that arresting nerve metabolism (for example, by hypothermia) can help to limit the spread of CNS damage after injury. Some years ago, we reported that anti-MBP T cells could reversibly inhibit nerve conduction in vivo. In support of this finding, we further showed that the anti-MBP T-cell line indeed produced a transient but significant inhibition of electrophysiological activity in the injured nerve, and that this inhibition of nerve conduction coincided with the peak of T-cell accumulation at the injury site. There is evidence that cytokines can affect the electrophysiological functions of neurons and glial cells directly. Thus, cytokines secreted by anti-MBP T cells at the injury site might induce a transient reduction in neuronal excitability, for example, by increasing inactivation of the Na⁺ current. These findings suggest that the anti-MBP T cells might reduce injury-induced secondary damage by inducing a transient resting state in the damaged nerve, thereby reducing its energy demands and enhancing its ability to cope with the stress that results from the injury. At this stage of study, we do not know whether the neuroprotection mediated by passive transfer of autoimmune T cells occurs directly or whether it is an indirect effect that involves other cells, such as macrophages.

Autoimmunity in the CNS

T cells that react specifically to CNS-myelin antigens have justifiably earned a bad reputation. Such autoimmune T cells cause the potentially lethal disease EAE in animals and are associated with multiple sclerosis in humans. Nevertheless, despite the classical teaching that potentially pathogenic T cells should not exist in healthy individuals, it is experimentally evident that MBP-responsive T cells can be isolated from healthy individuals and not only from patients suffering from multiple sclerosis. Natural autoimmunity to specific dominant self-antigens such as MBP was proposed to represent the immune system's positive picture of the individual self, the 'immunological homunculus'. Indeed, components of myelin appear to be prominent among the limited set of self-antigens to which autoimmunity naturally exists. Moreover, CNS trauma was found to elicit an autoimmune response against a component of CNS myelin. Spinal cord transection was shown to cause direct sensitization of the host immune system to MBP and, indeed, when injected intravenously into naive rats, systemic T cells isolated from spinal-cord-injured rats caused neurological deficits that were similar to EAE. Thus, it seems that autoimmunity is awakened in response to CNS injury. It is conceivable that the endogenous T cells that accumulate spontaneously at sites of CNS injury arise from an injury-triggered autoimmune response. It might be beneficial but too weak and in need of boosting, or inappropriate and in need of modification. The results presented above suggest that, under certain circumstances, autoimmunity might be beneficial in CNS maintenance. We infer that the spontaneous autoimmune response is not optimal with respect to what is needed to prevent secondary damage.

Recent evidence suggests that inflammation in the CNS is associated with an altered presentation of endogenous MBP, which results in activation of T cells that are specific for cryptic epitopes possibly hidden in intact nerves. Epitopes that are not accessible in the intact CNS might become sufficiently accessible after injury to be seen by receptor-bearing T cells. This might explain the similarity in the neuroprotective effects induced by the anti-p51–70 T cells and the anti-MBP T cells, despite their differing effects in the intact CNS. Accordingly, it might be worthwhile seeking ways to augment a beneficial autoimmune response therapeutically without triggering a persisting autoimmune disease. Such augmentation might be achieved, for example, by employing T cells that are specific to the self-antigenic epitopes normally sequestered in the intact CNS. These autoimmune T cells would not accumulate or interact with undamaged areas and, thus, would not induce disease, yet they might be able to assist in the repair of injured CNS tissue if the injury should expose the covert epitope.
The results presented in this article suggest that activated, anti-CNS T cells (which convey adaptive autoimmune) respectively on innate immunity, can help to sustain the injured CNS. Figure 2 summarizes how activated macrophages and autoimmunity and T cells might promote re-growth and protection from secondary degeneration in the CNS following white-matter injury. The challenge, then, is to learn how to manipulate the inability of the CNS to recover after injury is the price plate the potential for CNS repair. It is possible that the species that we now find ourselves able to contemplate were not available during most of vertebrate evolution. Furthermore, intensive care and life-support systems can help to sustain the healthy and intact CNS, but becomes dis-advantagous once the CNS has suffered injury. Our present theory is that immune privilege in the CNS had evolved to save the organism was seen to be viewed as a life protector; the immune system and how to supply the site. The present theory is that immune privilege is an optimal solution for ongoing maintenance of the healthy and intact CNS, but becomes dis-advantagous once the CNS has suffered injury. Furthermore, intensive care and life-support systems were not available during most of vertebrate evolution. It is only through the cultural evolution of the human species that we now find ourselves able to contemplate the potential for CNS repair. It is possible that the inability of the CNS to recover after injury is the price plate the potential for CNS repair. It is possible that the inability of the CNS to recover after injury is the price plate the potential for CNS repair.

References
19 Honda, D.A. et al. (1991) Brain Res. 567, 1–19
22 Yoshino, A. et al. (1997) Brain Res. 756, 106–119
38 Ben-Zeev-Drum, A. et al. (1990) Brain 23, 181–190
45 Moshal, G. et al. (FASEB J. (in press)
67 Eti Yoles for editorial assistance, and Igor Frank and Lin Fei for help with graphics. The authors’ research was supported in part by the Allen Brown Foundation for Spinal Cord Injuries (M.S.), by the US Army (M.S.) and by an Alan award (M.S.).

Acknowledgments

The authors’ research was supported in part by the Allen Brown Foundation for Spinal Cord Injuries (M.S.), by the US Army (M.S.) and by an Alan award (M.S.).