

arrest specific gene 1 is induced by Wnt and antagonises Shh signalling by binding to the N-terminus of Shh [24].

Concluding remarks

Just when it seemed that the upregulation of Hh signalling was bad news in cancer [4,16], van den Brink and co-workers [5] report that Ihh is potentially beneficial because of its differentiation-promoting and proliferation-inhibitory effects in the colonic epithelium [5]. This serves as an important message: in the increasing enthusiasm for specific mechanism-based cancer therapies [4,15,16], it is essential to factor in the different ways in which the Wnt and Hh signalling pathways interact in different tissues. In addition, it will be important to keep in mind the association between Ihh and hormonal regulation in epithelia, particularly because androgens can also regulate β -catenin–TCF-mediated transcription [25].

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Inflammation and apoptosis: linked therapeutic targets in spinal cord injury

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The secondary cascade of cell death that follows central nervous system (CNS) injury or ischemia has long been considered a target for neuroprotective agents aimed at sparing tissue and function. Recently, several

laboratories have shown remarkable protection and recovery of function in rodent models of spinal cord injury using treatments that target components of the CNS inflammatory response. The use of minocycline, an antibiotic that reduces microglial activation, antibody blockade of the CD95 (FAS) ligand and the blockade of glycosphingolipid-induced iNOS (inducible nitric oxide

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synthase) have recently been shown to reduce neuronal and glial apoptosis with concomitant improvement in neurological function, and appear to enhance the efficacy of cell transplantation strategies.

Traumatic mechanical injury to the spinal cord can kill cells and disrupt axons immediately, but also appears to be followed by a cascade of 'secondary' injury that involves both necrotic and programmed cell death [1,2].

Figure 1 presents a schematic of the development of secondary injury after spinal cord contusion. The gray matter at the region of impact immediately following injury contains disrupted cells and blood, but the surrounding white matter, and the gray matter rostral and caudal to the impact region, can appear remarkably intact. However, over a time-course of minutes to hours, the lesion is thought to spread centripetally, initially by the induction of necrotic cell death that is mediated, for example, by excitatory amino-acid-induced Ca^{2+} entry and energy failure, nitric oxide (NO) production, oxidative stress and membrane breakdown [1]. These events might also be exacerbated by the presence of inflammatory cells [3,4] and proinflammatory cytokines, such as tumor necrosis factor- α (TNF α) [5]. In addition, the injury induces a more slowly spreading cell death that is characterized by apoptotic neurons at the lesion margins and, even later, induces microglial activation and apoptosis of oligodendrocytes in areas with degenerating axons that were injured at the original lesion site [1,2]. It is, therefore, possible that anti-inflammatory treatments could have both short term and long-term effects on necrotic and apoptotic cell death, respectively.

New approaches to the treatment of secondary injury

Many of the pharmacologic approaches to central nervous system (CNS) injury have been aimed at cell death caused by excitatory amino acids (EAAs). The excess glutamate that follows CNS injury [6,7] has been linked to cell death [8] and, in animal models of stroke or injury, antagonists to ionotropic glutamate receptors have proven effective.

However, for a variety of possible reasons, clinical trials of EAA antagonists have not been efficacious [9]. Three recently reported studies illustrate new therapeutic approaches that might be effective, at least in part, by interfering with the acute CNS inflammatory cascade. In one, it was shown that injury induced the immune receptor CD95 (also known as FAS) and that blockade of this receptor produced a better recovery after experimental spinal cord injury (SCI) in mice [10]. In the second two, an antibiotic tetracycline derivative, minocycline, that has anti-inflammatory and anti-apoptotic actions provided substantial sparing of both neurons and glial cells and also resulted in better neurological outcomes in two different SCI models in rats [11,12]. Another recent paper reports a novel effective treatment that reduces the NO release that is associated with acute inflammation after SCI [13]. This new work is focused on the effects of anti-inflammatory and anti-apoptotic treatments on the longer-term secondary events. However, all the treatments that worked in these studies were given acutely, and in one case, 30 min before the injury [10]. The effects on apoptosis were manifest hours to days later, but there were also effects on lesion size, which were perhaps due to reductions in acute necrotic cell death. The results of each study indicated a reduction of axonal 'dieback' and hinted at enhanced regeneration.

In yet another recent study aimed at developing transplantation strategies for recovery from SCI, Pearse *et al.* [14] found that increases in cAMP enhanced the efficacy of Schwann cell transplants on recovery, but only if the cAMP levels were increased acutely after injury. And cAMP given acutely dramatically reduced the production of the inflammatory cytokine TNF- α . Again, these early inflammatory events seem linked to the longer process of secondary injury and repair that occur up two weeks later after injury. The long-term effects of acute treatment emphasize the complexity of the cascades of cellular events that are initiated by injury to the CNS. The short-term necrotic damage seems to set up the conditions for longer-term apoptosis in a way that reflects the

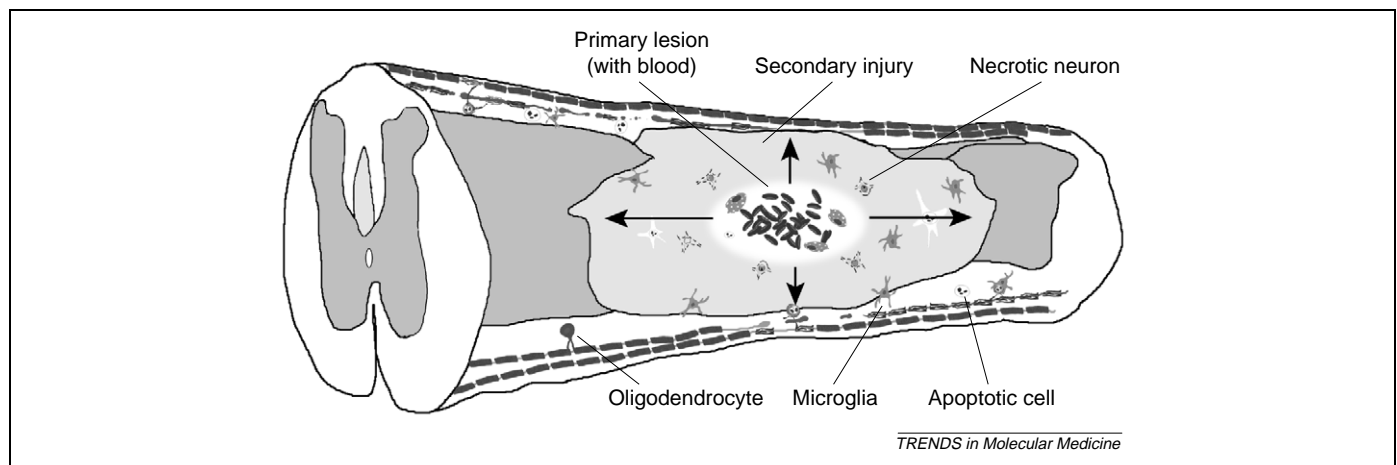


Figure 1. Secondary spinal cord injury. Initial trauma to the cord is directed to the central gray matter, where frank destruction occurs. The initial injury is followed by a cascade of biochemical events (secondary injury) that is thought to enlarge the area of cell death through necrosis and apoptosis. Neurons and glial cells at the margins of the expanding lesion represent a target for neuroprotection. When axons in the white matter are damaged, Wallerian degeneration can induce microglial activation and apoptotic cell death of oligodendrocytes long after the initial injury. This later microglial activation represents another potential target for treatments that reduce microglial activation and/or oligodendrocyte apoptosis.

patterning of axonal loss [5,13,15]. The longer-term events, including the delayed cell death of oligodendrocytes, seem very similar to the situation encountered in progressive neurodegenerative disorders, especially multiple sclerosis [16].

The role of CD95, TNF- α and microglia in CNS inflammation and apoptosis

CD95 is a member of the TNF receptor family, and CD95 ligand (CD95L) and TNF- α can initiate cell death in a variety of cell types, including oligodendrocytes, through the activation of CD95 and the TNF receptor 1 (TNFR1), respectively [3]. Microglia also express CD95 and TNFR1. The activation of microglia can cause these cells to release CD95L and TNF- α . An earlier study by Casha *et al.* [1] showed the upregulation of CD95 and p75 in oligodendrocytes after a compression injury to the cervical cord in rats. To determine whether CD95 was causally related to cell death in these cells, Demjen *et al.* [10] transected the dorsal two-thirds of the mouse spinal cord at the thoracic level, an injury that caused severe and persistent paralysis while sparing the ventral funiculus. This injury also produced apoptotic cell death at the lesion and in the surrounding tissue and resulted in an upregulation of CD95, CD95L and TNF- α during the first three days after injury. CD95 and CD95L were seen in neurons, microglia and oligodendrocytes after injury. To counteract the effects of CD95L and TNF- α , neutralizing antibodies against CD95L and TNF were administered i.p. at 30 min before lesioning and for four weeks afterwards (twice a week). The neutralization of CD95L, but not TNF- α , reduced the number of apoptotic cells at three-days post-injury. In addition, performance on a standard locomotor test [17] and several other behavioral tests was markedly improved in the four weeks after injury in mice receiving antibodies against CD95L. Finally, treatment with CD95L-neutralizing antibodies appeared to promote the return of protein markers for neurons and glia and to increase the sparing of neurons and oligodendrocytes close to the lesion margins. Overall, this is an impressive series of results. The authors suggest that CD95 might be being produced and released by activated microglia (Figure 2). The findings of increased CD95 and CD95L after SCI [1,10] suggest that both ligands and receptors are upregulated, perhaps not only on oligodendrocytes but also on microglia. This could result in the continued activation of microglia and the increased production of pro-inflammatory cytokines, NO and glutamate, resulting in a cascade of cell death. Of relevance, microglia themselves have been seen to undergo apoptotic cell death in the white matter after injury [1,18]. Indeed, it has been suggested that microglial apoptosis during inflammation in the CNS might serve as a protective mechanism to short circuit this damaging cascade [19]. If so, then CD95-induced microglial cell death might be a beneficial effect. Until this is proved, the current studies show that the presence of CD95 on oligodendrocytes is disastrous for these cells.

Minocycline reduces microglial activation and apoptosis in several models of CNS injury, including SCI [11], and two reports now show that this treatment can reduce neuronal and oligodendroglia apoptosis and improve

function. Stirling *et al.* [12] used a dorsal hemisection of the rat cervical cord and showed that minocycline reduced lesion size, reduced the number of apoptotic oligodendrocytes and neurons and spared function, as assessed by a grid-walking task. In addition, they saw more corticospinal tract axons growing past the lesion in treated rats. Teng *et al.* [11] used a thoracic contusion lesion model in rats to examine the suppression of apoptosis and cytochrome-c release using minocycline. They used the cytochrome c response as an index of minocycline efficacy and mechanism of action, and used the derived dose to treat injured rats and measure chronic outcomes. As in the other studies, the lesion size was reduced, oligodendrocytes were spared in the white matter and locomotor outcomes were improved in the treated rats. These authors emphasize the role of minocycline in the direct blockade of apoptosis, whereas others have emphasized its anti-inflammatory effects [20]. Its efficacy serves to focus again on the link between inflammation and, subsequently, apoptosis. And although these data, together with previous reports, suggest that a rapid assessment of its potential for therapy in humans is warranted, minocycline therapy remains controversial, with a recent review questioning its efficacy [18].

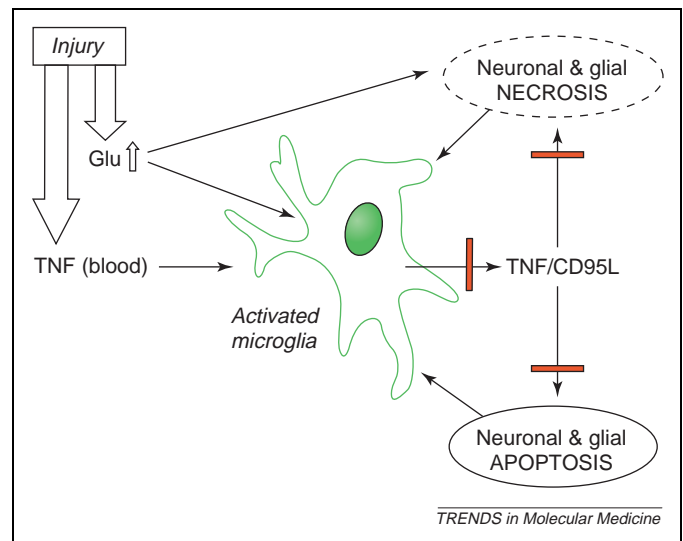


Figure 2. Activated microglia might be at the center of the injury cascade and are targets for treatments. Mechanical impact on cord tissue, accompanied by the breakage of blood vessels and extravasation of peripheral blood elements, brings tumor necrosis factor α (TNF α) and other inflammatory molecules into the lesion. Neuronal damage releases glutamate (Glu) into the extracellular space and high concentrations initiate an excitotoxic cascade with receptor-mediated increases in neuronal and glial Ca^{2+} that can lead to necrotic cell death. Glu might, in turn, activate microglia, which can release inflammatory molecules, such as TNF and CD95L. These can act with Glu to add to the early post-injury necrotic cell death. TNF and CD95L can also activate apoptotic pathways for long periods after injury. The delay in remote cell death suggests that these events initiate a long-term signaling sequence that results in increased susceptibility to apoptosis in oligodendrocytes, possibly through the activation of microglia by axonal degeneration. The blockade of the initial inflammatory cascade with antagonists to CD95L might reduce later oligodendrocyte cell death indirectly, by blunting the early damage, and reduce later vulnerability to ligand-induced apoptosis. Drugs such as minocycline, which has both anti-inflammatory and anti-apoptotic activity, might affect both acute and long-term degeneration. Points in the cascade that are related to actions of the treatments discussed herein are shown in red. Astrocytes (not shown) are also important as buffers of extracellular Glu and as potential sources of inflammatory molecules.

Interrupting the cycle of microglial-induced cell death?

The current literature implies that injury induces apoptosis, at least in part, by releasing CD95L and/or TNF, which then act on upregulated CD95 and/or TNFR1 receptors to induce the activation of caspases, the release of cytochrome c and terminal apoptosis. However, the *in vivo* models make it difficult to determine the sequence of events. What is the initiator of the release of CD95 (and other inflammatory) ligands? Does minocycline blockade of cytochrome c release account for its neuroprotective actions, or does its role in reducing microglial activation have a crucial role? And if microglial activation starts the cascade, what might be the trigger for microglial activation itself? One possibility is that the initial excitotoxic events that are associated with necrosis also activate microglia. Thus, glutamate has been shown to activate microglia (reviewed in [16]), producing pro-inflammatory cytokines. It is also notable that oligodendrocytes express α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors and are susceptible to excitotoxic cell death (both necrotic and apoptotic) [16]. Another intriguing possibility is that microglial activation might be due to an innate immunity response to CNS injury; much of the neuronal damage seen in lipopolysaccharide (LPS)-induced CNS injury models appears to be mediated through Toll-like receptors (TLR4) present only on microglia (at least in the uninjured CNS) [21]. Perhaps injury induces one of the proposed endogenous ligands for Toll-like receptors [22]. Other members of the TNFR family, such as p75, might also be involved [1,23].

Concluding remarks

Considerable evidence now suggests that an inflammatory response to injury in the CNS, mediated in part by microglia, might be crucial for inducing necrotic and apoptotic cell death. The papers discussed here support the suggestion that the blockade of several different aspects of the inflammatory cascade can benefit outcome after SCI. Furthermore, they link later apoptotic cell death in oligodendrocytes to these early inflammatory events and to dysfunction, because sparing these cells is associated with better neurological function. Together, this new work shows promising efficacy in preclinical models of SCI. However, they also point to the multitude of effects of such treatments and the complexity of the repair and recovery processes, including axonal growth and sprouting, cell replacement and remyelination.

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