

Degenerative and Spontaneous Regenerative Processes after Spinal Cord Injury

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ABSTRACT

Spinal cord injury results in acute as well as progressive secondary destruction of local and distant nervous tissue through a number of degenerative mechanisms. Spinal cord injury also initiates a number of endogenous neuroprotective and regenerative responses. Understanding of these mechanisms might identify potential targets for treatments after spinal cord injury in humans. Here, we first discuss recent developments in our understanding of the immediate traumatic and subsequent secondary degeneration of local tissue and long projecting pathways in animal models. These include the inflammatory and vascular responses during the acute phase, as well as cell death, demyelination and scar formation in the subacute and chronic phases. Secondly, we discuss the spontaneous axonal regeneration of injured and plasticity of uninjured systems, and other repair-related responses in animals, including the upregulation of regeneration-associated genes in some neurons, increases in neurotrophic factors in the spinal cord and remyelination by oligodendrocyte precursors and invading Schwann cells. Lastly, we comment on the still limited understanding of the neuropathology in humans, which is largely similar to that in rodents. However, there also are potentially important differences, including the reduced glial scarring, inflammation and demyelination, the increased Schwannosis and the protracted Wallerian degeneration in humans. The validity of current rodent models for human spinal cord injury is also discussed. The emphasis of this review is on the literature from 2002 to early 2005.

Key words: acute degeneration; axonal dieback; regeneration; scar formation; spinal cord injury

INTRODUCTION

SPINAL CORD INJURY causes severe and long-lasting neurological dysfunction and morbidity in humans. Currently, there is no successful treatment for any of the main types of dysfunction after spinal cord injury, in-

cluding motor and sensory deficits, chronic severe pain, bladder, bowel and sexual dysfunction, and autonomic dysreflexia. It is therefore essential to develop treatments in animal models that are relevant to any or all of these major problems. Spinal cord injury results in acute as well as progressive secondary tissue damage, but also

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initiates a number of neuroprotective and regenerative responses within the nervous system. It is expected that a better understanding of the mechanisms that underlie these degenerative and spontaneous regenerative processes might identify potential targets for developing treatments in humans. Such treatments could be neuroprotective, regenerative or could enhance plasticity of the uninjured systems. The understanding about degenerative and spontaneous regenerative processes clearly is also important for the current extensive research effort to promote long-distance regeneration. Here, we discuss recent developments in the spinal cord injury research field. As most of the research is performed in rodents, we also discuss the similarities and potentially important differences compared to what is known about the human pathology. This is meant to stimulate discussion within the spinal cord research community about the validity of current rodent models.

DEGENERATIVE PROCESSES AFTER EXPERIMENTAL SPINAL CORD INJURY

A traumatic impact to the spinal cord causes immediate death of cells in the vicinity of the injury site, including local neurons, astrocytes, oligodendrocytes and endothelial cells. Stretching of axons, particularly the larger myelinated ones, can cause membrane damage (Shi and Pryor, 2002), which in later stages can contribute to progressive axon degeneration. In addition, death of endothelial cells of local blood vessels results in a hemorrhage, disturbing the oxygen and nutrient supply to the damaged area and its surroundings. During the first acute phase of injury, the body responds by activating the complement cascade and with a usually massive infiltration of neutrophils into the lesion site and nearby parenchyma (Schnell et al., 1999; Saville et al., 2004). Vascular damage and inflammation result in edema of the spinal tissue soon after the insult.

The hemorrhagic area at first is restricted to the highly vascularized gray matter and at later stages (minutes to hours) expands into the peripheral white matter and in a rostral and caudal direction. This spread of the secondary injury continues during the second sub-acute phase and sometimes continues for months, even years during the chronic phase. Continuing cell death further increases the level of amino acids such as glutamate in the extracellular fluid, which contributes to excitotoxicity (Park et al., 2004). Many local and distant microglia become activated and their reactive state remains high for up to 4 weeks after the injury. Lipid peroxidation and formation of free radicals contribute to further damage of nearby nervous tissue. Different mechanisms may cause the ini-

tial necrotic cell death; excitatory amino-acid-induced Ca^{2+} entry and energy failure, nitric oxide (NO) production, oxidative stress and membrane breakdown (Casha et al., 2001). During the first week, monocytes, macrophages, and T-lymphocytes start to invade the damaged area. The peak of the lymphocyte invasion is slightly earlier than that of the monocytes and macrophages, of which the bulk is present around the 7th day (Popovich et al., 1997). The accumulation of especially macrophages and lymphocytes in the damaged nervous tissue can worsen the necrosis-mediating events (Popovich, 2000). Oligodendrocytes die due to apoptotic events (Casha et al., 2004). During this secondary phase, a scar forms at the impact site consisting mainly of invading meningeal fibroblasts and surviving reactive astrocytes, which express extracellular axonal growth-inhibitory proteoglycans.

During the chronic phase of injury most of the processes that started during the subacute phase continue and result in atrophy of the spinal cord. Apoptotic cell death is still present at and away from the site of impact. Demyelination due to oligodendrocyte death also continues for extended times. The scar remains present and often appears to enclose the injury site. The loss and clearance of tissue results in the formation of one or more fluid-filled cysts.

It is obvious that an injury to the spinal cord initiates a plethora of destructive events. Some of the cells and molecules present at the site of injury can contribute to protection and possibly repair, but the ultimate outcome is loss of tissue and function of various systems, including motor and sensory systems. Neurological function spontaneously recovers to a level that depends on the severity of the injury. This recovery appears in large part to be the result of plasticity (re-organization) in existing circuitries in the lower cord levels. Following is an assessment of the current status and the latest developments in our knowledge of the main events that take place during injury-induced degeneration of the spinal cord in animal models (Fig. 1). The second main section of this review will describe recent studies that highlight the spontaneous regenerative processes found in animal models. The third main section will discuss the human pathology.

Vascular Responses

The initial damage to the local blood vessels is thought to be decisive for the evolution of the destructive events that ensue during the secondary injury phase (Mautes et al., 2000). Loss of blood flow at the actual injury site and impaired blood flow in the surrounding spinal tissue bring about anoxia and edema. Moreover, the breakdown of the blood-spinal cord barrier causes

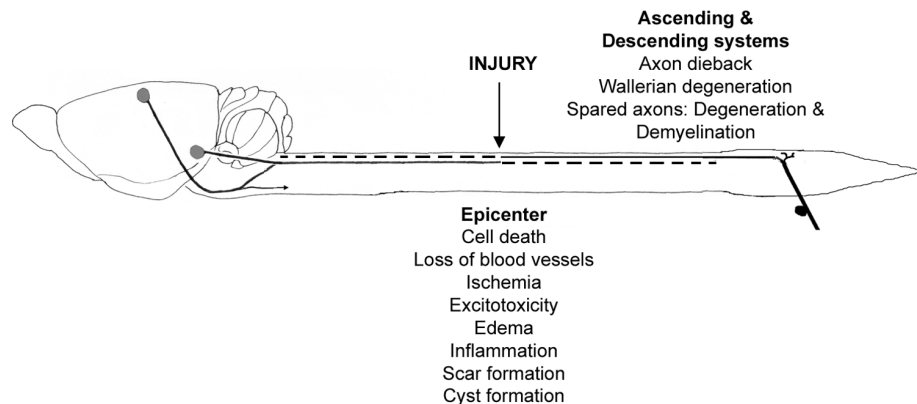


FIG. 1. Schematic representation of the injured spinal cord. Indicated are the major degenerative processes that occur following an injury. At the injury site, cell death, loss of blood supply, ischemia, excitotoxicity, edema, and inflammation all result in tissue loss, which result in the formation of cysts surrounded by a glial scar. The proximal ends of damaged axons die back and their distal ends undergo Wallerian degeneration. Spared axons may lose their myelin due to oligodendrocyte death.

the influx of inflammatory cells, first neutrophils and later T-lymphocytes and macrophages (Bareyre and Schwab, 2003). It was demonstrated that matrix metalloproteinase-9 plays an important role in the dysfunction of the blood–spinal cord barrier (Noble et al., 2002). The spinal tissue close to the impact site is seriously damaged because its blood supply is compromised due to impaired autoregulation (Krassioukov et al., 2003) and vasospasm (Attar et al., 2001; Yuceer et al., 2002). Nitric oxide may be one of the molecular regulators in the maintenance of spinal arteriolar tone in the injured cord (Ishikawa et al., 2002). This idea was supported by the finding that nitric oxide synthase (NOS) inhibition by N(G)-nitro-L-arginine-methyl ester (L-NAME) in rabbits exacerbated ischemic spinal cord damage (Matsumoto et al., 1999).

It is important to realize that spinal cord injury also causes deleterious systemic effects, such as neurogenic shock (Tator and Fehlings, 1991), bradycardia, and hypotension, all of which can further contribute to spinal cord tissue damage. Recently, it has been demonstrated that the mean arterial blood pressure significantly decreases after a transection at the ninth thoracic (T9) spinal cord level in adult rats (Guizar-Sahagun et al., 2004). Also, the same study established a clear association between spinal cord injury and a significant decrease in microvascular blood flow in organs such as liver, spleen, muscles, which could be indicative for organ dysfunction.

The importance of blood flow after spinal cord injury has long been recognized. During the last years our understanding of this particular issue has made some progress. Following spinal cord injury new blood vessels are formed. Loy et al. (2002) demonstrated the existence

of a bi-phasic angiogenic response following contusion injury of the spinal cord in rats, of which the first phase (3–7 days post-injury) (Casella et al., 2002) but not the second phase (28–60 days post-injury) corresponded to the reported time course of changes in locomotor function. It is evident that the angiogenic responses do not have a significant effect on the final outcome of spinal cord injury but it may demonstrate the ability of the spinal cord for self-repair. Glutathione monoethyl ester stabilizes blood flow in the spinal cord when administered immediately after a spinal cord contusion (Guizar-Sahagun et al., 2005). In this study an improved functional outcome and increase neuronal survival in the red nucleus were also observed, suggesting a relationship between blood flow and improved outcome after spinal cord contusion. A similar relationship was observed after neutralization of the chemoattractant CXCL10 that results in improved angiogenesis at the injury site and improved functional outcome (Glaser et al., 2004). It is generally thought that the development of treatments that focus on increasing angiogenesis and the restoration of normal blood flow soon after injury would be crucial for repair of the spinal cord (Loy et al., 2002). Such treatments would limit injury-induced tissue loss, which in turn would limit the loss of motor and sensory function (Carlson and Gorden, 2000). Restoration of normal blood flow after injury might also set the stage for greater improvements due to spontaneous recovery and/or interventions aimed at repair.

Ischemia

During the ischemic period, reactive oxygen species are produced which through their cytotoxicity contribute

substantially to the observed loss of tissue following spinal cord injury. Castro et al. (2004) proposed that the large number of infiltrating neutrophils do not significantly contribute to the formation of reactive oxygen species. However, most cell types produce these reactive oxygen species, as well as other free radicals, resulting in the formation of peroxides through lipid peroxidation, which damages cell membranes.

The crucial role of free radicals in the progressive loss of tissue after spinal cord injury is suggested by a recent study where treatment with the free-radical scavenger melatonin resulted in improved functional recovery (Cayli et al., 2004). Over the last years, a large group of drugs have been identified with a positive effect on ischemia-induced spinal cord damage (Usul et al., 2004). However, the precise mechanisms underlying the observed improvements are generally obscured by the multifaceted actions of these drugs. More in-depth research into their mechanisms of action may allow for more effective use of these drugs. Another important question that remains to be answered is whether early intervention targeting ischemia following spinal cord injury would benefit functional and/or histological outcome after long post-injury times.

An alternative destructive mechanism associated with ischemia is the associated lack of glucose and oxygen. Neurons and central myelinated axons are critically dependent on a continuous supply of oxygen and glucose. For instance, it has recently been demonstrated that oxygen and glucose deprivation followed by reoxygenation induced apoptosis (rather than necrosis) in cortical neurons (Tanaka et al., 2005). This effect could be suppressed by overexpression of Bcl-2, which indicates mitochondrial impairment as one of the mechanisms of apoptosis (Tanaka et al., 2005). It was also shown that several pro-apoptotic proteins such as cytochrome c, apoptosis-inducing factor, and endonuclease G were released from mitochondria during the reoxygenation phase (Tanaka et al., 2005). Interestingly, these apoptotic changes could be attenuated by inhibitors of a DNA damage-sensor, poly(ADP-ribose) polymerase-1 (PARP-1), such as 1,5-dihydroxyisoquinoline and benzamide. This suggests that PARP-1 plays a principal role in inducing mitochondrial impairment (Capriani et al., 2005) which ultimately leads to apoptosis of neurons after ischemia (Tanaka et al., 2005).

Excitotoxicity

Injury-induced accumulation of excitatory amino acids, such as glutamate and aspartate, seems to contribute to additional cell death (Park et al., 2004). Excess

of glutamate may be the result of glutamatergic loops that involve the exocytosis of calcium-dependent glutamate containing synaptic vesicles and release of intracellular glutamate as a result of cell lysis (Choi, 1992; Matyja et al., 2005). This could cause excitotoxic cell death through hyperactivation of the NMDA (*N*-methyl-D-aspartate) receptors or the α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)/kainite type glutamate receptors. All types of neural cells in the injured spinal cord are susceptible for excitotoxic cell death. Neurons may die after overactivation of AMPA and NMDA receptors, which results in water influx and lysis through depolarization and increase of intracellular sodium (Matyja et al., 2005), or as a result of apoptosis (Das et al., 2005; Tarabal et al., 2005). Glial cells are also susceptible to excitotoxic cell death (McDonald et al., 1998) often via the AMPA and kainate receptors. Oligodendrocytes are especially vulnerable because of their high permeability for calcium of the AMPA and kainate receptors (Rosin et al., 2004).

Although the role of excitotoxic cell death in spinal cord injury has been well established, its magnitude has been a subject of discussion. For example, it was recently demonstrated that the loop of glutamate release–cell death–glutamate release may not be a significant contributor to cell death following injury (McAdoo et al., 2005). Clearly, there are additional mechanisms that result in cell death after spinal cord injury.

Edema

Very soon after spinal cord injury, edema or swelling through build-up of fluids can be seen at the site of impact and this can exacerbate the tissue damage when the swollen tissue is compressed against fractured vertebra. Compression may also lead to increased ischemia. Edema is caused by various events that all result directly or indirectly from the actual injury. The main contributor is the increased permeability of blood vessels around the site of impact. Vascular endothelial growth factor (VEGF) is known to significantly increase the blood vessel permeability in the spinal cord and cause tissue damage (Benton and Whittemore, 2003). VEGF levels increase in the injured spinal cord (Skold et al., 2000). In fact, inhibition of Src kinases downstream of VEGF by the pyrazolopyrimidine PPI results in reduced water content and tissue loss in the injured spinal cord (Akiyama et al., 2004). Although these results may implicate VEGF in secondary spinal cord injury, it should be recognized that PPI also inhibits TNF α , IL-1 β , and inducible NO synthase in macrophages, which could reduce tissue loss. Edema is also affected by inflammatory mechanisms. For example, TNF α antiserum can reduce swelling of the injured spinal cord (Sharma et al., 2003).

Inflammation

Inflammation also clearly contributes to the destruction seen after spinal cord injury and, conversely, intervention in inflammatory events can improve functional outcome (Demjen et al., 2004; Stirling et al., 2004). However, some components of the inflammatory response are also known to be protective. One of the main cellular contributors in the inflammatory response is the macrophage, which unmistakably has a dual role in spinal cord injury and repair (Bethea, 2000). Macrophages produce and secrete a number of cytokines such as transforming growth factor- β 1 (Wyss-Coray et al., 1997) that maintain the inflammatory process and contribute directly or indirectly to further cell death. Also, macrophages and activated neutrophils produce reactive oxygen species, which can result in lipid peroxidation and the subsequent formation of peroxides that cause severe membrane damage. The resident microglia are also involved in the inflammatory response. Activated microglia are found near the lesion and within fiber tracts that undergo Wallerian degeneration. Activated microglia can cause oligodendrocyte death (Emery et al., 1998), possibly via peroxynitrite produced by inducible nitric oxide synthase and the superoxide-generating enzyme NADPH oxidase (Li et al., 2005). Activated microglia also produce proinflammatory cytokines, NO, glutamate, which may be sustained through CD95 and TNF α production and release (Demjen et al., 2004). Both CD95 and TNF α could activate their respective receptors and activate caspases to induce apoptosis. After spinal cord injury, CD95 and its ligand and TNF α are also upregulated in neurons and oligodendrocytes (Demjen et al., 2004), which suggests that these two cell types could contribute to their own death. On the other hand, the implication of TNF α as a neuroprotectant (Guo et al., 2004) raises the possibility that the neuronal and oligodendrocyte responses are self-protective.

There is ample evidence that macrophages are involved in injury-induced destructive events in the spinal cord. However, macrophages are also strongly implicated in constructive events, of which the underlying mechanisms are still under investigation. Macrophages secrete molecules that could promote repair after injury and they are normally involved in the removal of cellular debris. Especially the latter function of macrophages has been applied in spinal cord repair. In a series of studies, it was demonstrated that grafting into the injured cord of macrophages activated in the peripheral nerve resulted in improved functional outcome (Schwartz and Yoles, 2005). The improvement of function may have resulted from an increased axonal regeneration response within the injured cord after an accelerated clearance of debris,

including oligodendrocyte myelin fragments that may contain axonal growth-inhibitory molecules, by the implanted macrophages.

Demyelination

Following injury, oligodendrocytes may die directly or indirectly via any of the above described mechanisms. Oligodendrocyte death is not only present at the site of injury but also occurs distant from the impact site (Emery et al., 1998). The ensuing demyelination severely hampers normal functioning of spared axons. The demyelination is thought to contribute substantially to the overall functional consequences of spinal cord injury. An important cell type in this process is the activated microglia, which following injury has been demonstrated to induce apoptosis in oligodendrocyte (Emery et al., 1998; Li et al., 2005). Another mechanism that also may result in apoptotic death of oligodendrocytes is the loss of trophic support by the axons after their degeneration (Barres et al., 1993). Oligodendrocyte death may also be caused by the injury-induced increases in NGF, derived from astrocytes and microglia (Krenz and Weaver, 2000), which can induce oligodendrocyte apoptosis through the low-affinity nerve growth receptor p75 (Casaccia-Bonnel et al., 1996; Beattie et al., 2002).

Axonal Degeneration and Dieback

Many fibers remain intact immediately after a contusion injury but degenerate over the next month. Part of this progressive injury may be caused by tissue loss (i.e., by loss of vascular and glial support). Axonal damage may allow for an ionic imbalance with an influx of calcium (Iwata et al., 2004), which results in calpain activation and mitochondrial injury/swelling (Wingrave et al., 2003) resulting in release of cytochrome c, activation of caspases and subsequent exacerbation of the damage to the axon (Bao and Liu, 2003).

Axonal degeneration may also be caused by injury-induced mechanisms inherent to the neurons. An exciting finding was published by Araki et al. (2004) in a study using Wallerian degeneration slow (wlds) mice. In these mice, degeneration of distal parts of severed axons is delayed because of overexpression of a chimeric protein (Wlds) composed of the ubiquitin assembly protein, Ufd2a and the nicotinamide adenine dinucleotide (NAD) biosynthetic enzyme, Mmnat1. In this particular study it was revealed that Mmnat1 was responsible for the axon sparing activity of the Wlds protein. Moreover, they demonstrated that the downstream effector of increased Mmnat1 activity that leads to axon protection is SIRT1 (Araki et al., 2004). SIRT1 is the mammalian ortholog

of yeast Sir2, a NAD-dependent deacetylase that plays an essential role in epigenetic silencing at the silent mating type loci and telomeres, thereby connecting transcription, metabolism, and aging (North and Verdin, 2004). These results suggest that an increased supply of NAD and/or SIRT1 may be effective in sparing axons following traumatic spinal cord injury.

The dieback of the severed axons and its contribution to the overall consequences after spinal cord injury is sometimes overlooked. The retraction of axons away from the site of injury may decrease their ability to respond to a growth-promoting intervention such as cell transplantation at the site of injury. Following an injury to the spinal cord, severed axons will form swellings at their tip due to axoplasmic stasis. These bulbous axonal tips, which can be found as soon as 15 min after injury, contain organelles such as mitochondria and smooth endoplasmic reticulum, cytoskeletal proteins, and many enzymes. The latter will be released upon rupture of the bulbous ends and contribute to further damage of the nervous tissue. The formation of these degenerative structures and the dieback of injured sensory and corticospinal axons can be reduced by local administration of neurotrophins or FGF1 (Guest et al., 1997; Sayer et al., 2002). This suggests that the increase of trophic factors seen after spinal cord injury is not fast or large enough.

An interesting observation is that the distance between the site of axotomy and the cell body is crucial for the outgrowth of their damaged axon for some but not all types of neurons. Spinal motor neurons regenerate their axon regardless of a proximal or distal axotomy, whereas rubrospinal neurons survive and can grow their axon after a cervical but not thoracic injury (Fernandes et al., 1999), which may have been caused by a significant decrease in the receptor for brain-derived neurotrophic factor (Kwon et al., 2004). Hains et al. (2003) demonstrated that a proportion of the corticospinal motor neurons undergo apoptotic death after a thoracic spinal cord lesion, indicating that this may be one mechanism underlying the poor corticospinal regeneration seen after spinal cord injury even after grafting a growth-permissive environment.

Scar Formation

Although at first the immediate environment at the site of injury is not necessarily inhibitory to axonal growth (Fawcett and Asher, 1999), gradually a non-permissive glial scar forms that may frustrate the early attempts of severed axons to regenerate. The glial scar walls off the lesion site and contains reactive, hypertrophic astrocytes. This response most likely serves to reestablish a barrier between the CNS and the rest of the body. In fact, when

this glial response is reduced after spinal cord injury, the extent of cavitation is greatly increased (Faulkner et al., 2004). On the other hand, the reactive astrocytes express outgrowth-inhibitory molecules such as chondroitin sulphate proteoglycans (CSPGs). In addition, the injured spinal cord contains inhibitory molecules (such as NOGO-A, MAG, and OMpg) in myelin debris, the inhibitory chondroitin sulphate proteoglycan NG2 on oligodendrocyte precursors, and inhibitory semaphorins on meningeal fibroblasts (Fawcett and Asher, 1999). The latter are a major component of scars that develop in response to spinal cord lacerations. The three-dimensional structure of the scar may present a molecular and mechanical barrier to axonal regeneration. On the other hand, axons can regenerate within such environments when growth-promoting molecules such as laminin are present (Jones et al., 2003). The glial scar is discussed in detail in several other reviews in this journal issue.

In conclusion, it is clear that many degenerative processes contribute to the tissue loss and dysfunction after experimental spinal cord injury. This might suggest that neuroprotective treatments should consist of multiple approaches. However, many of these degenerative events seem to be part of injury cascades. Therefore, it is possible that single interventions at certain times after the injury could result in substantial functional recovery. Not everything is doom and gloom after spinal cord injury. The spontaneous regenerative processes discussed in the next section (Fig. 2) may provide an opportunity to further enhance functional recovery.

SPONTANEOUS REGENERATIVE PROCESSES AFTER EXPERIMENTAL SPINAL CORD INJURY

Spontaneous Axonal Sprouting

Santiago Ramon Y Cajal already more than 100 years ago described the process of abortive sprouting that is seen after CNS injuries in adult mammals. The conclusion was, and still is to a large extent, that spontaneous axonal regeneration in the adult mammalian CNS is rare, minimal and does not lead to substantial functional recovery. Such a transient or abortive sprouting response is probably not present in all systems and types of injuries. For example, axonal sprouting in the retina is rapid and continues for at least 100 days (McConnell and Berry, 1982). Corticospinal axons can grow new sprouts that persist for at least 13 weeks after an electrolytic lesion (Li and Raisman, 1995) and 3 months after a contusion injury (Hill et al., 2001) in adult rats. In adult mice, serotonergic axons grow sprouts up to 6 months after a

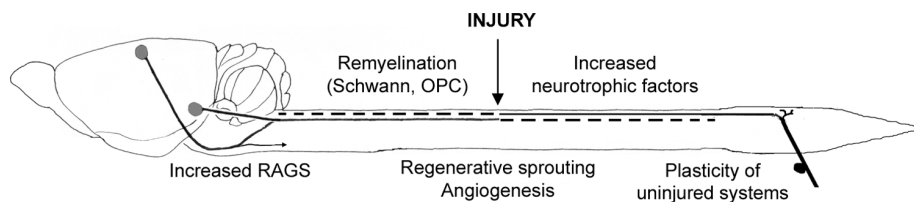


FIG. 2. Schematic representation of the injured spinal cord. The major spontaneous regenerative processes that occur following an injury are indicated. Within the neuronal cell bodies regeneration associated genes may be up regulated, while near the injury site where the endogenous levels of neurotrophic factors increase regenerative sprouting and formation of new blood vessels (angiogenesis) takes place. Furthermore, Schwann cells from the periphery may invade the injured cord and remyelinate central axons. Oligodendrocyte precursor cells (OPC) may further differentiate into mature myelinating oligodendrocytes and remyelinate axons. Some plasticity may occur in uninjured systems.

hemisection (Camand et al., 2004) and a crush injury (Inman and Steward, 2003) of the adult spinal cord. Catecholaminergic and serotonergic axons regenerate over 15 weeks after a focal ischemic lesion in adult rats (Von Euler et al., 2002). Recently, we have found evidence for sprouting and structures that might be growth cones in the ascending sensory tract as early as 24 h after a dorsal column transection at T9 in adult rats (Hagg et al., unpublished data). Sprouting of damaged axons may be facilitated by local peri-wound inflammation and macrophages (Batchelor et al., 2002), which was demonstrated for the corticospinal system (Li and Raisman, 1995). Thus, there are regenerative responses in long projecting axonal pathways after spinal cord injury which may not be abortive but, at least in some systems, progress over time. The extent of functional recovery due to spontaneous regeneration, however, is still very limited. Several of the reviews in this issue will deal with treatment strategies to enhance the natural ability of CNS neurons to regenerate following spinal cord injury.

Neuronal Regeneration-Associated Genes

CNS neurons mount a transient regenerative response as is evidenced by the expression of regeneration-associated proteins or genes. One of the first regeneration or growth-associated proteins (GAP) identified was GAP-43 (Skene and Willard, 1981; Bulsara et al., 2002). Its involvement in regeneration has been studied extensively and may include the regulation of membrane mobility and F-actin polymerization associated with lipid rafts (Schmidt, 2004; Golub and Pico, 2005). β 1-tubulin and β II-tubulin upregulation can be seen in neurons after injury and can be induced by neurotrophin treatment (Kwon et al., 2002a; Storer et al., 2003). Two weeks after a complete transection of the spinal cord, local cells such as interneurons show an increased expression of a variety of genes, including neuronal annexins that may be involved

in survival responses, and HB-GAM, which is implicated in neurite outgrowth during development (Liu et al., 2004; Zhang et al., 2004).

A transient expression of regeneration-associated proteins for approximately 2 weeks after injury has been demonstrated (Tetzlaff et al., 1991) and corresponds well with the temporal pattern of sprouting of long projecting tracts such as the rubrospinal projections in the spinal cord. GAP43 and the tubulins are structural components and therefore expected to be increased during the growth phase. Surprisingly, corticospinal motor neurons do not express regeneration-associated genes after spinal cord injury, but do so after an axotomy close to the cell body (Tetzlaff et al., 1994; Hains et al., 2003; Mason et al., 2003). However, such lesions close to the perikaryon result in extensive neuronal death.

The knowledge about regeneration-associated genes expressed by neurons following injury continues to increase. One new member may be glypican-1, which is one of the few genes that are induced by both central and peripheral injury to the sensory afferents (Bloechlinger et al., 2004). Glypican-1 is a co-receptor for regeneration-promoting molecules such as FGF and laminin. Another new member may be galectin-1, which is expressed by injured central neurons (McGraw et al., 2004) and is known to facilitate PNS regeneration by activating the release of neurotrophic factors from macrophages (Horie et al., 2004). The transcription factor CTIP2 has recently been implicated in corticospinal tract development and may also prove to be important for regeneration (Arlotta et al., 2005).

Various genes which may be needed for sustained outgrowth have been associated with regenerating systems (e.g., facial motor neurons) as compared to non-regenerating ones (e.g., red nucleus) 7 days after injury (Schmitt et al., 2003a). Not surprisingly, proteins such as GAP-43 were not differentially expressed between these systems, as both show increases after injury. At present, the in-

tracellular signals that lead to spontaneous growth after injury are not clear. Identification of such signaling pathways would help to develop drugs that enhance or reduce their activation. The pathways may include the ATF3/c-jun heterodimer transcription factor complex (Mason et al., 2003), which is needed for outgrowth in the PNS (Pearson et al., 2003; Lindwall et al., 2004). Differential ATF3 expression in response to injury is seen between CNS and PNS non-neuronal cells (Hunt et al., 2004). An intracellular signaling molecule that recently has received much attention is cAMP (Filbin, 2003), which may mediate some of its regeneration-promoting activity by regulating CREB and CRE-mediated transcription of genes needed for regeneration (Gao et al., 2004). The number of genes potentially regulated by CREB is large (Impey et al., 2004). It should be noted that the time course of the sprouting response of these various types of neurons after traumatic spinal cord injuries has not been investigated, making it difficult to compare the various gene expression profiles to the sprouting response. Such an effort might enable the identification of genes that are associated with spontaneous axonal regrowth.

Integrins are transmembrane receptors for a variety of extracellular matrix molecules and are known to be involved in neurite outgrowth. For example, $\alpha7\beta1$ integrin contributes to neurotrophin-induced outgrowth of sensory neurons in vitro (Gardiner et al., 2005). This integrin could play a role for sensory sprouting as most normal DRG neurons express it, despite the fact that it is upregulated after nerve but not after CNS injury (Wallquist et al., 2004). Of interest is the finding that c-jun, which is required for outgrowth, regulates $\alpha7\beta1$ expression (Raivich et al., 2004). There may be growth substrate differences between different classes of sensory neurons depending on the types of integrins they express (Guan et al., 2003). Another interesting observation is that αv integrin appears to be needed for long-term axonal maintenance (McCarty et al., 2005), raising the possibility that it could play similar roles after spinal cord injury.

External Changes Influencing Spontaneous Outgrowth

The responses in the injured spinal cord tissue also have given insight into the mechanisms that lead to the limited outgrowth response. After spinal cord injury, local expression of neurotrophic factors (such as FGF-2, IL-6, TGF- β , GDNF, CNTF, VEGF, NGF, and BDNF) is increased (Logan and Berry, 1993; Oyesiku et al., 1997; Satake et al., 2000; Bareyre and Schwab, 2003; Madiyai et al., 2003; Brown et al., 2004; Zhang et al., 2004). NGF is responsible for sprouting of nociceptive TrkA ex-

pressing sensory afferents (McMahon et al., 1995; Gwak et al., 2003). The increase in some of these factors, such as BDNF, is as rapid as 6 h after injury, but apparently not sufficient to overcome inhibitory mechanisms or not long enough to promote a maintained regenerative response of longer projecting fibers or across injury sites. Other reviews in this journal issue will discuss the recent advances made in neurotrophic factor treatments of the injured spinal cord.

Extracellular matrix molecules also play a role in stimulating regenerative sprouting after spinal cord injury (Grimpe and Silver, 2002). Laminin is known for its neurite-promoting activity and is increased in the injury site after spinal cord contusion due to deposition by new blood vessels and Schwann cells (Casella et al., 2002; Loy et al., 2002; Jones et al., 2003). Axons growing into fibroblast grafts have been shown to associate with laminin and L1 on Schwann cells after spinal cord injury (Jones et al., 2003). L1 may actually mediate the preference of axons for Schwann cells over astrocytes (Adcock et al., 2004). Neuronal laminin containing the $\gamma1$ chain (Hagg et al., 1989; Yin et al., 2003) is involved in axonal growth in the hippocampal formation (Grimpe et al., 2002). Another extracellular matrix molecule that may play a role in spontaneous axonal growth following injury is collagen, which can often be found in the fibroblast rich core that forms in the injured mouse spinal cord (Inman and Steward, 2003). However, collagen has also been implicated in sequestering growth inhibitory molecules and therefore indirectly in axonal growth inhibition. The role of collagen in spinal cord injury is reviewed elsewhere in this journal issue.

Metalloproteases are involved indirectly in axonal sprouting in the injured nervous system. Matrix metalloproteases (MMPs) remodel the extracellular matrix, including degradation of inhibiting molecules, and other metalloproteases such as the ADAMs can activate cleavage-dependent proteins such as certain growth factors and other molecules involved in axonal outgrowth (McFarlane, 2003; Dzwonek et al., 2004). MMP-2 and 9 may play a role in modifying inhibitory parts of the wound area after spinal cord injury in adult rats to permit growth of axons (Duchossoy et al., 2001). MMP-9 can degrade the neurite growth inhibitor NG2 (Larsen et al., 2003). MMP 1, 2, and 9 are similarly involved in optic nerve regeneration (Ahmed et al., 2005). After spinal cord contusion, MMP-9 increases rapidly and is reduced to control levels again after 14 days, whereas MMP-2 appears at 7 days and remains upregulated for at least 3 weeks (Goussev et al., 2003). These changes could coincide with spontaneous axonal outgrowth.

MMP-9 is also involved in early detrimental vascular events after spinal cord injury and its inhibition by ge-

netic or pharmacological means leads to better functional recovery (Noble et al., 2002). MMP-5 reportedly is involved in the development of neuropathic pain (Komori et al., 2004). Clearly, MMPs play a variety of roles, which are beneficial or detrimental to regenerative events in the injured spinal cord.

In general, the positive contributions of neurotrophic factors and some of the extracellular matrix molecules is most likely counteracted by a number of inhibitors that are expressed after spinal cord injury. Such inhibitors are discussed elsewhere in this issue.

Remyelination after Injury

The lack of significant functional recovery is thought to also be caused by the lack of appropriate remyelination of spared fibers. Demyelination is common after spinal cord injury in rodents (Cao et al., 2005). Interestingly, besides their known role in scar formation, reactive astrocytes appear to protect against demyelination after spinal cord injury and also have other neuroprotective effects (Faulkner et al., 2004). In the spinal cord, after a focal demyelinating lesion, spontaneous remyelination by Schwann cells and oligodendrocytes has been demonstrated (Gilson and Blakemore, 2002). However, this remyelination process most often results in the formation of thin myelin sheaths, which would still not lead to optimal signal conduction by axons. It was demonstrated by Talbot et al. (2005) that remyelination by oligodendrocytes requires the presence of astrocytes, suggesting that the latter contribute myelination-inducing agents. Such myelin-inducing molecules could include CNTF, which is made by astrocytes and has been shown in a viral demyelination model to cause an autocrine release of FGF2, which in turn induces remyelination (Albrecht et al., 2003). MMP-9, which is increased after spinal cord injury (Noble et al., 2002), may play a role in remyelination by degrading NG2 (Larsen et al., 2003).

Plasticity of Uninjured Systems

Adult humans and animals with partial injuries often undergo spontaneous recovery that can progress over long times after the initial injury. At least part of this recovery may be due to collateral sprouting of non-injured axons (Hagg, *this issue*). For example, retrograde tracing studies showed collateral sprouting distal to an injury months after spinal cord hemisection in adult monkeys (Aoki et al., 1986). Also, after a transection of the dorsal corticospinal tract in adult rats, the uninjured ventral corticospinal pathway sprouts and contributes to improved locomotor behavior (Weidner et al., 2001). Collateral sprouting can also occur from spared corticospinal fibers on the denervated side in the lumbar cord after par-

tial transection of the both corticospinal tracts at the level of the pyramids (Hagg et al., 2005). Evidently, in many tracts undamaged axons have the inherent ability to form collaterals in response to injury of their neighboring axons.

Is it possible that new circuits are formed after spinal cord injury and could these be involved in functional recovery? After a transection of the hindlimb corticospinal tract in adult rats, it was demonstrated that the injured axons sprouted in the cervical gray matter to contact propriospinal neurons which in turn bridged the injury site and contacted lower motor neurons (Bareyre et al., 2004). In this particular study the effects of the new circuits on functional recovery were not evaluated. However, several studies have demonstrated that plasticity in the spinal cord itself can improve motor functions after incomplete and complete transections (Raineteau and Schwab, 2001; Edgerton and Roy, 2002; Edgerton et al., 2004). Reorganization after spinal cord injury also occurs in the somatosensory cortex of monkeys (Kaas and Collins, 2003). So far, the exact mechanisms underlying the functional recovery seen in association with these plastic changes in the injured spinal cord are still unknown. Also, it should be kept in mind that plasticity in the injured spinal cord does not always result in a positive outcome. For instance, sprouting of primary afferents after spinal injury is associated with autonomic dysreflexia (Marsch et al., 2002; Weaver et al., 2002) and pain (Gwak et al., 2003). These are common and debilitating consequences of spinal cord injury and a potential hurdle for therapies that promote regeneration of other systems.

HUMAN SPINAL CORD INJURY AND THE VALIDITY OF ANIMAL MODELS

The following description of the known natural history and neuropathology of spinal cord injury in humans is largely based on two recent comprehensive reviews (Kakulas, 2004; Norenberg et al., 2004). The morphological responses in humans are qualitatively very similar to that seen in rodent models. However, there are differences that should receive more attention, as they might challenge the current experimental development of therapies (Table 1). The inflammatory component is much less pronounced in humans, with only a minor response by neutrophils. On the other hand, the rapid and transient expression of cytokines is very similar to that seen in rats with spinal contusions (Streit et al., 1998; Yang et al., 2004). The astroglial response is markedly delayed and reduced compared to rodents, and only a mild astroglial scar develops (Puckett et al., 1997; Buss et al., 2004). CSPGs are expressed after spinal cord injury but are primarily associated with other

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TABLE 1. SIMILARITIES AND DIFFERENCES BETWEEN THE HUMAN AND RODENT SPINAL CORD IN RESPONSE TO INJURY

	<i>Rodent</i>	<i>Human</i>
Degenerative processes		
Vascular response	Hemorrhage, angiogenesis	Hemorrhage, angiogenesis
Inflammation	Extensive	Much less pronounced, despite similar cytokine expression
Demyelination	Yes	Yes, but perhaps less pronounced
Axonal degeneration	Some die-back and Wallerian degeneration	Wallerian degeneration much more protracted
Glial scar	Extensive, with astroglial CSPG	Not extensive, CSPGs mostly in blood vessels
Cyst formation	Rat yes; mouse no	Yes
Schwann cell response	Some invasion	Extensive Schwannosis
Regenerative processes		
Sprouting	Yes	Yes
Remyelination	Yes	Yes
Plasticity of uninjured systems	Yes	Yes

CSPG, chondroitin sulphate proteoglycan.

cells and not astrocytes. In fact, most CSPG staining is seen in blood vessel walls and occasional astrocytes, and in Schwann cells (Bruce et al., 2000), which invade the spinal cord as part of the pronounced Schwannosis that develops in the majority of SCI cases. Schwannosis is present in humans but less prominent in rodents. Wallerian degeneration is very protracted in humans and found years after the injury (Buss et al., 2004, 2005). Of interest is the finding that the inhibitory protein NOGO-A is rarely seen in the peri-axonal myelin sheath and that the inhibitor MAG disappears between 14 days and 4 months after the injury in humans (Buss et al., 2005). These axonal growth inhibitors remain present on the outer sheath for long times, which raises the possibility that axons could be made to regenerate more readily inside the hollow tubes of myelin of spared oligodendrocytes. Lastly, there has been a sense that very little demyelination of spared axons is present in human cases (Kakulas, 1999). One of the problems with the pathology results is the lack of high resolution analysis that is needed to detect demyelination. A recent immunocytochemical study provided evidence for substantial numbers of demyelinated axons in 13 humans with spinal cord injury (Guest et al., 2005). It will be important to provide electron microscopic analysis of important systems such as the corticospinal and sensory tracts, as this would establish the need and potential for oligodendrocyte replacement therapies. Oligodendrocytes undergo apoptosis in injured humans, but these are associated with injured systems (Yamaura et al., 2002; Buss et al., 2005).

In humans, there is some evidence of endogenous regeneration in the injured spinal cord, as in animals, most clearly by sensory afferents. The neuropathology under-

lying such spontaneous recovery has been reviewed earlier (Tator, 1998). The spinal cord circuitry has been shown to undergo plastic changes, which may include growth of sensory fibers (Edgerton and Roy, 2002; Edgerton et al., 2004; Calancie et al., 2005). The cortical circuitry also shows plastic changes and reorganization in humans with SCI (Curt et al., 2002; Corbetta et al., 2002; Mikulis et al., 2002; Crawley et al., 2004).

Remyelination by Schwann cells has been demonstrated to occur in humans with spinal cord injury. Angiogenesis in the injured human spinal cord appears to develop over a similar time frame as in injured rodents (Kakulas, 2004; Norenberg et al., 2004). CNS neurons most likely respond similar to injury in humans as they do in rodents. For example, the regeneration associated genes GAP43 and c-jun are transiently expressed in Clarke's nucleus after a human spinal cord injury (Schmitt et al., 2003b).

A variety of experimental animal models have been used to investigate responses to spinal cord injury and potential treatments (Kwon et al., 2002b; Stokes and Jakeman, 2002; Eaton, 2003). The contusion model appears to be closest to the most common form of injury in humans. A contusion injury with the dura intact leaves a rim of spared white matter after several weeks, which is also known to happen in humans. Interestingly, with less than 10% spared white matter at the injury site, both humans and animals can have spared motor functions (Nashmi and Fehlings, 2001). Obviously, the percentage is not as important as the location of the spared fibers. Laceration (transection) models are also commonly used and are relevant, as human injuries can be pure laceration.

tions or often are a combination of laceration and contusion (e.g., as a result of the common burst fracture). Similarly, compression, alone or in combination with laceration and contusion, is seen in humans and has been used in animal models. Purely ischemic models are also relevant, as ischemia is a component of human spinal cord injury.

There are many different and perhaps relevant injury models for human spinal cord injury. The problem with most injury models are that they are applied from the dorsal surface after a laminectomy. This standardizes the injury severity and location, but does not mimic the majority of human injuries, which mainly are the result of a ventral impact and are associated with compression within a closed vertebral canal. In addition, the impact speed of contusion devices is much slower than that of most human injuries. Should one develop high-velocity impact injuries that mimic the complicated and variable human injuries, possibly requiring numerous animals per data point? Perhaps the answer is that if a treatment does not work in defined and minimal injuries in the laboratory, then it would not be effective or relevant for the more severe and varied injuries in humans. Ultimately, such treatments will need to be tested in a variety of models and, if effective in all, would predict their success in humans. Given the potentially important differences in neuropathological responses between humans and rodents, the correct choice of species for laboratory investigation has not been resolved. Even the rat and mouse and different strains respond differently to spinal cord injury. We suggest (as do others) that, in cases where treatments are effective in different models in both mice and rats, human trials are warranted only if the treatments are expected to have a minimal amount of risk. Otherwise, testing in an intermediate species is advised. Therefore, perhaps it is time to identify an animal model that more closely mimics the human injury response, preferably a primate. Marmosets may be a good choice as they are small, breed well and can be available in large numbers, and have already been used in spinal cord injury research (Fouad et al., 2004; Iwanami et al., 2005). Moreover, their genome is being sequenced, which may help to identify genes and proteins that explain potential differences and similarities in responses to spinal cord injury between the humans and marmosets.

CONCLUSION

In conclusion, it is clear that many temporally and spatially distinct degenerative processes underlie the tissue loss and dysfunction caused by spinal cord injury. It is also clear that much remains to be discovered, as no success-

ful treatments based on the current knowledge exist. An increased understanding of these processes might facilitate the rational design of neuroprotective treatments which target individual components of degeneration. The general view is that this will require multiple treatment approaches. However, since many of the degenerative events are part of cascades, it is possible that single interventions could result in substantial functional preservation or recovery if started at certain crucial times after the injury. The spontaneous regenerative processes after spinal cord injury, including sprouting of injured axons, remyelination and plasticity of uninjured systems, are more extensive than were thought a few decades ago. These also provide an opportunity to further enhance neurological recovery. It will be important to further investigate the molecular regulators of these regenerative mechanisms. A better understanding of the degenerative and spontaneous regenerative processes also is essential for the ongoing efforts to promote long-distance regeneration in the spinal cord. Lastly, an important and unresolved question in the spinal cord injury research field (as in any field) is the relevance of the current animal models for injured humans. Clearly, much more needs to be done to understand the neuropathological processes and molecular changes in humans after spinal cord injury.

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