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Spinal cord injury – scientific challenges for the unknown future

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Abstract

The history of spinal cord injuries starts with the ancient Egyptian medical papyrus known as the Edwin Smith Surgical Papyrus. The papyrus, written about 2500 B.C. by the physician and architect of the Sakkara pyramids Imhotep, describes "crushed vertebra in his neck" as well as symptoms of neurological deterioration. An ailment not to be treated was the massage to the patients at that time. This fatalistic attitude remained until the end of World War II when the first rehabilitation centre focused on the rehabilitation of spinal cord injured patients was opened.

Our knowledge of the pathophysiological processes, both the primary as well as the secondary, has increased tremendously. However, all this knowledge has only led to improved medical care but not to any therapeutic method to restore, even partially, the neurological function.

Neuroprotection is defined as measures to counteract secondary injury mechanisms and/or limit the extent of damage caused by self-destructive cellular and tissue processes. The co-existence of several distinctly different injury mechanisms after trauma has provided opportunities to explore a large number of potentially neuroprotective agents in animal experiments such as methylprednisolone sodium succinate. The results of this research have been very discouraging and pharmacological neuroprotection for patients with spinal cord injury has fallen short of the expectations created by the extensive research and promising observations in animal experiments. The focus of research has now, instead, been transformed to the field of neural regeneration. This field includes the discovery of regenerating obstacles in the nerve cell and/or environmental factors but also various regeneration strategies such as bridging the gap at the site of injury as well as transplantation of foetal tissue and stem cells. The purpose of this review is to highlight selected experimental and clinical studies that form the basis for undertaking future challenges in the research field of spinal cord injury. We will focus our discussion on methods either preventing the consequences of secondary injury in the acute period (neuroprotection) and/or various techniques of neural regeneration in the sub-acute and chronic phase and finally expose some thoughts about future avenues within this scientific field.

Introduction

The medical history of spinal cord injuries starts with the ancient Egyptian medical papyrus known as the Edwin Smith Surgical Papyrus (1, 2). This medical treasure was written about 2500 years B.C., i.e. at the time of the great pyramid building. The construction of the pyramids made it possible for the first time to study CNS trauma, due to a high incidence of accidents. The papyrus, written by the physician and architect of the Sakkara pyramids Imhotep, presents 48 trauma cases in-

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cluding six of vertebral injuries ranging from mild to severe deterioration. In the well-known case 33, “Instructions concerning a crushed vertebra in his neck”, the author’s statement “having a crushed vertebra in his neck” is considered as “an ailment not to be treated”. Imhotep describes in that case symptoms associated with spinal cord injury (SCI) such as loss of motion in arms and legs (tetraplegia), loss of sensation below level of injury and of bladder control.

It lasted until Hippocrates (about 460–377 B.C.) before vertebral injuries were correlated with paralysis. He introduced the “Hippocratic Board” to reduce spinal deformities and advocated, in the treatment of the SCI patients, a diet consisting of 4-9 pints of donkey milk combined with honey and a special mild white wine from Mendez in Egypt. Hippocrates also described the difficulties for the patients with paralysis such as constipation, dysuria, skin problems and edema. The Greek Galen (130–201) was the first to perform animal experiments. He described that a longitudinal incision to the spinal cord did not give any loss of function but a transverse incision at cervical level resulted in loss of both motor and sensory function below level of injury. The surgeon Paulus (625–690) from the Greek island of Eagina introduced 500 years later the laminectomy; i.e. the removal of the vertebral arch in order to decompress the dural sac and spinal cord. Andreas Vesalius published in 1543 one of the greatest medical books ever written – entitled “Seven books on the fabric of the human body” in which the human nervous system for the first time was illustrated in detail.

During the 19th century famous persons such as Lord Nelson and the US president James Garfield died following a spinal cord injury. The bullet was not found, in the latter, despite serious attempts from Alexander Graham Bell to localize it with the help of a special metal detector device. The president died after a few months due to infections and internal bleedings.

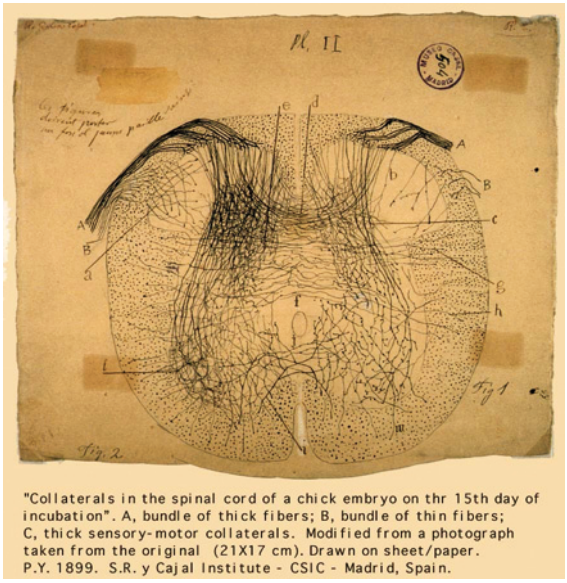

Figure 1. Transection of chicken spinal cord from the collections of Ramon Y Cajal.

The "father of neuroscience" Santiago Ramon y Cajal was rewarded with the Nobel Prize in medicine 1906 for the description of the central nervous system structure. In 1911 Reginald Allen introduced the experimental weight-drop technique starting the "modern era" of SCI research. Both these researchers were aware of, already at that time, the contribution of secondary injury mechanisms to the final neurological deficit after an injury to the spinal cord. During that period, including the First World War era, the mortality was still up to 80 % within the two first weeks following a spinal cord injury at the cervical level. This lead to a continued fatalistic attitude towards treatment of SCI during the inter world war period. However, the modern era of rehabilitation started in 1943 when Sir Ludwig Guttmann opened the Stoke-Mandeville National Spinal Centre. The creation of rehabilitation clinics that focused on the special program associated with SCI together with the introduction of antibiotics led to a big step forward for this group of patients. In the following decades an improved health care resulted in a substantial improvement in quality of life and life expectancy. For a person with SCI the life expectancy is today close to the non-injured population.

Our knowledge about the pathophysiological process has increased in parallel with the improved medical management. The concept of primary and secondary mechanisms has been accepted both experimentally and in the acute care of these patients. All this knowledge, however, has only led to improved medical management but not to any therapeutic method to restore, even partially, the neurological function.

The prevention of the secondary damage or self-destruction of neurons following an injury and the desirable effort to rescue tissue is summarised under the context of neuroprotection. A variety of therapeutic agents has been used targeting one or more mechanisms of secondary injury in order to confer neuroprotection and prevent unnecessary tissue damage. However, the focus of research has now been transformed to more long-range consequences of injury to the spinal cord due to the unsuccessful result of the neuroprotective efforts. The present and future research includes the results from the field of neural regeneration. The breakthrough in this field was the work of David and Aguayo in 1981 (3) concluding that if axon regeneration should occur both intrinsic cellular and CNS environmental factors have to be approached. In conclusion, the purpose of this review is to highlight selected experimental and clinical studies that form the basis for undertaking future challenges in the research field of spinal cord injury. We will focus our discussion on methods either preventing the consequences of secondary injury in the acute period (neuroprotection) and/or various techniques of neural regeneration in the sub-acute and chronic phase and finally expose some thoughts about future avenues within this scientific field.

Neuroprotection

Neuroprotection is defined as measures to counteract secondary injury mechanisms and/or limit the extent of damage caused by self-destructive cellular and tissue processes. The hypothesis of secondary events was, during the 1970s, focused on vascular

Table 1. Reported positive effects of MPSS in animal experimental studies

Improves energy metabolism	Reduces oedema formation by maintaining the blood-brain barrier
Reduces posttraumatic ischemia	Reduces degeneration of nervous structures
Stabilises phospholipid-membrane structures	Counteracts formation of free radicals
Reduces inflammatory response	Reduces neurological deterioration

damage leading to oedema, free radical formation and norepinephrine release while the calcium processes, opiate receptor mechanisms, cytokine involvement and nitrous oxide formation and finally lipid peroxidation were highlighted during the 1980s. The knowledge of apoptosis, energy metabolism, inflammation and excitoxicity has increased markedly during the 1990s. The co-existence of several distinctly different injury mechanisms after trauma has provided opportunities to explore a large number of potentially neuroprotective agents in animal experiments.

Methylprednisolone sodium succinate (MPSS) is the most extensively studied agent of these substances and table 1 summarizes reported positive effects of the substance in animal experiments.

Neuroprotection research has, besides characterization of injury mechanisms and testing of different substances in animal experiments, been focused on a number of clinical studies aimed at minimizing neurological deficits after trauma (4). Baptiste and Fehlings published a survey of 10 randomised, prospective and controlled neuroprotective studies, considered by the authors to be scientifically reliable (table 2).

Although numerous other attempts have been made to support the spinal cord´s own neuroprotective potential, these studies are the most quoted. Even if we will concentrate our review on experimental studies, it should be emphasized that all types of immediate therapeutic interventions also have as an underlying objective to support the organism's intrinsic neuroprotective potential. A common and remaining problem for all treatment in the acute stage is the absence of knowledge about the width of the so-called therapeutic window, i.e. within which time frame after injury must treatment start to become effective? Extensive efforts have been invested to translate experimental data on secondary injury mechanisms to help defining therapeutic windows in the clinical situation.

In a series of clinical studies, see table 2, the National Acute Spinal Cord Injury Studies (NASCIS I-III), MPSS was given alone or in combination with either the opioid antagonist naloxone or the lipid peroxidation inhibitor, tirilazad mesylate (5, 6). In addition, based on beneficial effects in animal experiments, clinical studies have been carried out with GM-1-ganglioside (7), thyrotropin releasing hormone (TRH), the calcium channel blocker Nimodipine and the NMDA-receptor antagonist Gacyclidine. Although none of these studies has shown significant beneficial clinical effects, administration of MPSS within 8 hours after injury is today considered as a "treatment option" (8).

Also, non-pharmacological interventions have been claimed to contribute to neuro-

Table 2. Summary of ten randomised, prospective and controlled neuroprotective studies (from Baptiste and Fehlings, 2006)

	Study	Year	Properties
1	NASCIS-I	1984	Antioxidative/antiinflammatory: see table 1
2	MPSS NASCIS-II MPSS, naloxone or placebo	1990	Naloxone improves spinal cord conduction and reduces oedema formation
3	NASCIS-III MPSS or NMPSS and tirilazad mesylate	1997	Tirilizad mesylate prevents lipid peroxidation, stabilises phospholipids membranes and restores endogenous vitamin E level
4	Japanese MPSS study	1994	
5	Maryland ganglioside study (GM-1)	1991	GM-1 stimulates axonal growth through the site of injury
6	Sygen® ganglioside study (GM-1)	1998	
7	Thyrotropin releasing hormon (TRH) TRH or placebo	1995	TRH counteracts the effect of excitatory amino acids and endogenous opioids and has antioxidant and membrane stabilising properties
8	Nimodipine, Nimodipine, MPSS, in combination or placebo	1998	Calcium-channel blocker
9	Gacyclidine study Gacyclidine or placebo	1999	Neuroprotective (NMDA-receptor antagonist)
10	Decompression	1997	Decompression performed less than 72 h, or more than 5 d, respectively, after trauma

protection (9). Animal experiments have shown that early decompression reduces neurological deficits after spinal cord trauma, and a prospective, large scale multi-center study, STACIS, addressing these problems, has now been started. A major reason for this is the widespread reluctance to refrain from an operative intervention, which is commonly regarded to help recovery and mobilisation after acute spinal cord injury. Similarly, lowering whole body or local spine temperature has not been proven to influence the outcome after spinal cord injury. In summary, pharmacological neuroprotection for patients with spinal cord injury has fallen short of the expectations created by the extensive research and promising observations in animal experiments.

Regeneration

The functional consequences of spinal cord injury are determined by the level and extent of damage to pathways coursing in the white matter (Figures 2 and 3).

With the exception of certain areas on the neck, gray matter destruction over a few

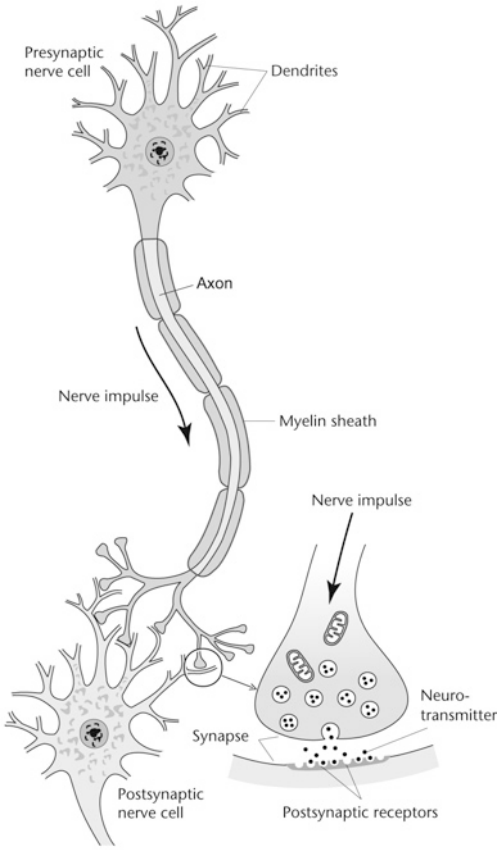

Figure 2. Intact nerve cell making synaptic connections with a postsynaptic neuron. The enlarged figure to the right shows the synaptic complex with the presynaptic terminal, release of its neurotransmitter and the postsynaptic site where the neurotransmitter is bound to specific postsynaptic receptors.

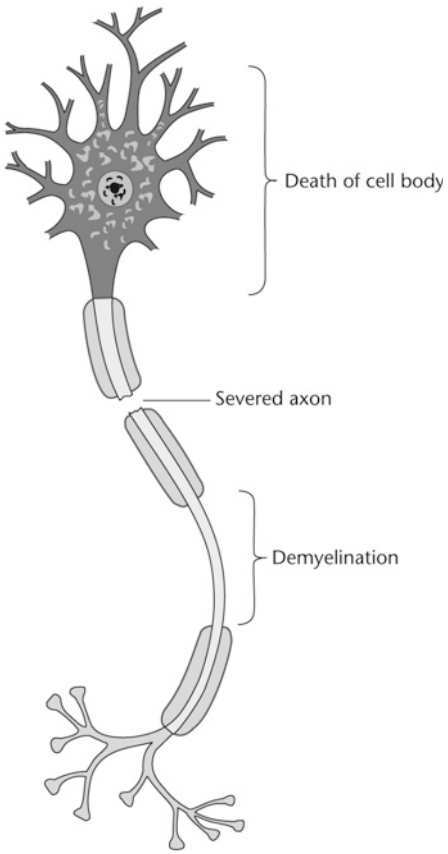

Figure 3. The main degenerative events following neuronal injury are: death of the neuron, interruption of its axon leading to Wallerian degeneration of the distal stump, and loss of myelin internodes (demyelination).

segments is usually followed by only modest functional deficiencies. Interruption of descending and ascending white matter pathways, i.e. separation of axons from their nerve cell bodies, results in Wallerian degeneration of axons and myelin. Axon injury may also cause death of the parent nerve cell body, particularly if the lesion is close to it. In order to restore lost functions, injured neurons have to survive, and their axons need to regenerate across or around the lesion site and make functionally useful connections caudal (descending tracts), or rostral (ascending tracts) to it. As a result of mechanical compression, myelinated axons may lose their myelin (demyelination), and hence their ability to propagate nerve impulses. These axons need to become remyelinated in order to resume impulse propagation.

The failure of injured spinal cord axons to regenerate was originally demonstrated by Ramon y Cajal in the end of the 19th century. The dogma that spinal cord

neurons are unable to regenerate their injured axons dramatically changed with the results of the experiments by David and Aguayo in the early 1980s (3). Their experiments clearly demonstrated that central nervous axons are able to regeneration provided they are exposed to a permissive environment (peripheral nerve tissue), but cease to elongate when confronted with CNS tissue. During the subsequent two decades several regeneration obstacles have been identified. Based on these findings, various regeneration strategies have been developed with the aim of modifying the growth inhibitory properties of the spinal cord environment. Here, we present some regeneration obstacles followed by a selection of interesting regeneration strategies.

Regeneration obstacles

The adult central nervous system environment contains a variety of mechanisms that actively inhibits axonal growth. Various processes, in the acute as well as in the chronic stage, following a SCI counteracts the potential of injured axons to cross the level of injury and finally to reconnect with nerve cells below the injury site. These obstacles include an insufficient growth response by the injured nerve cells (nerve cell disability) and environmental factors in the nerve cell surrounding (table 3).

Nerve cell disability

Following axon injury, signals to the affected nerve cell body induces a shift in neuronal gene expression (10, 11). In contrast to the situation in the peripheral nervous system, central neurons are able to maintain the expression of regeneration-associated genes only for a limited period, presumably due to the absence of sufficient growth stimulating factors in their environment. As a result, the injured neurons gradually enter an atrophic state, and may eventually degenerate and die.

Environmental factors

The injured axons are surrounded by factors which inhibit axon growth. These include NOGO-A (12) and myelin-associated glycoprotein (MAG; 13), which are both produced by oligodendroglial cells. Astrocytes, microglial cells, oligodendrocytes and meningeal cells contribute to the formation of a scar at the injury site. The scar presents a mechanical barrier, but the main obstacle for axon growth is the presence of a chemical barrier composed of proteoglycans and collagens (14).

Table 3. Regeneration obstacles

Nerve cell disability	Environmental factors
Nerve cell body response	Presence of growth inhibiting factors Scar tissue formation Formation of cavities and cysts at, below and above the level of injury

The addition of extensive necrotic and apoptotic cell death in the injury region enhances the development of cavities and cysts in the spinal cord itself. When the scar is fully formed, it undoubtedly provides a mechanical obstacle to axon growth. However, this process requires many weeks to complete. The question therefore remains – why are axons unable to grow across the injury site before the scar has formed? The most plausible explanation is that a growth inhibitory environment is created early after the injury, including the expression of myelin-associated inhibiting components, as a result of the combined activity of local glial cells and invading hematogenous cells. Finally, the emergence of a scar tissue adds further obstructive properties to the injured spinal cord.

Regeneration strategies

In the light of these considerations, successful regeneration in the spinal cord requires the combination of several approaches, which will be discussed in the following text.

I. Promoting the intrinsic neuron capacity for regeneration.
II. Counteracting the early inhibitory mechanisms on axon growth in the environment of the injured neurons.
III. Overcoming the late inhibitory mechanisms on axon growth in the environment of the injured neurons.

I Promoting the intrinsic neuron capacity for regeneration

Neurons are endowed with a normally inactive regeneration "program", which becomes activated following injury. In order to restore neuronal capacity following damage the injured axons need to activate the regeneration-associated genes (RAG). This activation program is markedly stimulated by treatment with appropriate growth factors (Table 4).

The first of these factors was identified in the 1960s, termed nerve growth factor (NGF). Nerve growth factors which influence the development and maintenance of neurons are often referred to as neurotrophic factors ("nourishing" neurons). Subsequent studies showed that NGF is a member of the neurotrophin family of growth factors, which also include brain-derived neurotrophic factor (BDNF), neurotrophins 3 (NT-3) and 4 (NT-4). Neurotrophic growth factors are small molecules

Table 4. Examples of growth factors (neurotrophic factors)

Neurotrophic factors	Abbreviation
Nerve growth factor	NGF
Neurotrophin 3 och 4	NT-3/NT-4
Brain-derived neurotrophic factor	BDNF
Fibroblast growth factor	FGF
Glia cell line derived neurotrophic factor	GDNF

with a wide range of activities, including the promotion of neuron survival and axonal outgrowth, as well as the regulation of neuron-target interactions (Table 5; 15, 16). The most interesting activities of these factors in the context of spinal cord injury seem to be their ability to stimulate survival of injured neurons, as well as to regulate the expression of RAGs in a way that increase the regeneration capacity of injured axon (17–20).

Although several growth factors have well documented effects on axonal growth, there are problems with their application in spinal cord injury. The specificities of the growth factors are incompletely known in terms of their effect on neuronal populations and peripheral tissues, e.g. muscle. Several growth factors stimulate axons that convey nociceptive information, which can lead to increased pain experiences (21). Different growth factors also appear to stimulate neuronal growth at different stages in the repair process, and sometimes in an antagonistic manner (Table 5; 22). It is therefore a challenging task for the future to determine which growth factors are optimal for promoting survival and regeneration of different types of neuron populations, and at what time point they should be administered. Moreover, growth factors do not pass the blood-brain barrier, which raises the issue of how to deliver them in an efficient and controlled way. Intrathecal delivery via osmotic pumps containing genetically modified neurotrophin releasing cells, e.g. fibroblasts, the implantation of slow-release cell-free systems, or gene therapy (23) are possible options. Taken together, there are a number of well-defined growth factors with established positive effects on axon growth, that might be used in future treatment of SCI.

II Counteracting the early inhibitory mechanisms on axon growth in the environment of the injured neurons

We have chosen to define "Strategies counteracting the early inhibitory mechanisms on axon growth in the environment of the injured neurons" as measures that can be performed as long as formation of scar tissue is estimated to take place at the level of injury.

Axonal regeneration is counteracted in the early period after an injury by the emergence of myelin-associated inhibitors and by factors that accelerate the formation of scar tissue in the environment surrounding the nerve cell. Here, we discuss some of the main strategies that have been adopted to counteract the effects of early inhibitory mechanisms in the nerve cell environment (Table 6).

Table 5. Strategies stimulating the neuronal intrinsic regenerating capacity

Target/mechanism	Delivery	Effect
CNS-tissue (neurons)		
Stimulating regeneration associated genes	Intrathecal injections Local deposition and others	Re-growth of damaged axons Functional improvement

Table 6. Strategies to counteract the early inhibitory mechanisms of the CNS environment

Factor	Target	Delivery	Effect
Antibodies (IN-1)	NOGO-A receptors on regenerating axons	Pumps Pills?	Blocked inhibition of axonal outgrowth immunisation
4 – amino pyridine	Demyelinated axons	Intravenous Intrathecal	Restored signal transmission
Monoclonal antibody	ICAM	Immunisation	Reduced oedema Increased SCBF
	Interleukins	Systemically Ip	Switches of the inflammatory response
Chondroitinase ABC	Proteoglycans	Local infusion	Digestion of scar Functional improvement

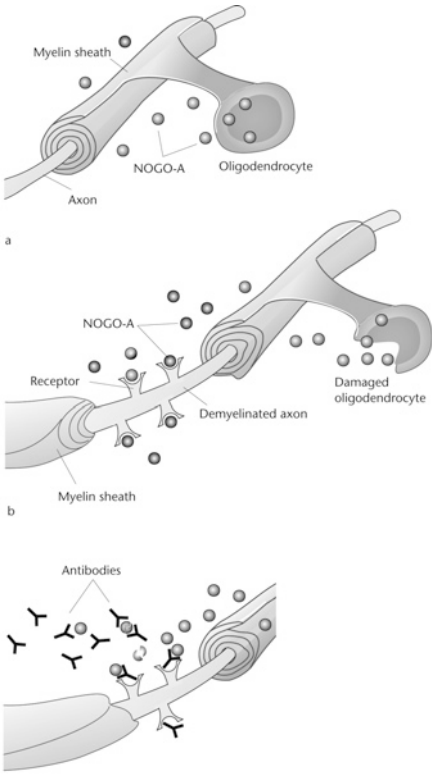

Figure 4. Blocking the growth inhibitory influence of NOGO-A by administration of NOGO-A antibodies.

a) NOGO-A is produced and released by oligodendrocytes.
b) Binding of NOGO-A to specific receptors on injured neurons inhibits axon elongation.
c) NOGO-A antibodies bind NOGO-A as well as its receptor, thereby allowing the injured axon to grow.

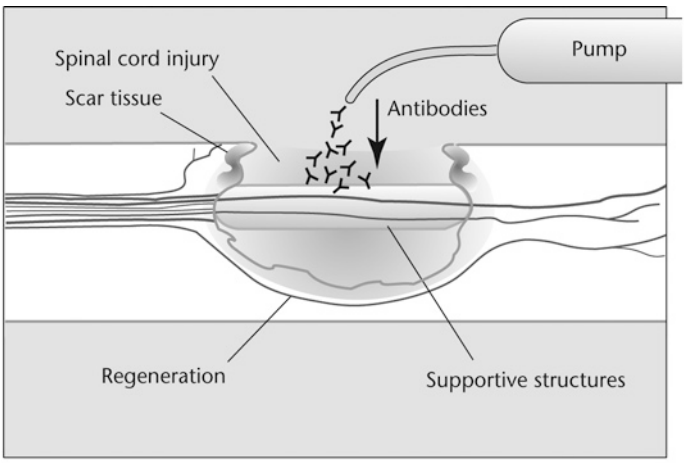

Figure 5. Pharmacological or bioactive agents, e.g. NOGO-A antibodies can be infused intrathecally via a thin tube connected to an osmotic minipump.

Blocking axonal growth inhibitory molecules

The spinal cord of healthy uninjured adults contains powerful inhibitory substances that prevent neurite growth. These factors are vital since they stop axonal growth after the axons achieve contact to other nerve- and muscle cells. Following trauma, the same factors creates a major obstacle at the molecular level by delaying and/or preventing the onset of the regenerating process. The pioneering work by Schwab and collaborators led to the identification of a myelin growth-inhibiting factor, NOGO-A, a myelin protein produced by oligodendrocytes (table 6; 12). NOGO-A exerts its inhibitory effect by blocking specific NOGO-A-receptors on the surface of the axons (fig 4 a–c; 24, 25).

The interaction between NOGO-A and the NOGO-A-receptor results in a receptor mediated inhibition of axon growth. This mechanism is prevented by the administration of antibodies (IN-1) that binds to the NOGO-A itself (neutralising antibodies; 26) or acts as antagonists to the NOGO-A-receptor (27). Thus, intrathecal infusion of these neutralising antibodies blocks the effect of NOGO-A, and thereby indirectly stimulates axon regeneration (fig 5).

In addition to NOGO-A, other axonal growth inhibitory proteins, such as MAG (myelin associated glycoprotein) and OMP (oligodendrocyte myelin glycoprotein) also exerts their effect at the receptor level. Thus, the presence of myelin-associated inhibitors to regeneration is fully recognized. Besides the studies with IN-1 antibodies against NOGO-A, experimental studies has been performed using a passive or active vaccination approach towards myelin inhibitory molecules (fig 6, 28). Substantial anatomical regeneration and functional recovery have been reported using this approach (29). However this approach is controversial, since other studies have reported that passive or active immunization increases structural damage and functional impairment following SCI (30, 31). Recent studies have shown that the growth inhibitory influence of myelin-associated proteins can be overcome by increasing the levels of cAMP in the injured axons and promote axonal elongation in the injured spinal cord (32, 33).

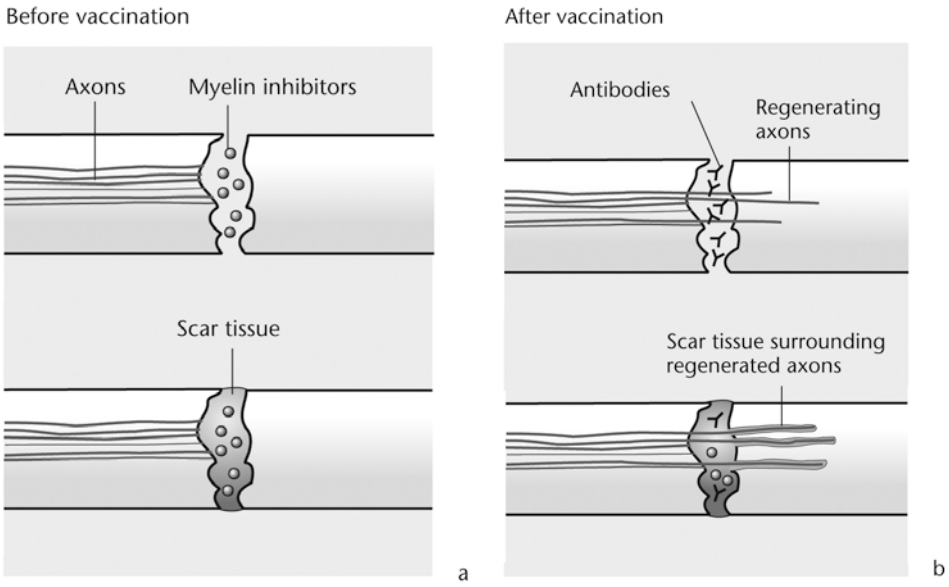

Figure 6. Vaccination.
a) Myelin-associated growth inhibitors first prevent injured axons to cross the injury site; subsequently, the scar tissue provides additional growth inhibition.
b) Circulating antibodies bind these inhibitors, thereby allowing growth of injured axons across the lesion site.

Restoration of impulse propagation by modulating axon membrane properties

Contusion damage to the spinal cord is the most frequent pathological consequence of spinal cord trauma. Transection of the spinal cord rarely occurs and the majority of spinal cord injured patients probably have some axons that survived the acute mechanical damage as well as the effects of the secondary injury mechanisms. In both situations, degeneration of oligodendrocytes, largely by apoptosis, occurs, resulting in demyelination and thereby insecure impulse propagation or complete conduction failure. The myelin that covers the axons is partially or totally lost resulting in insecure or completely lost impulse propagation. In addition, the propagated electrical impulses could be spread between demyelinated axons like a short-circuit in an electrical cable when the outer insulation is damaged. When an axon is demyelinated following injury a large number of potassium channels are exposed and potassium (K+) leaks into the extracellular space resulting in conduction failure (Figure 7).

Infusion of the fast voltage-sensitive potassium channel blocker Fampridine-SR (4-amino-pyridine, 4-AP) blocks the potassium channels at those gaps and makes it possible for the demyelinated axon to propagate action potentials (table 6; 34). This agent has been tested in phase-2 studies and trials are ongoing to give the substance in the early stage of the injury (35).

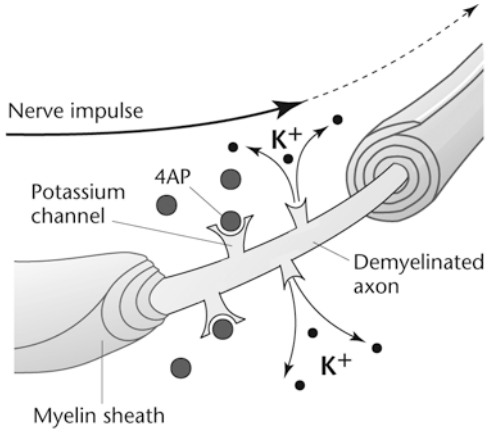

Figure 7. Fampridine-SR (4-AP) blocks open potassium channels and helps to maintain a resting potential.

Modulating the immune system

Modulation of the immune system is a potentially useful strategy to reduce the inflammatory response after trauma, and in this way improve the environment for neuron survival and regeneration. The inflammatory response after spinal cord injury encompasses degenerative as well as reparative processes (36-38). Cellular debris and breakdown products from degenerating neurons, glial cells and haematogenous cells are eliminated through of variety of processes, thereby promoting the restoration of a beneficial environment for surviving cells. Inflammatory cells and their mediators also help to build novel structural components, including the scar, which isolates the trauma area from surrounding healthy tissues.

The inflammatory response can be divided into three overlapping phases: the phase of initiation (the phase of disintegration), the phase of maintenance (the scar forming phase), and the phase of shutting off. All tissues harbour mononuclear cells, which together form the mononuclear phagocyte system, and have the ability to rapidly transform into macrophages in trauma and disease. Macrophages have long been known to play a key role in inflammation, including the repair of injured peripheral nerves (39). Recent studies indicate that an inadequate macrophage response in the injured central nervous system is an important factor behind the failure of axon regeneration in the spinal cord. In the injured spinal cord, most of the macrophages originate from microglia, the intrinsic members of the mononuclear phagocyte system. Local factors in the central nervous system from e.g. astrocytes exert a much tighter control of the activation of microglia to fully competent macrophages compared to the situation in other tissues. While this control may serve to minimize the extent of secondary damage from a fully developed inflammation, it also appears to hamper the neuroregenerative response.

To facilitate the entry of monocytes into the degenerating spinal cord white matter and subsequent differentiation to competent macrophages may therefore promote tissue repair and axon growth.

Circulating monocytes first have to attach to endothelial cells in the capillaries

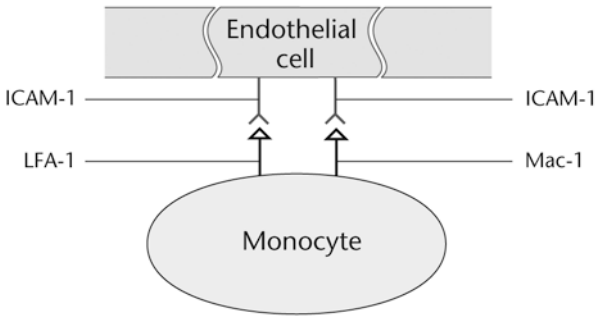

Figure 8. Intercellular cell adhesion molecule (ICAM)-mediated adhesion of monocytes to endothelial cells.

in order to be able to enter the spinal cord tissue. The attachment and subsequent entry process is regulated by a set of cell adhesion molecules (CAMs) located at the cell surface (40, 41); fig 8.

Intercellular cell adhesion molecule (ICAM) is expressed on the surface of endothelial cells and is a ligand for the CAM L1, which is expressed by T-cells, neutrophils and monocytes. ICAM has also been shown to be a ligand for macrophages, and has been suggested to play a significant role in the early phase of the inflammatory response. By modulating ICAM expression, it might therefore be possible to regulate the monocyte-mediated components of inflammation following spinal cord injury. Ideally, sufficient macrophage activity should be present in the early phase to rapidly remove cell debris and breakdown products, after which the macrophage response should be attenuated to reduce their scar tissue promoting activities. This sequence of events occurs following injury to peripheral nerves, and is considered to be an important factor underlying peripheral nerve regeneration (42). An indication that such modulation is feasible and beneficial is shown by observations that administration of monoclonal anti-ICAM antibodies plays a role in regulating the presence of macrophages as well as neutrophils at the site of injury (table 6; 43).

The interleukin (IL) family of cytokines play a key role in growth and function of many cell types. Some of the ILs, e.g. IL-1, IL-6 and IL-10 are important regulators of immune and inflammatory responses, and are induced or up-regulated following neural trauma (44). Whereas IL-1 and IL-6 are considered pro-inflammatory, IL-10 plays a role in switching off the inflammatory response, although the precise mechanism of action is incompletely known. By giving IL-10 at an early stage following spinal cord injury, the inflammatory response can be attenuated and the secondary injury process mitigated (table 6). An advantage with interleukins is that they can be administered systemically, even intraperitoneally (ip), in contrast to the growth factors discussed above. Interleukins were shown to reduce oedema formation and extent of secondary damage, as well as improved local blood flow and neurological recovery in experimental models of spinal cord injury.

In summary, ICAM and interleukins are examples of molecules which participate in the inflammatory response after spinal cord injury and which are potential targets for pharmacological treatment in spinal cord injury. However, their pos-

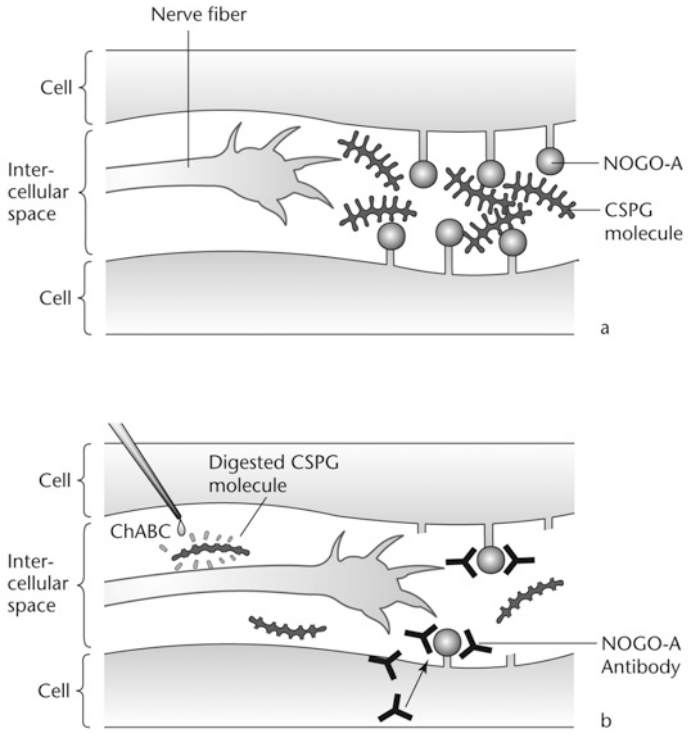

Figure 9. Chondroitinase ABC (ChABC) cleaves newly produced chondroitin sulfate proteoglycans, a major component in the induced scar tissue.

sible use is complicated by the difficulties in determining at what time point these molecules should be administered in order to interfere in an efficient way with the inflammatory process (45).

Chondroitinase ABC – a molecular machete

The presence of certain molecules in the extracellular matrix is considered to counteract axonal regeneration. Proteoglycans, collagens and adhesive proteins are the main components in the extracellular matrix (46). Chondroitin sulfate proteoglycans (CSPG) are the main contributors to the scar formation following injury and therefore largely responsible for the failure of injured axons to grow beyond the level of injury. The scar formation is hostile towards axonal regeneration but a natural response after injury. A bacterial enzyme was discovered 2002 with a capability to digest the chondroitin sulfate. In this pioneering work by Bradbury and colleagues, bacterial chondroitinase ABC (ChABC) was administer intrathecally following a trauma to the posterior horn in adult rats (47). In their experiment ChABC digested the CSPG at the level of injury reducing the scar formation (table 6 and fig 9).

The enzyme acts like a molecular machete and reduces or eliminates the scar tissue barrier that mechanically and chemically counteracts nerve regeneration. The amount of regeneration associated proteins was increased and regeneration of afferent sensory axons as well as efferent corticospinal axons was supported. The synaptic activity was restored below the level of injury and minor functional recovery

in movement and proprioception was observed. These experiments have later been duplicated and we are now waiting for exciting "trials" in a clinical setup.

Erythropoietin

Erythropoietin (EPO), the prime stimulator of erythroid progenitor cell proliferation, has been found to exert potent neuroprotective effects by diminishing lipid peroxidation and inflammation, as well as counteracting apoptosis. The agent carbamyl erythropoietin (CEPO) retains neuroprotective properties without having hematopoietic potential. Administration of CEPO within the first 24 hours after experimental spinal cord injury reduces the neurological deficits compared to control (48, table 6).

RHO pathway antagonists

Rho is a GTPase-associated signalling protein, which transduces extracellular signals to alterations in the actin cytoskeleton, thereby influencing cell motility (49). Injury to CNS axons induces increased Rho activity, which correlates with growth cone collapse and neurite retraction. The administration of Rho-associated kinase inhibitors such as C 3 in the early period after spinal cord injury in rats, promotes neurite extension, improves spinal cord blood flow, and results in improved locomotion (50, table 6). Currently, phase 1–2 multicentre studies are under way, using extradural administration of the Rho-associated kinase inhibitor cethrin during the first two weeks after injury.

Activated autologous macrophages

Activated macrophages are considered to play a significant role in the process of peripheral nerve regeneration, by efficient removal of myelin-associated inhibitors and the production and release of growth factors. CNS macrophages, in contrast, are significantly less activated, which may contribute to regeneration failure after spinal cord injury. Implantation of peripherally derived macrophages to the environment of the injured spinal cord has neuro-protective effects and stimulates axon regeneration, thereby reducing spinal cord cyst formation and promoting recovery of motor function (51, 52, table 6). ProCord consists of macrophages isolated from the patient`s own blood activated through a special procedure. Currently, a clinical phase 2 trial is ongoing, in which ProCord is injected directly into the injury epicentre of the spinal cord within 14 days after injury.

III Overcoming the late inhibitory mechanisms on axon growth in the environment of the injured neurons

The late inhibitory strategies start once the scar-formation has been established. In the late stage of an injury the upper and lower stumps of the injured spinal cord are separated by a gap composed of scar tissue and/or liquid filled cyst formation. Injured axons are unable to traverse this area and have to be guided through or around it by biological or biosynthetic "bridges" or supportive structures, often used in

combination with growth factors (table 7). The term "filling the gap" is often used in the literature to describe different methods (strategies) for bridging the mechanical obstacle created by the scar formation and/or liquid filled cyst-formation.

Although supportive structures provide a permissive pathway for axonal elongation, the growing axons typically fail to re-enter the spinal cord above or below the lesion. Thus, supportive structures are only part of the solution to restore functional connections. A promising way to reach this objective is therefore to combine supportive "bridging" structures with cell replacement using transplantation of various forms of foetal tissue (table 9), or more recently, stem cells (table 10).

Supportive structures

Supportive structures can promote axonal growth by serving as a scaffold for growth factors and/or as a substrate for growth permissive interactions with regeneration axons (tables 7, 9, 10). Since injured CNS axons are able to grow for extended distances in a transplanted peripheral nerve graft, peripheral nerve tissue or components of it are rational sources as growth supporting structures.

a) Peripheral nerves. Of the numerous studies carried out with peripheral nerve grafts, the most remarkable results are the ones reported by Cheng and Olson (53). Intercostal nerve grafts were positioned to reach from grey to white matter in descending as well as ascending directions between the stumps of a complete spinal cord transection. The graft was stabilized with fibrin glue allowing the slow release of acidic fibroblast growth factor (aFGF) (table 7). Axonal regrowth across the lesion site and recovery of hindlimb sensorimotor functions were demonstrated. Despite these promising results, the procedure has not been possible to implement in the clinical setting.

b) Schwann cells. Since Schwann cells are the essential growth promoting cellular element of peripheral nerve, a logical alternative to whole peripheral nerve tissue is to use isolated Schwann cells, or growth promoting Schwann cell molecules, as guidance channel for injured spinal cord axons (Fig 10).

Bunge and co-workers used "guidance channels" filled with Schwann cells and growth factors. They were able to demonstrate an increased axonal growth, reduced secondary degeneration of axons as well as functional recovery in animal models

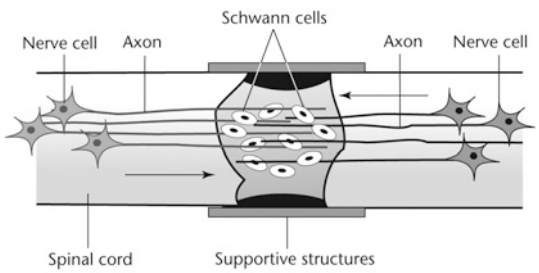

Figure 10. "Bridge" created by Schwann cells and extracellular matrix components provides a growth supportive substrate for injured axons.

(table 7, 54). The problems when using Schwann cells is the hostile astrocytic reaction towards the Schwann cells in the damaged area which decreases their ability to produce myelin.

c) Olfactory ensheathing cells. Olfactory ensheathing cells (OECs) have emerged as an attractive source for supportive structures (55). OECs envelop olfactory sensory axons along their way to the target neurons in the olfactory bulb. There is a continuous growth of axons into the olfactory bulb from newly formed olfactory sensory neurons in the olfactory mucosa. OECs are continuously supporting this growth, and are fully integrated in the adult CNS, properties that make them interesting with regard to spinal cord injury repair (56). OECs release growth factors, as well as other growth promoting molecules, and are able to produce myelin around regenerated CNS axons (table 7). Locally implanted OECs stimulate re-growth of damaged axons in the spinal cord as well as growth of axons through areas with scar-tissue formation. Furthermore, functional recovery of sensory and postural functions was found. The mechanisms underlying functional improvement associated with transplantation of OECs are incompletely understood, but appear not only to be the result of their supportive and guiding properties, but also of their ability to promote synaptic plasticity (57). Furthermore, stem cells from the olfactory mucosa may be present in OEC transplants, and contribute to structural and functional repair (58). The capacity of OECs to fully integrate in the CNS environment and migrate through connective tissue makes them more favourable candidates than Schwann cells for supporting axonal regeneration within the injured spinal cord.

OECs can easily be harvested under local anaesthesia from the human olfactory mucosa, grown in vitro and thereafter used for transplantation. Clinical trials with autologous transplantation of OECs into the spinal cord have been initiated in patients with chronic, complete spinal cord lesions in Brisbane, Australia. The technique and imaging results using magnetic resonace imaging (MRI) one year after transplantion have been reported (59).

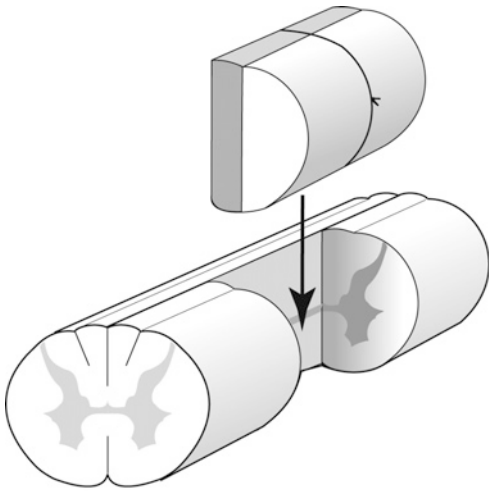

Figure 11. A multi-component polymer system is designed to fit into the cavity created by two spinal cord hemisections and the removal of the intercalated tissue.

d) Artificial supportive structures. Various artificial structures have been presented (60). As an example Schneider and colleagues (61) grafted a multi-component polymer into the gap of the hemisected spinal cord (fig 11). The inner part of the polymer was filled with stem cells and the outer part contained a suitable substrate for axons to grow beyond the level of injury. Grafted animals showed better hindlimb functional recovery than controls (table 7).

Transplantation

a) Transplantation of embryonic/foetal neural tissue for spinal cord repair. Embryonic/foetal neural tissue contains undifferentiated neurons with a potential to survive transplantation to the mature CNS and develop into mature neurons and make functional synaptic connections in the host spinal cord. In addition, embryonic/foetal tissue contains stem cells and non-neuronal cells, which may provide trophic and substrate support for transplanted immature neurons as well as for the injured neurons in the host spinal cord. Thus transplantation of embryonic/foetal tissue has four prime objectives:

- to replace specific cells in order to restore lost functions
- to minimize neuron degeneration and scar formation
- to promote regeneration and plasticity by providing a scaffold and trophic influence for axonal growth

Table 7. Strategies to overcome the late inhibitory mechanisms of the CNS environment – supportive structures

Factor	Target	Delivery	Effect
Peripheral nerve + aFGF	Axotomised axons	Locally with glue	Structural and functional recovery.
Schwann cells (SC) + Growth factors	Axotomised axons	Placement of SC transplants in guidance channels	Reduced secondary degeneration. Functional improvement.
Olfactory ensheathing glial cells (OEC)	Axotomised axons	Local deposition	Reduced secondary degeneration. Functional improvement.
Noncellular elements + stem cells	Cut axons Scar forming glia	Placement of stem cells in transplanted polymers	Improved tissue sparing. Reduced scar formation. Functional improvement.
Erythropoietin (EPO)		Systemically	Retain neuroprotective properties.
Rho	Actin cytoskeleton		Improves SCBF and locomotion.
Activated macrophages		Injury epicentre	Improved motor function. Reduces cyst formation.

- to serve as a relay station in which descending or ascending impulses can terminate and subsequently be transferred via axons from transplanted cells to neurons caudal/rostral to the lesion

Embryonic/foetal tissue has a number of properties, which make it attractive in spinal cord injury (table 8).

However, ethical aspects complicate the use of such tissue for experimental and clinical purposes. Different strategies have been used when transplanting embryonic/foetal tissue in experimental spinal cord injury studies (table 9; 62).

b) Animal tissue to animal recipients. Solid embryonic tissue transplanted to a liquid-filled cavity in the rubrospinal tract was found to counteract retrograde cell death of neurons within the red nucleus probably due to a release of growth factors (table 9). An increase in local re-innervation as well as a functional recovery were seen after transplantation of embryonic brainstem tissue to a spinal cord segment below the level of injury.

c) Human tissue to animal recipients. Human embryonic spinal cord tissue can survive, grow, differentiate and become morphologically integrated following transplantation into the animal spinal cord. Human foetal tissue, including spinal cord tissue, harvested from early abortions (5–8 weeks) has been used to fill experimentally induced posttraumatic cavities and these transplants were found to promote survival of injured neurons and axons as well as to provide a bridge for axonal growth (table 9; 63, 64).

d) Human tissue to patients with spinal cord injury. Embryonic/foetal tissue has been used in humans in the treatment of Parkinson´s disease, diabetes, leukemia and in blindness due to macula degeneration. Given the observations that human embryonic/foetal tissue was able to counteract cyst expansion in experimental spinal cord injuries, the question arose whether implanting such tissue into the posttraumatic cyst-cavity in humans could counteract further cyst expansion in patients with posttraumatic syringomyelia. The transplant might consist of solid tissue or a suspension of cells (65–67). Injection of cell suspensions is associated with less injury to the surrounding tissue but solid transplants have the advantage to be harvested from older foetuses.

Table 8. Properties of embryonic tissue

1	Rapid growth and cell division.
2	Ability to develop to similar cells as in surrounding tissue.
3	Less often rejected.
4	Contain factors stimulating in-growth of vessels as well as a higher proportion of growth factors promoting survival when transplanted.
5	Less sensitive to ischemia and thus able to survive in surroundings with lowered oxygen levels.

Table 9. Strategies to overcome the late inhibitory mechanisms of the CNS environment – transplantation of embryonic/foetal tissue

Factor	Target	Delivery	Effect
Solid embryonic/ foetal tissue	Red nucleus axons	Transplantation to cavity	Rescue axotomised neurons
Brain stem fragments	Denervated terminal field	Injection	Local reinnervation Functional restitution
Solid embryonic/ foetal tissue	Axotomised axons	Transplantation to cavity	Axonal elongation into and out of transplant
Human embryonic/ foetal tissue	Axotomised axons	Transplantation	Feasibility Cyst obliteration

Experience of transplanting human embryonic/foetal tissue to patients with spinal cord injuries has provided information on transplant survival, how the transplant fills the cavity and whether rejection is provoked (table 9). The final objective is that the embryonic/foetal tissue will integrate with the host spinal cord, and provide structural and molecular support for cyst retraction. It is important to stressing this context that embryonic tissue transplant research of today is not focused on functional recovery; it is only for study of feasibility purposes and the use of embryonic tissues in transplantation research has provoked strong emotions and created ethical discussions since the tissue is harvested from aborted foetuses.

e) Transplantation of stem cells for spinal cord repair. Stem cell research has opened a new arena for regenerative science. Stem cells are the source of all cells in the organism and have the potential to differentiate to functionally competent cells of different types. They provide a repair system and are theoretically able to divide without limitations and substitute other cells throughout an individual's whole life. The first publication on isolation of neural stem cells was published in 1994 (68), and the first report on the use of stem cells in spinal cord injury was published in 1999 (69). In this study, embryonic stem cells from mice were injected into the damaged rat spinal cord, and the results indicated some functional recovery. The injected cells differentiated into neurons, oligodendrocytes and astrocytes. The results proved that transplanted embryonic stem cells are able to survive and differentiate in the adult spinal cord. Stem cell research is the only field where clear evidence has been demonstrated that cell therapy might repair damage within the central nervous system (70–72).

Embryonic stem cells. Embryonic stem (ES) cells are derived from in vitro fertilized eggs and thereafter donated to research with permission from the donor. Consequently, ES cells are not derived from eggs fertilized within the female body (fig 12 a och b). ES cells are obtained from the inner cells of a blastocyst, corresponding to a 4–5 days old fertilized egg and thereafter transferred to a solution with special substrate. ES cells kept in this solution without differentiation for 6 months are called pluripotent (fig 12a).

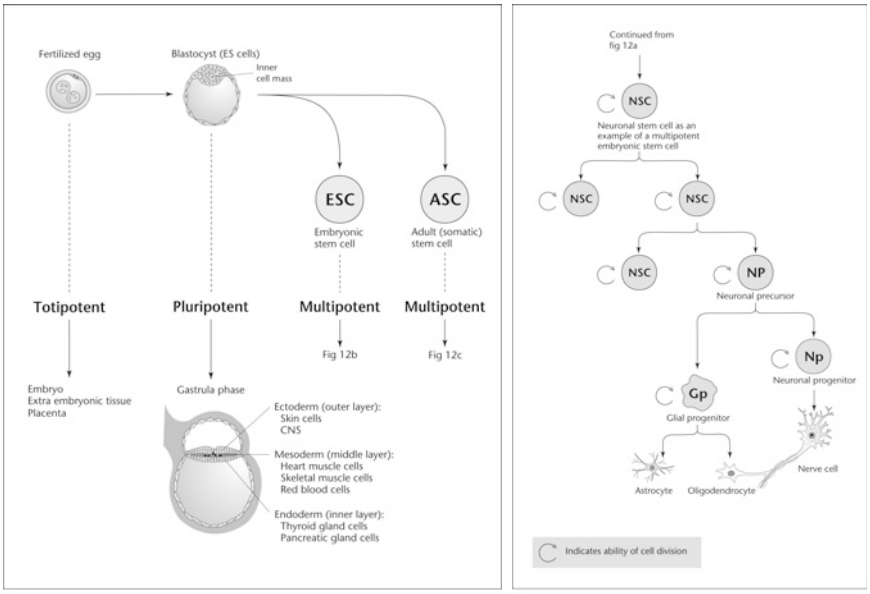

Figure 12.
a) Scheme of the principal types of stem cells and their developmental potential. With ongoing maturation of the embryo, stem cells become restricted in their developmental potential.
b) Stem cells from the embryonic nervous system give rise to all intrinsic neural cells, except microglia.

The term pluripotent is used to describe stem cells able to differentiate to cells of all of the 3 embryonic layers, ecto-, meso- and endoderm. The neural stem cell is an example of a multipotent stem cell (fig 12b). When the cells in a culture are genetically identical and contain a normal set of chromosomes, they are called embryonic stem cells (fig 12a). When a cell line is established it can be stored frozen. New ES cell lines have continuously been produced. In 2001 it was reported that it was possible to control the differentiation of ES cell lines in vitro towards a certain type of cells. Thus, it is possible to isolate human ES cells from blastocysts, maintain these cells as pluripotent in vitro for extended periods of time, and possibly, control their differentiation to desired cell type(s). However, the risk of tumour formation by transplanted ES cells needs to be seriously considered (73, 74).

In summary, ES cells:
i. Are derived from the inner cells of the blastocyst
ii. Have possibilities to undergo an unlimited number of symmetrical divisions without differentiation over a long period of time
iii. Exhibit and keep a stable and full set of chromosomes
iv. Are undifferentiated and might generate differentiated types of cells to any of the 3 primary layers within the embryo
v. Have the capacity to integrate in all kinds of foetal tissue that is under development
vi. Have clonogenic properties and can produce a colony of genetically identical cells

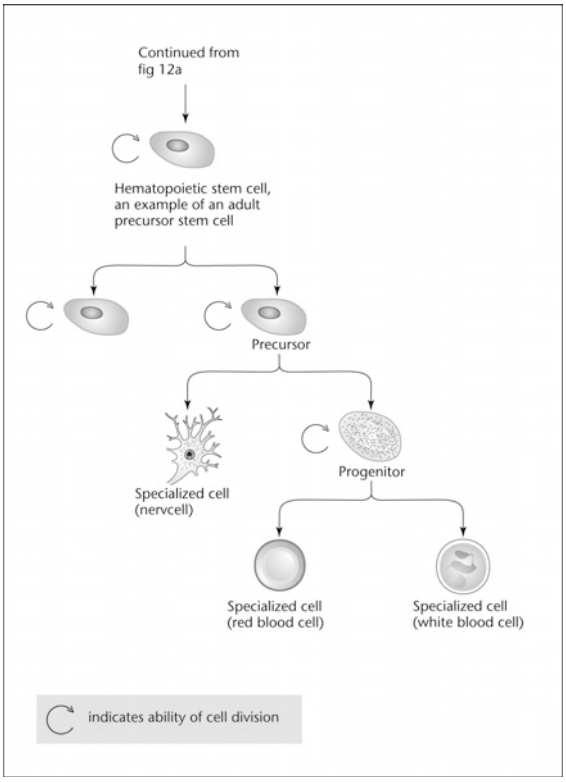

Figure 12
c) Stem cells from the adult nervous system.

Adult stem cells. Adult stem cells are able to proliferate during long periods of time and generate identical copies without undergoing differentiation (fig 12 c). In response to specific extrinsic factors, adult stem cells differentiate into functional cells, which are appropriate for the surrounding tissue.

Some adult stem cells even have the ability to differentiate into cells typical for other than the surrounding tissue although, there is still no evidence that adult stem cells are pluripotent. This phenomen is denoted plasticity. Two fundamental strategies for the use of stem cells in the repair of nervous system diseases and trauma are used. Firstly, the use of cells prepared in vitro with the ultimate aim of making them suitable for implantation in patients. Secondly, the use of growth factors and other molecules to stimulate the patients´ own stem cells to repair the injury, e.g. by migrating to the injury and differentiate to the appropriate types of cells for that region. In reality stem cell mediated positive outcomes are likely to depend on complex mechanisms, as was discussed above with regard to implantation of embryonic/foetal neurons. Thus, much of the morphological and functional improvements reported in experimental neural stem cell research on the injured or diseased nervous system may be the result of neuroprotective and/or neurotrophic mechanisms, rather than specific cell replacement (75).

f) Stem cells for treatment of spinal cord injury. In conditions such as Parkinson's disease only one type of cells may need to be replaced, the dopamine producing cells,

in order to achieve long term alleviation of the symptoms. In a traumatically injured spinal cord, the repair procedure will be much more complex as many different kinds of cells are affected and, hence, in need of being replaced. The primary aim of the stem cell research is to restore/repair diseased white matter, often referred to as partial repair of spinal cord injury (table 10).

The grey matter damage is of less interest since it only produces peripheral loss of function within the injured segment. The predominating functional defects following spinal cord injury are caused by interruption of axonal continuity and/or by focal demyelination as a result of oligodendrocyte degeneration (76). In both pathologies, the affected axons are unable to propagate impulses to their axon terminals. Experimentally, it has not yet been possible to achieve more than limited long distance axon regeneration of injured axons in the spinal cord. However, to repair a site of demyelination appears to be a more realistic goal. Therefore, the primary goal in the use of stem cells in spinal cord injury is to learn how to replace lost oligodendrocytes with cells that are able to make sufficient myelin for impulse propagation to resume.

McDonald and co-workers (76–78) used embryonic stem cells aimed at transplantation and initiated the cells to generate progenitor cells that differentiated to astrocytes, oligodendrocytes and neurons. The neurons had inhibitory and excitatory properties including the ability to form synapses. One million embryonic stem cells were injected into injured spinal cords in immunosuppressed rats. A large number of the cells died but some survived and the cells were able to dislodge in both directions in the spinal canal. Ten percent of the cells stayed in the injured area and differentiated to neurons. After the transplantation the animals showed improved motor function.

Aims with stem cell research

An important goal for stem cell research is to learn how to control differentiation of human pluripotent embryonic stem cells to a certain kind of cells, for example neurons. It is also important to learn how to identify these differentiated cells. By using growth factors or by changing the chemical composition of the surface that the cells are growing on, it is possible to stimulate stem cells to differentiate to neurons. Another method is to introduce new genes in the stem cell so they might differentiate towards the cell type of interest.

Laboratory studies it have been shown that human embryonic stem cells are able to differentiate into different kinds of cells, such as cells building vascular structures as well as neurons producing dopamine. However, it is still not known how stem cells are

Table 10. Strategies to overcome the late inhibitory mechanisms of the CNS environment – transplantation of stem cells

Factor	Target	Delivery	Effect
Neural stem cells + NT 3	Axotomised axons	Transplantation to cavity	Reconstitute cellular matrix. Supporting axonal growth

able to divide without differentiating to more specialised cell types and whether this is influenced by genetic changes. Nor is it known at which level of differentiation the human embryonic stem cells are optimal for transplantation.

Much research today is focusing on production of stem cells for use in transplantation of dopamine producing cells in patients with Parkinson´s disease, beta cells in patients with diabetes mellitusand heart muscle cells in patients with heart failure. However, in the near future ES cells will be used in spinal cord injury research rather than in human therapy.

Future challenges

In this review selected examples of the research within spinal cord injury neuroprotection and neural regeneration have been presented. The amount of knowledge regarding the posttraumatic events has increased tremendously, particularly during the recent 3 decades, but the challenge to cure paralysis still remains. The achievement of functional recovery in experimental models, although limited, raises hope for the future. The examples of research and development described here show very clearly that knowledge about the pathological processes is a prerequisite for future research and development and hopefully, in a later stage, treatment options to the spinal cord-injured patients. No single therapy will reduce the secondary injury mechanisms or increase the capability for regeneration of injured or transected axons. In order to achieve the goal of minimising the neurological deficit following SCI a combined treatment of several strategies must be used within the fields of neuroprotection and neural regeneration. The "golden drug" or "treatment" has not yet been developed and new questions arise as soon as a new piece of knowledge is acquainted.

Every additional increase in knowledge of SCI will be an important piece in the puzzle that creates the prerequisite for future development of new treatment strategies. "Future challenges" involve first of all further increased knowledge within every major field of SCI research ranging from experimental basic science to pharmacological treatment and medical management. Our expectations also include improved diagnostic measures such as MRI that further will improve the therapeutic possibilities for the patients.

The general impression is, after reviewing the medical literature, the need for combined approaches in order to achieve additional functional improvement. In this review strategies such as "filling" and/or "closing" the gap within the injured spinal cord are discussed, but at the same time the necessity of closing/filling the gap between researchers within various fields could not be emphasised enough. To build bridges between scientists in the field of basic science and clinicians interested in research is a challange in itself. The interpretation of new information to "common interest" demands good relationship between these groups. The main impression, however, is that the most important future challenge must be to create a multidisciplinary approach to basic science and clinical management thus enabling us together to highlight various hypothesis from a broad angle.

Early treatment in the acute stage after a SCI is advocated in most countries in the

western world today. Patients sustaining spinal cord injury are treated in intensive care units and submitted to early surgery in order to avoid unnecessary neurological deterioration and to establish early mobilisation. This is, according to our opinion, an accepted and established treatment for the majority of these patients. Of course, additional questions arise that need to be answered through a multidisciplinary approach, as to whether this is the future treatment for all patients;

What is actually the importance of all secondary mechanisms in relation to future neurological deterioration and could our knowledge about the secondary pathophysiological processes indicate the exact time for any eventual "regeneration strategy"?

Do the results from the regeneration research implicate changes of the treatment protocol in the acute stage?

What is the optimal tissue treatment in the acute stage in order to facilitate the "regeneration treatment", i.e. how do we optimize the chemical environment surrounding the nerve cells in order to maintain the nerve tissue that survived in the acute stage in as good condition as possible?

Is decompression of the dural sac and fixation of the spinal column in the acute stage not enough to optimize the possibility for the spinal cord to recover?

Should, if such is not the case, the dural sac be opened and the subarachnoid space be exposed and flushed in order to eliminate blood accumulated subarachnoidally? Could such a therapeutic measure for instance decrease or eliminate the inflammatory response and minimize the accumulation of proteoglycans and finally reduce the scar formation at the level of injury?

Is it meaningful to decompress and stabilize all patients, or should we in the future, for instance, use foetal tissue, stem cells, OECs, or scaffold structures with or without neural growth factors already in the acute stage as a complementary treatment option in some cases?

Should we expect with the surgical procedure until we artificially induce the regeneration procedure in a later stage and if so, when is the most appropriate time for this combined "treatment"?

Hypothetically, should a two-step surgical procedure be performed in the acute stage? Let us assume that the patient has a spinal cord injury at the cervical level due to a burst fracture compressing the spinal cord anteriorly. Theoretically, the procedure initially starts with an anterior decompression of the dural sac followed by a stabilizing procedure. The patient is then rotated and placed in a prone position. The dural sac is exposed posteriorly following a laminectomy after which the dura sac is opened through a midline incision visualizing the spinal cord. The spinal cord is probably covered with various blood products and inflammatory debris. These products have to be eliminated through careful rinsing since they are the prerequisites for scar formation and at the end an obstacle for the regeneration procedure. An artificial dura sac is applied in such a fashion that it counteracts the compression from posteriorly extradural-located blood. This is now followed by infusion intradurally of factors that counteracts scar formation and increases the speed of regeneration. This infusion cocktail includes, according to our present knowledge neurotrophic growth factors, antibodies against NOGO-A receptors, 4-amino-pyridine, monoclonal antibodies against ICAM-1, and finally chon-

droitinase ABC. This infusion can start, theoretically, immediately after the application of the artificial dural sac or percutaneously among the conservatively treated patients. The treatment period is extended until an estimated scar formation period, usually 2-6 weeks, is ended. The inflammatory response is stopped by adding interleukines when we estimate that the injured area is clean from damaged spinal cord tissue and debris.

Various scaffold structures could now be prepared during the infusion period depending on the level and severity of injury. A second posterior approach is performed and the artificial dural sac is opened. Scaffold structures could be attached with the addition of Schwann cells, neurotrophic factors and stem cells. By using a special scaffold-like gel these factors could be released gradually. OECs could, in addition, be administrated either through infusion or as a cell suspension and the infusion of a concentration of macrophages from the injured patient itself counteracting the inflammatory response is an exciting alternative now tested clinically. Maybe, will the infusion in this period will also include roliprane acting as cAMP preservers.

Our knowledge will hopefully increase in fields of more controversy, such as stem cell and foetal tissue research. Ethical and juridictional questions have to be answered parallel to the medical development. Will it be possible in the future to create stemcell- or featal tissue banks or will purified adult stem cells harvested from the injured patient be used as replacement tissue for the spinal cord? Will it be possible to use such cells already at the first operation? Thoughts like this are today very close to medical science fiction. Our knowledge today is not enough to give the spinal cord-injured patients the ultimate treatment to restore neurological treatment. The route to that goal is paved with many controversial issues. Our knowledge has increased tremendously, however, during the latest 2–3 decades indicating some hope to solve one of the most prestigious medical challenges, i.e. how to regenerate transected and/or contused axons and create the possibility to regain function, although limited, below the level of injury.

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