Published in final edited form as:

Neuroscience. 2009 February 6; 158(3): 1039–1048. doi:10.1016/j.neuroscience.2008.07.036.

THE ROLE OF MACROPHAGES IN OPTIC NERVE REGENERATION

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Abstract

Following injury to the nervous system, the activation of macrophages, microglia, and T-cells profoundly affects the ability of neurons to survive and to regenerate damaged axons. The primary visual pathway provides a well-defined model system for investigating the interactions between the immune system and the nervous system after neural injury. Following damage to the optic nerve in mice and rats, retinal ganglion cells, the projection neurons of the eye, normally fail to regenerate their axons and soon begin to die. Induction of an inflammatory response in the vitreous strongly enhances the survival of retinal ganglion cells and enables these cells to regenerate lengthy axons beyond the injury site. T cells modulate this response, whereas microglia are thought to contribute to the loss of retinal ganglion cells in this model and in certain ocular diseases. This review discusses the complex and sometimes paradoxical actions of blood-borne macrophages, resident microglia, and T-cells in determining the outcome of injury in the primary visual pathway.

Keywords

optic nerve; regeneration; oncomodulin; monocytes; central nervous system; inflammation

The optic nerve has long served as a model for understanding regenerative success or failure in the CNS (Benowitz and Yin, 2008). Under normal circumstances, mature retinal ganglion cells (RGCs) fail to regrow axons beyond the site of optic nerve injury, and if axotomy occurs within the orbit, most RGCs go on to die within a few weeks (Mey and Thanos, 1993; Berkelaar et al., 1994). Regenerative failure is not inevitable, however. In his classic work, "Degeneration and Regeneration of the Nervous System," Ramon y Cajal (DeFelipe and Jones, 1991) described Tello's discovery that mature RGCs can regenerate axons through a peripheral nerve graft sutured to the cut end of the optic nerve, and concluded that "the regenerative failure of the central paths is ... an accidental condition due to the neuroglial environment." Expanding on this observation, Aguayo and colleagues (Richardson et al., 1980; David and Aguayo, 1981; Aguayo et al., 1991) carried out many studies demonstrating that mature RGCs and other CNS neurons retain the potential to regenerate their axons in a more favorable environment, sparking renewed interest in the factors that support or inhibit nerve regeneration. Recent data show that the immune response dramatically affects the outcome of injury to the optic nerve. While neuro-immune crosstalk in CNS diseases is covered in another article in this issue by Kerschensteiner et al., 2009, this review discusses current knowledge on the role of blood-

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derived macrophages, resident microglia, and T-cells in RGC survival and/or axonal regeneration after injury to the primary visual pathway.

THE IMMUNE RESPONSE IN THE CNS AND PERIPHERAL NERVOUS SYSTEM (PNS)

The immune system operates via two separate but closely interacting subsystems: innate immunity as the antigen-independent arm and adaptive immunity as the antigen-specific arm. Inflammatory responses during traumatic and degenerative CNS diseases are dominated by cells of the innate immune system, most importantly resident microglia and blood-borne macrophages (Schroeter and Jander, 2005). After phagocytosing cellular debris, microglia/macrophages present antigens to lymphocytes, thereby activating the antigen-specific arm of the immune response (Suzumura et al., 1987; Abromson-Leeman et al., 1993; Ulvestad et al., 1994). Inflammation is the key component of host defense responses to injury, tissue ischemia, autoimmune reactions or infectious agents (Allan and Rothwell, 2003; Lucas et al., 2006). In general, the effects of inflammation are complex, and depending on the mode of stimulation, inflammation can have neuroprotective or neurotoxic effects or even a mixture of the two (David et al., 1990; Huitinga et al., 1990, 1995; Lu and Richardson, 1991; Dijkstra et al., 1992; Minghetti et al., 1999; Stoll et al., 2002; Wolf et al., 2002; Yin et al., 2003; Byram et al., 2004; Correale and Villa, 2004; Butovsky et al., 2005).

The inflammatory response in the CNS is attenuated relative to that in the PNS and other tissues. In fact, the CNS had long been thought to be an immune-privileged site, stemming from the properties of the blood–brain barrier (BBB) in restricting the passage of large molecules and cells into the CNS, the lack of draining lymphatics, and the apparent immunoincompetence of microglia in the CNS (Shrikant and Benveniste, 1996). However, recent evidence shows that both T and B lymphocytes can cross the BBB (Kajiwara et al., 1990; Hickey et al., 1991; Knopf et al., 1998; Becher et al., 2000), and that the CNS is far from passive in its interactions with the immune system (Ransohoff et al., 2003; Carson et al., 2006). Nonetheless, relative to the PNS, cellular infiltration is delayed and relatively weak in the CNS following traumatic injury or as a result of inflammatory responses (Andersson et al., 1992a,b; Avellino et al., 1995; Perry et al., 1995; Reichert and Rotshenker, 1996; Moalem et al., 1999; Allan and Rothwell, 2001; Jander et al., 2001). Following a crush injury of the optic nerve, infiltration of blood-borne macrophages into the degenerating distal segment of the nerve is considerably less than is seen in the sciatic nerve (Perry et al., 1987); this is also the case after the optic nerve is cut (Reichert and Rotshenker, 1996). One of the major roles that macrophages play after nervous system injury is to remove myelin debris (Stoll et al., 1989), thus facilitating axon regrowth and remyelination. The high capacity of PNS neurons to regrow injured axons is paralleled by the recruitment of large numbers of macrophages to the degenerating area, whereas the poor regenerative capacity of CNS neurons is correlated with a more limited presence of macrophages at the injury site. The phagocytic activity of macrophages or brain-derived microglia is enhanced upon exposure to sciatic nerve segments, but is inhibited by exposure to optic nerve segments (Zeev-Brann et al., 1998). The low microglia/macrophage activity in the CNS after injury may be related to the presence of specific inhibitors or to an absence of chemoattractants. A greater accumulation of T cells in the injured sciatic nerve than in the injured optic nerve has also been reported (Moalem et al., 1999).

INTRAVITREAL INFLAMMATION STIMULATES OPTIC NERVE REGENERATION

Prior to undergoing apoptotic cell death, axotomized RGCs show transitory axon sprouting at the injury site but almost no long-distance regeneration (DeFelipe and Jones, 1991; Ramon y

Cajal, 1991). Macrophage-derived EphB3 mediates this sprouting (Liu X et al., 2006). The transfer of peripherally activated macrophages into the visual system was previously reported to enhance axonal regeneration (David et al., 1990; Lazarov-Spiegler et al., 1996; Rapalino et al., 1998). In more recent studies, the direct induction of an inflammatory response in the eye was shown to have dramatic effects on RGC survival and axon regeneration (Leon et al., 2000; Yin et al., 2003; Lorber et al., 2005; Pernet and Di Polo, 2006; Luo et al., 2007b; Fig. 1). As a result of either injuring the lens or injecting zymosan, a yeast cell-wall preparation, into the eye and subsequent activation of macrophages, the survival of axotomized RGCs increases eightfold to 10-fold and axon regeneration beyond the site of optic nerve injury increases 100-fold or more, with some axons extending almost as far as the optic chiasma (Leon et al., 2000; Fischer et al., 2001; Yin et al., 2003; Lorber et al., 2005; Pernet and Di Polo, 2006). It is important to note that this regeneration occurs through the optic nerve itself, a CNS environment that is normally hostile to axon regeneration. In addition, if one simultaneously counteracts the inhibitory effects of myelin and the glial scar that forms at the injury site, the amount of regeneration obtained increases several-fold, with numerous axons growing right through the scar (Fischer et al., 2004a,b). The effects of intravitreal inflammation on optic nerve regeneration have also been seen after treatment with another macrophage activator, oxidized galactin-1 (Okada et al., 2005). Earlier studies had demonstrated massive increases in RGC survival after vehicle injection into the eye (Mansour-Robaey et al., 1994) or implanting fragments of pre-injured peripheral nerve into the vitreous (Berry et al., 1996, 1999). These effects are probably due, at least in part, to intravitreal macrophage activation (Lorber et al., 2008). Lens injury and other methods of inducing inflammation in the eye also greatly increases the growth of RGC axons through the more permissive environment of a peripheral nerve grafted onto the cut end of the optic nerve (Fischer et al., 2000; Yin et al., 2003; Luo et al., 2007b).

The magnitude of RGC protection and axon regeneration depends upon the timing and extent of macrophage influx (Yin et al., 2003; Luo et al., 2007b). Macrophages stimulated by zymosan at the time of optic nerve injury or 3 days afterward cause dramatic axon regeneration, whereas zymosan injections either 1 week before or 1 week after optic nerve injury have little value (Yin et al., 2003). After activation by zymosan, macrophages produce both beneficial and detrimental molecules (Yin et al., 2003), and it is possible that the outcome of an inflammatory response depends upon the balance between the two (Fig. 2). Both macrophages and microglial cells are the sources and targets of numerous factors, including pro- and anti-inflammatory cytokines (Giulian et al., 1986; Essner et al., 1989; de Waal Malefyt et al., 1991; Ballou and Lozanski, 1992; Brenneman et al., 1992; Rappolee and Werb, 1992; Kiefer et al., 1996; Schroeter et al., 1997; Asakura and Rodriguez, 1998; Batchelor et al., 1999; Dougherty et al., 2000; Leskovar et al., 2000; Hanisch, 2002; Tesseur et al., 2006; Campbell et al., 2007). Among the known factors with beneficial effects on neurons are brain-derived neurotrophic factor (BDNF), IL-6, PDGF, and glial-cell-line-derived neurotrophic factor (GDNF), whereas those with deleterious effects include nitric oxide, TNF- α and IL-1 β (Ballou and Lozanski, 1992; Dougherty et al., 2000; Leskovar et al., 2000; Rappolee and Werb, 1992). Thus, in principle, the timing of macrophage activation, and/or activation under different pathological conditions, may alter the levels of various molecules that are produced, with profoundly different consequences on RGC survival and/or axonal regeneration. It is also possible that the proportions of different macrophage sub-populations may vary under different pathological conditions. There are at least three different populations of activated macrophages that possess different biological markers and distinct biological functions, including the production of different cytokines, chemokines, and cytotoxic free radicals such as NO and O₂⁻ (Mosser, 2003). Rats with neonatal thymectomies show a less sustained macrophage activation after injecting zymosan into the eye than normal rats, and this transitory response is associated with a reduction in axon regeneration, despite elevated RGC survival (Luo et al., 2007b). These data suggest that the early stage of macrophage activation may be sufficient to provide strong RGC

protection, but that prolonged macrophage activation may be required for axonal regeneration. Macrophages normally become abundant at the site of an optic nerve crush (Berry et al., 1996). As in the spinal cord, macrophages accumulate at the core of the injury site in the optic nerve and are surrounded by fibroblasts and astrocytes (Blaugrund et al., 1992; Sellés-Navarro et al., 2001; Silver and Miller, 2004). These macrophages may provide some trophic support for RGCs, but it is clearly not enough to stimulate these cells to regrow axons, in view of the regenerative failure that normally occurs after nerve crush alone.

IDENTIFICATION OF ONCOMODULIN AS NOVEL MACROPHAGE-DERIVED GROWTH FACTOR

The small Ca^{2+} -binding protein, oncomodulin, plays a key role in stimulating RGCs to regenerate their axons following intravitreal inflammation. Macrophages that enter the vitreous within 24 h of lens injury express high levels of oncomodulin mRNA and protein, whereas macrophages that enter the eye later show reduced levels. Concomitantly, Western blots reveal a strong elevation of the protein in the vitreous by day 1 and a decline to more modest levels by day 7 (Yin et al., 2006, and in press). Oncomodulin binds to a high-affinity receptor on RGCs in a cAMP-dependent manner. In the presence of elevated $[\text{cAMP}]_i$ and mannose, another essential co-factor, oncomodulin stimulates more outgrowth from RGCs in cell culture than ciliary neurotrophic factor (CNTF), BDNF, GDNF, or several other trophic factor known to act on these cells (Yin et al., 2003, 2006). Most strikingly, oncomodulin, when delivered from slow-release polymeric beads together with a cAMP analog, promotes extensive axon regeneration through the optic nerve itself (Yin et al., 2006). Depletion of oncomodulin from media conditioned by a macrophage cell line eliminates the axon-promoting effects of the conditioned media (Yin et al., 2006), and a peptide that competes with oncomodulin for receptor binding strongly reduces axon regeneration *in vivo* after lens injury (Yin et al., in press). These results indicate that oncomodulin plays an essential role in mediating the effects of intravitreal inflammation on optic nerve regeneration. However, additional inflammation-associated factors are presumably responsible for elevating intracellular $[\text{cAMP}]$, thereby enabling oncomodulin to act, and perhaps for maintaining high levels of RGC survival as well.

Another group has recently claimed that the pro-regenerative effects of intravitreal inflammation are not mediated by either macrophages or oncomodulin (Müller et al., 2007; Hauk et al., 2008). The sources of disagreement between these authors and us are apparent in some cases but not others. Hauk et al. (2008) found that either a partial elimination of blood-borne macrophages or a local elimination of macrophages in the eye enhanced rather than reduced regeneration after lens injury. These studies used clodronate liposomes, which cause apoptotic death of blood monocytes and tissue macrophages when phagocytosed in sufficient numbers (van Rooijen et al., 1996). However, because these authors injected liposomes peritoneally, they would not be expected to enter the bloodstream in large numbers, and we have previously shown that limited macrophage activation produces better RGC survival than strong activation (Yin et al., 2003), presumably by producing a more favorable balance between cytotoxic and pro-regenerative products in the eye. Direct injection of clodronate liposomes into the eye may similarly shift the balance between positive factors that get released before macrophages die, including oncomodulin, and negative factors. Using the peripheral nerve graft model, we have found that eliminating blood monocytes by repeated i.v. injections of clodronate liposomes blocks the pro-regenerative effects of lens injury almost completely (Cui et al., in press). Müller et al. (2007) also reported an inability to diminish axon regeneration after injecting a polyclonal anti-oncomodulin antibody into the vitreous following lens injury, but this reagent has not been shown to act as a neutralizing antibody (Cui et al., 2008a). All in all, the evidence for oncomodulin being critical for the effects of lens injury seems compelling, i.e. the dramatic elevation of the mRNA and protein after lens injury, the ability of oncomodulin

plus a cAMP analog to induce regeneration *in vivo*, and the effect of a competitive peptide in blocking the effects of lens injury on axon regeneration.

POSSIBLE PRO-REGENERATIVE EFFECTS OF MACROPHAGES IN OTHER TREATMENTS

The cytokine CNTF strongly enhances the ability of RGCs to regenerate axons through a peripheral nerve graft (Cui et al., 1999; Cui and Harvey, 2000), and high concentrations or viral delivery of CNTF have been reported to cause considerable (Leaver et al., 2006a,b; Müller et al., 2007), modest (Lingor et al., 2008) or no (Leon et al., 2000; Pernet and Di Polo., 2006) axon regeneration through the optic nerve itself. The effects of CNTF are enhanced by elevating intracellular cAMP levels (Cui et al., 2003; Park et al., 2004). Müller et al. (2007) have proposed that CNTF is the principal factor that mediates the effects of lens injury on optic nerve regeneration, based in part upon the observation that co-culturing retinal explants with lens or with zymosan increases CNTF expression in retinal astrocytes. However, these authors did not investigate whether this CNTF gets secreted, nor whether this astrocyte-derived CNTF or even exogenous CNTF can induce axon outgrowth in these cultures. It is unclear whether CNTF gets secreted from uninjured astrocytes (Stöckli et al., 1989; Winter et al., 1995), and exogenous CNTF exerts only modest effects on mature RGCs in dissociated culture (Lorber et al., 2002; Yin et al., 2003, 2006; Lingor et al., 2008) and little or no effect on retinal explants (Cohen et al., 1994; Cen et al., 2007). Recent results provide a likely resolution of the discrepancies concerning the role of CNTF *in vivo* and in culture. CNTF is a chemoattractant for blood-derived macrophages, and depletion of macrophages via i.v. or intravitreal application of clodronate liposomes nearly abolishes the effects of CNTF on RGC survival and axon regeneration *in vivo* (Cen et al., 2007).

Müller et al. (2007) report that once RGCs have been stimulated to regenerate their axons by injuring the lens *in vivo*, an anti-CNTF antibody or an inhibitor to the Jak-STAT signaling pathway partially diminishes axon regeneration when RGCs are placed in culture. However, other studies have reported that a function-blocking antibody to the CNTF receptor subunit gp130 did not diminish regeneration stimulated by exposing RGCs to factors derived from the injured lens despite its ability to block the effects of CNTF on rat RGC neurite outgrowth in culture (Lorber et al., 2002, 2008); and that an inhibitor of the Jak/STAT signaling pathway actually augments RGC survival and axon regeneration after optic nerve injury (Luo et al., 2007a). As with CNTF, this latter treatment stimulates macrophage entry into the eye, and removing macrophages with clodronate liposomes significantly reduced the effects of blocking this signaling pathway on RGC survival and axon regeneration (Luo et al., 2007a). Fischer et al. (2008) recently reported that crystallin can stimulate axon regeneration after lens injury, but crystallins also stimulate inflammation in the eye.

ROLE OF LOCAL MICROGLIA IN RGC SURVIVAL AND AXONAL REGENERATION

Blood- and bone marrow-derived monocytes can cross the BBB under physiological conditions and take up residence in the CNS parenchyma as microglial cells (Ling, 1979; Hailer et al., 1997; Wu et al., 2000; Hailer, 2008). Traumatic or ischemic injuries in the CNS result in the activation of local microglia that become morphologically and immunochemically similar to activated macrophages, losing their ramifications and acquiring the ability to migrate. The relationship between phagocytic cells in the retina and axon regeneration following optic nerve injury is unclear. Increased numbers of OX42 or NDPase-positive microglia/macrophages are commonly seen in the retina after optic nerve axotomy (Garcia-Valenzuela et al., 2005; Cen et al., 2007; Luo et al., 2007a; Sobrado-Calvo et al., 2007). While resting

microglia are relatively inactive in secretion, activated ones produce a large number of substances including cytokines, proteases, free radicals and various trophic factors (Hanisch, 2002; Hailer, 2008). Owing to their secretion of proinflammatory cytokines and other neurotoxic substances, microglia are generally thought to aggravate neuronal damage after acute or chronic CNS disorders. In the developing retina, microglia-derived nerve growth factor (NGF) causes the death of RGCs (Frade and Barde, 1998). In the adult, treatment with Thr-Lys-Pro (also referred as tuftsin 1–3 or macrophage inhibitory factor, MIF), a tripeptide that inhibits microglia/macrophage activation, has been reported to retard axotomy-induced neuronal degradation and enhance axonal regeneration through a peripheral nerve graft; conversely, a tetrapeptide Thr-Lys-Pro-Arg (also referred as tusftin or macrophage stimulating factor, MSF) that stimulates monocytes has been reported to augment the devastating effects of optic nerve axotomy on RGCs (Thanos et al., 1993). Consequently, others have used tuftsin 1–3 to optimize RGC axon regeneration in peripheral nerve grafts and innervation of the superior colliculus (Whiteley et al., 1998; Avilés-Trigueros et al., 2000; Raibon et al., 2002). It is interesting to note that, when axotomized RGCs are protected from dying by intentionally enhancing inflammation or attaching a PN graft onto the axotomized ON, microglial activation is suppressed (Leon et al., 2000; Raibon et al., 2002). These data suggest that the activation of microglia may be secondary to RGCs becoming compromised, rather than being a primary factor that causes RGC loss. Recently, the Nogo receptor (NgR) has been found to be expressed on microglia/macrophages at PNS (Fry et al., 2007) or CNS (David et al., 2008) injury sites, and it is possible the presence of this receptor on phagocytic cells modulates the inflammatory responses and secondary damage after CNS injury (David et al., 2008).

ROLE OF MONOCYTES/MACROPHAGES IN GLAUCOMA

In other models of retinal disease, the role of monocytes can be quite different from that seen after optic nerve injury. Intraretinal microglia become activated after elevation of intraocular pressure (IOP), a model commonly used to study glaucoma, and this activation correlates with degeneration of RGCs (Naskar et al., 2002; Zhang and Tso, 2003). Nakazawa et al. (2006) showed that increasing IOP in mice by surgically impeding the flow of aqueous fluid leads to a rapid increase in TNF- α mRNA and protein in the eye. This increase is followed shortly afterward by increased microglial activation. Oligodendrocytes in the optic nerve begin to die after 2 weeks or so, and by 4 weeks, RGCs begin to die. The loss of oligodendrocytes and RGCs can be mimicked by injecting TNF- α into the eye. Conversely, injecting a function-blocking anti-TNF- α antibody into the eye or deleting the genes that encode TNF- α or its receptor, TNFR2, prevents the loss of oligodendrocytes and RGCs after elevating IOP. The deleterious effects of elevated IOP are also eliminated by deletion of genes required for monocyte activation (Nakazawa et al., 2006).

In contrast to what was seen after optic nerve injury, blood-derived macrophages in the eye play a deleterious role following acute elevation of IOP, a model that mimics ischemic/mechanical injury in acute glaucoma (Huang et al., 2007a). Inhibition of the PI3K/akt and JAK/STAT3 pathway resulted in macrophage recruitment into the eye following acute elevation of IOP, but the action of these blood-derived macrophages was detrimental to RGC survival under these conditions (Huang et al., 2007b, 2008).

Depending upon the nature of the injury, the modes of RGC death rendered by optic nerve axotomy versus acute IOP elevation are different (Berkelaar et al., 1994; Neufeld et al., 2002; Samardzija et al., 2006). The timing of macrophage activation and/or activation under different pathological conditions may lead to the production of different levels of beneficial and detrimental molecules, resulting in different actions on RGC survival and/or axonal regeneration. It is also possible that the proportions of different macrophage sub-populations vary under different pathological conditions.

ROLE OF T-CELLS IN RGC SURVIVAL AND AXONAL REGENERATION

Activated T-cells can enter the parenchyma of the CNS (Hickey et al., 1991) and accumulate at the site of injury (Popovich et al., 1996; Hirschberg et al., 1998; Raivich et al., 1998; Ling et al., 2006), including in the visual system (Moalem et al., 1999; Fisher et al., 2001; Johnson et al., 2007). T-cells have been widely implicated in inflammatory responses leading to axonal damage and neuronal death, probably via the production of factors such as proinflammatory cytokines, chemokines and nitric oxide (Brow and Bal-Price, 2003; Giraudon et al., 2005; Schroeter and Jander, 2005). However, CNS-infiltrating T-cells may be neuroprotective under certain circumstances (Hammarberg et al., 2000) depending on the subtype of T-cells involved and their activation state (Jones et al., 2002; Kipnis et al., 2002; Wolf et al., 2002). One way in which T-cells can be protective is through the production of neurotrophins (Ehrhard et al., 1993; Kerschensteiner et al., 1999; Moalem et al., 2000). The positive and negative effects of different lymphocyte subpopulations in stroke have been reviewed by Becker, 2009.

After optic nerve crush injury in rats and mice, T-cell-related autoimmunity has been reported to protect those RGCs whose axons were spared from degenerative events, though the effect of T-cells on the axon-injured RGC population was not determined (Moalem et al., 2000; Kipnis et al., 2001; Yoles et al., 2001). On the other hand, in evaluating the actions of CD4⁺ CD25⁺ regulatory T-cells, Kipnis et al. (2002) counted the survival of pre-labeled RGCs, whether their axons were injured or not. These latter T cells were found to be detrimental to RGC survival after optic nerve crush and to impair locomotor activity after spinal cord contusion injury (Kipnis et al., 2002).

In our own recent studies, we did not see an accumulation of T-cells in the eye after optic nerve injury in adult rats (Cui et al., 2007; Luo et al., 2007b), and neonatal thymectomy, which eliminates the production of T-cells, strongly enhanced RGC survival after axotomy (Luo et al., 2007b). These results suggest that, although some populations of T-cells may be protective, the overall effect of T-cell activation after axotomy appears to be detrimental to RGC survival. This result was further supported by observations *in vitro* showing that T-cells killed RGCs via a cell–cell contact mechanism in retinal explants (Luo et al., 2007b).

Activated monocytes present antigens to T-cells, and both types of cells produce molecules that influence each others' functions. T-cell heterogeneity in macrophage activation is well established (Bach et al., 1997; Gordon, 2003; Ghasemlou et al., 2007). Conversely, macrophage-dependent modulation of T-cell activity has also been amply demonstrated, though mostly in other systems (Gallina et al., 2006; Siciliano et al., 2006). In the optic nerve model, where intravitreal zymosan induces a dramatic invasion of activated macrophages into the eyes, this invasion is not sustained if T-cells have been eliminated at birth by thymectomy (Luo et al., 2007b). Interactions of neurons with T-cells have also been reported (Liu Y et al., 2006), and hence the interactions among the cells of the immune and nervous systems become even more complex.

STRAIN DIFFERENCES IN REGENERATION

The differences in regeneration that are seen between strains of rats or mice provide another example of how the immune system influences outcome after CNS injury. Fischer 344 (F344) and Lewis rats are two inbred rat strains that are, respectively, resistant and susceptible to experimental allergic encephalomyelitis (EAE). EAE is an autoimmune disease model that reflects the functioning of the hypothalamic–pituitary–adrenal (HPA) axis (Wilder et al., 2000). In the disease-resistant F344 rats, intravitreal zymosan injections produce prolonged activation of macrophages in the eye, and, as noted above, a dramatic increase in RGC survival and axonal regeneration after optic nerve injury. In Lewis rats, the same treatment has no effect on RGC survival and a small detrimental effect on axon regeneration (Luo et al., 2007b). A

similar correlation between EAE resistance and the ability of RGCs to survive after axotomy has been observed among different strains of mice (Kipnis et al., 2001). In another injury model, acute elevation of IOP results in a rapid and dramatic and prolonged increase in intravitreal macrophages in Lewis rats, but a more gradual and mild increase in macrophages in F344 rats (Huang et al., 2007a). In parallel to these differences in the inflammatory response, EAE-resistant rats showed better RGC survival than EAE-vulnerable rats (Bakalash et al., 2002; Huang et al., 2007a).

CONCLUSIONS

We have tried to convey some of the complexity of the interactions among cells of the nervous system and the immune system that influence outcome after neural injury in the primary visual pathway. As a whole, the field seems to be moving away from a simplistic debate of whether inflammation is good or bad for neural survival and axon regeneration, to a more nuanced discussion about which cells become activated under different circumstances, the profiles of gene expression induced in these cells, and the very complex interactions that take place among the various cell types. The optic nerve of mature rats or mice represents an instance in which inflammation can, under certain experimental conditions, dramatically enhance the survival and regenerative potential of injured neurons. Although we have identified oncomodulin as one molecule that plays a key role in this phenomenon, it is clear that other molecules participate in enabling RGCs to respond to oncomodulin by elevating intracellular cAMP and perhaps by enhancing cell survival. Studies in which the thymus is ablated at birth provide further insights into the complexity of the cellular interactions involved. On the other hand, it should be noted that the vitreous is a highly specialized environment that is normally highly suppressive to inflammation (Streilein, 2003; Kaplan, 2007), and it is possible that the combination of the anti- and pro-inflammatory molecules present when one injures the lens, injects zymosan, or carries out other manipulations creates conditions that are not readily reproduced in the parenchyma of the CNS. Like many other issues, this remains an empirical question. It is clear that further studies of how immune cells respond *in vivo* under different stimulatory conditions will be a rich area for future research, perhaps ultimately enabling us to fine-tune the immune response to optimize outcome after neural injury.

Abbreviations

BBB	blood–brain barrier
BDNF	brain-derived neurotrophic factor
CNTF	ciliary neurotrophic factor
EAE	experimental allergic encephalomyelitis
F344	Fischer 344
GDNF	glial-cell-line-derived neurotrophic factor
IOP	intraocular pressure

PNS

peripheral nervous system

RGC

retinal ganglion cell

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Fig. 1.

Intravitreal macrophage activation stimulates optic nerve regeneration. Macrophages were activated in vitreous by injecting zymosan into the eye after optic nerve crush in adult rats. Retina sections (a, c) or optic nerve sections (b, d) were immunostained with GAP-43 antibody to visualize regenerating RGCs and axons (green in a–d) and with ED-1 antibody to visualize macrophages (red in a, c) 2 weeks after the surgery. (a) Following optic nerve crush alone, there are no macrophages in retina and no GAP-43 immunostaining in RGCs (arrowheads), and (b) very few axons growing beyond the nerve crush site (asterisk). However, after zymosan treatment, (c) many macrophages are seen in the vitreous and near RGCs (arrows, red) and GAP-43 is dramatically upregulated in RGCs (arrowheads, green cells). As a result of this, (d) many GAP-43⁺ axons cross the nerve injury site and extend into the distal optic nerve. Scale bar=50 μ m (a, c); 200 μ m (b, d) (Yin et al., 2003).

Fig. 2.

A possible schematic time course of factors released by macrophages and RGC responsiveness. Based on the results of our previous studies, we hypothesize that (i) the net positive effect of macrophage activation peaks shortly after macrophages are activated; and (ii) RGCs show an increased responsiveness to positive-acting factors a few days after axotomy. (a) A long delay between macrophage activation and axotomy results in no axon growth. (b) Macrophage activation 3 days after axotomy produces strong regeneration.