■ REVIEW ARTICLE

The response to injury in the peripheral nervous system

S. Hall

From King's College
London, London,
England

Peripheral nerve injuries, particularly in the upper limb, may have devastating consequences. The outcome of injury is determined by the amount of cellular damage, and by variables which include the site of the lesion, the degree of disruption of the connective-tissue sheaths that surround the nerve, the extent of associated injuries, particularly vascular injuries (Fig. 1), and the age and health of the patient.

Meticulous surgical repair cannot guarantee full functional recovery because the surgeon does not control the 'biological battlefield' which rages inside a damaged nerve.¹ Therapeutic manipulation of the response to injury at the molecular level has the potential to keep more neurones alive, encourage their axons to cross longer interstump gaps, to maximise the accuracy of target re-innervation and to manage neuropathic pain. For now, these objec-

tives remain aspirational – they are predicated on a more detailed understanding of the way in which the peripheral nervous system responds to injury than is presently available. This brief update on the peripheral arm of the response to injury in the peripheral nervous system highlights areas where translational research may prove to be beneficial, for information on axotomy-related *central* phenomena, the reader is referred to earlier reviews.²⁻⁴

The injury response in the peripheral nervous system – an overview

Peripheral neurones are some of the largest and most spatially complex cells in the body. Their size means that they are unable to function without the structural and metabolic support provided by approximately ten times as many glial cells. It also means that a focal axonal injury which occurs centimetres from a neu-

Fig. 1

Mean and SD values for the ratios of each of the measured variables in three different injury groups observed six months after immediate repair of a defect of 3 cm in the median nerve coupled with a defect of 5 cm in the brachial artery (using a freeze-thawed muscle graft, (FTMG) and a vein graft, respectively). The y-axis is the ratio of the variable measured on the repaired side to the measurement made at the same site on the normal contralateral side of the same animal. Injury groups are: simple, nerve repaired with FTMG; cavity, reproducible cavity injury (see below); and V-graft, nerve repaired using FTMG and artery repaired using vein graft ($n = 5$ sheep in each group). The variables which were measured are: Vmax, peak conduction velocity in the nerve (m/s); Flow, blood flow in the nerve (arbitrary flux units); Axon, axonal diameter (mm); Fibre, fibre diameter (mm); Myelin, thickness of the myelin sheath (mm). A cavity injury was produced in three stages, and involved crushing surrounding muscle to produce areas of bruising and devitalised tissue, then abrading soft tissues and finally painting the tissues with creosote to provide widespread inflammation (reproduced with permission from Glasby et al, *J Hand Surg* 1998;23-B:354-9).

■ S. Hall, PhD, DSc,
Professor & Head
Department of Anatomy &
Human Sciences
King's College London,
School of Biomedical
Sciences, Guy's Campus,
London SE1 1UL, UK.

Correspondence should be
sent to Professor S. Hall;
e-mail:
susan.standring@kcl.ac.uk

©2005 British Editorial
Society of Bone and
Joint Surgery
doi:10.1302/0301-620X.87B10.
16700 \$2.00
J Bone Joint Surg [Br]
2005;87-B:1309-19.

Fig. 2

Diagrams showing the sequence of cellular events triggered by transection of a myelinated axon. Figure 2a – A normal myelinated axon associated with a longitudinal chain of Schwann cells and enclosed within a continuous basal lamina (dotted line). Figure 2b – Local injury producing dissolution of myelin sheaths and degeneration of axoplasm distally, and sealing of the tip of the proximal stump of the axon. Figure 2c – The Schwann cell tube is invaded by macrophages (m) which breach the basal lamina; Schwann cells (Schw) distal to the injury proliferate; axon sprouts emerge from the proximal stump. Figure 2d – Axon sprouts elongate within the Schwann tube and associate with the Schwann cells therein. Since the injury shown caused minimal separation of the two ends of the damaged axon, there is no axonal misrouting. Figure 2e – Daughter Schwann cells remyelinate the regrowing axon. The new myelin sheaths are thinner and the internodal lengths are shorter than their proximal counterparts. Changes also occur in the cell body (the response shown here is stereotypical and is known as chromatolysis. The changes are not always so marked) (reproduced with permission from Lundborg. *Nerve injury and repair regeneration*. 2005. Elsevier Inc.).

ronal cell body induces a response that involves the entire cell and its associated glia. Centrally, some axotomised neurones die, primarily as a consequence of a loss of target-derived neurotrophic support.⁵ Numbers quoted in the literature vary according to the experimental model used, but can be substantial, e.g. approximately 30% to 40% of the small diameter sensory neurones which contribute to a dorsal root ganglion apoptose after nerve transection. Proximal injuries produce more marked neuronal loss than distal injuries,⁶ and groups of neurones are differentially susceptible to injury, for example cutaneous afferents appear to be more vulnerable than muscle afferents.⁷ Persisting neurones switch to a 'survivor' phenotype and the expression of hundreds of genes^{8,9} is changed to compensate for the loss or diminution of target-derived neurotrophic factors,¹⁰ and in order to regrow their axons across the site of the injury and back into the periphery. Proximal changes, such as synaptic reorganisation in the cortex¹¹⁻¹³ and spinal cord, occur upstream of axotomised first-order motor and sensory neurones, and may influence the functional outcome months or even years later.¹⁴⁻¹⁶

Distal to the injury, a series of molecular and cellular events, some simultaneous, others consecutive, and collectively called Wallerian degeneration, is triggered throughout the distal nerve stump and within a small reactive zone at the tip of the proximal stump (Fig. 2).¹⁷⁻¹⁹

Axonal degeneration

Within hours of physical interruption, the ends of the severed axons are sealed. The mechanism is probably similar to that used to seal invertebrate giant axons.^{20,21} Anterograde axoplasmic transport continues in the proximal stump, and retrograde transport persists for several days in the distal stump. As a consequence the ends of the sealed axons swell as they fill with organelles which are unable to progress beyond the site of the lesion. Potent vasoactive peptides such as CGRP (calcitonin gene-related peptide) accumulate in the axonal end-bulbs in the proximal stump, and probably contribute to local hyperaemia around the site of the injury.²² Loss of the axoplasmic cytoskeleton begins one to two days after injury in small laboratory rodents, and within seven days in humans: the different latencies are approximately directly proportional to the size of the animal.²³ Increased intracellular Ca^{2+} is both necessary and sufficient to induce axonal degeneration, and to activate axonal calcium-dependent proteolytic enzymes, such as phospholipases and calpains, which mediate cytoskeletal breakdown.²⁴⁻²⁹ The rate of axonal degeneration has been slowed experimentally by exposing axons to lower extracellular concentrations of Ca^{2+} and by cooling within 12 hours of injury.^{29,30}

Until recently it was assumed that axons withered away because they were no longer supported by their cell bodies. That view, although intuitive, is no longer tenable. It was challenged first by work on a spontaneous mouse mutant *Wallerian degeneration, slow, Wld*,³¹⁻³⁴ which displays

retarded axonal degeneration for up to three weeks after transection or crush. More recent studies on normal nerves have revealed that disconnected axons destroy themselves, probably through a local caspase-independent process^{35,36} triggered by events which occur before any morphological or electrophysiological changes can be detected. It has been suggested that these very early events, which may include activation of m-calpain²³ and/or the ubiquitin-proteasome system,³⁷ 'light a fuse'²⁹ which ultimately leads to cytoskeletal disintegration. Therapeutic manipulation of processes which occur within minutes of an injury to a nerve is an unrealistic clinical goal except perhaps in the case of intra-operative injuries in which a nerve has been bruised but not cut, when focal application of selective antagonists, perhaps aided by cooling, might stabilise the axonal cytoskeleton sufficiently to prevent subsequent axonal degeneration. Until axolemmae break down, some conduction may be maintained in disconnected nerves for at least a week after injury, although there is no response to direct stimulation of the injured nerve distal to the lesion during this time.^{38,39}

The inflammatory response in Wallerian degeneration

T-cells, neutrophils and macrophages infiltrate the site of an injury within two days;⁴⁰⁻⁴² the neutrophil response is very limited in both time and extent. Within hours, endoneurial levels of the early inflammatory cytokines, tumour necrosis factor alpha ($\text{TNF-}\alpha$) and interleukin ($\text{IL-1}\alpha$), secreted mostly by Schwann cells, start to increase in the distal nerve stump. Within days, this network has been amplified by cytokines, chemokines and other bioactive molecules released by recruited macrophages, mast cells and activated endothelial cells.⁴³⁻⁵¹ Some of these molecules influence the behaviour of the Schwann cells (e.g. macrophage derived IL-1 regulates nerve growth factor (NGF) synthesis by Schwann cells)⁵² and others may play a role in the generation and/or maintenance of neuropathic pain. Any or all may therefore be appropriate targets for therapeutic manipulation. $\text{TNF-}\alpha$ and nitric oxide have attracted particular attention as potential mediators of the type of pain which is reported soon after injury,^{22,53-60} whereas cyclo-oxygenase-2 expressed by macrophages which remain within the endoneurium after the initial phase of clearance of debris, may mediate chronic post-traumatic neuropathic pain.⁶¹ $\text{TNF-}\alpha$ has several functions in Wallerian degeneration, e.g. activation of the neuroprotective transcription factor nuclear factor-kappa B.¹⁰

Macrophages. There are two populations of macrophages in an injured peripheral nerve, resident and recruited. Resident endoneurial macrophages, which constitute approximately 4% of the endoneurial cellular population in a normal nerve, respond extremely rapidly to injury.⁶² They are joined by haematogenously-derived osteopontin-negative,⁶³ CCR2-positive (chemokine (c-c motif) receptor 2)^{42,64} macrophages which flood into the endoneurium

Table I. Proteins upregulated in acutely-denervated Schwann cells

p75 ^{NTR} low-affinity neurotrophin receptor; ^{182,183} growth factors NGF ¹⁸⁴ and BDNF; ^{185,186} pro-protein convertases such as PC1, which may play a role in the processing of pro-BDNF ¹⁸⁷
neu receptors c-erbB2 and c-erbB3, involved in the regulation of the differentiation and proliferation of Schwann cells through cerbB-neuregulin signalling; ^{117,120,188,189} the transcript encoding LP(A1), the receptor for an extracellular signalling phospholipid which regulates the morphology and adhesion of Schwann cells <i>in vitro</i> ¹⁹⁰
cell-adhesion molecules N-CAM and L1; ^{191,192} ninjurin 1 and 2 ¹⁹³ and galectin-1; ^{194,195} laminin; ¹⁹⁶ J1/tenascin; ¹⁹⁷ $\alpha 5\beta 1$ fibronectin; ¹⁹⁸ $\beta 4$ integrin subunit; ¹⁹⁹ and p200, a heparin-binding adhesive glycoprotein, implicated in Schwann cell-extracellular matrix interactions ²⁰⁰
netrin-1, implicated in growth cone and axon guidance; ²⁰¹ GAP-43 ^{202,203}
cytokines and chemokines e.g. IL-6; ^{204,205} leukaemia inhibitory factor TNF- α and TNF- α converting enzyme; ²⁰⁶ TGF- $\beta 1$ ^{207,208} which may induce expression of LIF ²⁰⁹
erythropoietin, a neuroprotective cytokine ^{99,210,211}
connexin 46, a gap-junction protein which may mediate junctional coupling in dedifferentiated Schwann cells, facilitating the rapid diffusion of signalling molecules between cells downstream of the site of injury ⁹³
hemopoexin, an acute phase protein ²¹²
matrix metalloproteinases MMP-2 and MMP-9, ¹⁰² whose many functions include remodelling of the BNB; TIMP-1, the tissue inhibitor of some MMPs, which may protect basal laminae from dissolution during degeneration ⁴⁵

three to four days after injury by crossing a temporarily leaky blood-nerve barrier. Recruited macrophages are attracted by locally produced chemokines, including monocyte chemotactic protein-1 expressed by denervated Schwann cells,^{41,42,64} and are presumably activated by a neutrophil-independent pathway.

Macrophages penetrate the tubes of the Schwann cell, degrade the myelin sheaths contained therein and phagocytose the debris.⁶⁵⁻⁶⁸ Removal of debris is not just endoneurial 'good housekeeping', but an essential stage in Wallerian degeneration. Degradation of myelin and the subsequent clearance of myelin debris, whether by macrophages or by Schwann cells⁶⁹ liberates a Schwann-cell mitogen from the debris,⁷⁰⁻⁷⁸ and removes myelin-associated molecules, such as myelin-associated glycoprotein (MAG),⁷⁹ which would otherwise inhibit axonal growth. Treatment with anti-MAG antibodies enhances regeneration,⁸⁰ whereas axonal elongation is slowed when recruitment of macrophages is impaired experimentally.^{41,64,81-83} Macrophages also secrete ApoE (apolipoprotein E), which may scavenge lipids from axonal and myelin debris, and re-deliver them to Schwann cells for re-use during regeneration.

Lipid-laden macrophages leave the Schwann tubes by passing through the basal laminae. Many remain free within the endoneurium for several weeks, but most eventually disappear, as a result either of local apoptosis or by migrating to draining lymph nodes and the spleen.⁸⁴

Mast cells. There is a sustained increase in the number of mast cells within both the epineurium and endoneurium distal to a traumatic injury. Mast cell-derived vasoactive agents, together with matrix metalloproteinases secreted by endothelial cells, and TNF- α and IL- β secreted by macrophages, mediate the increased permeability of the blood-nerve barrier in early Wallerian degeneration, and so facilitate recruitment of macrophages.⁸⁵ Mast cells may also participate in the generation of pain and itching. In truth, relatively little is known about their possible contribution to Wallerian degeneration and subsequent regeneration in the peripheral nervous system.

Schwann cells

In an intact nerve, chains of Schwann cells either myelinate axons of large calibre (> 2 μ m) on a one-to-one basis, or wrap groups of small (< 2 μ m) unmyelinated axons in pockets of cytoplasm. They secrete a basal lamina which surrounds each Schwann-cell-axon unit, isolating individual units from their neighbours, and from the endoneurial extracellular matrix.

The functional relationship between axons and their ensheathing Schwann cells is normally tightly regulated by reciprocal signalling.⁸⁶ The way in which Schwann cells respond to the loss of that relationship, whether as a result of segmental demyelination, when axonal integrity is maintained but myelin is removed,⁸⁷ or of axonal degeneration after crush or transection, is a critical component of the response to injury in the peripheral nervous system.

Acutely-denervated Schwann cells. Within 48 hours of injury, denervated myelinating Schwann cells downregulate expression of genes encoding myelin-associated proteins, e.g. myelin proteins MBP (myelin basic protein), P0 (myelin protein zero) and PMP22 (peripheral myelin protein 22),⁸⁸⁻⁹¹ and proteins such as connexin 32,⁹²⁻⁹⁴ E-cadherin, which is important for maintaining the complex structural organisation of nodes, paranodes and incisures,^{95,96} and caveolin-1,⁹⁷ which may regulate the transport of cholesterol. The extent to which Schwann cells participate in the breakdown of their own myelin sheaths is uncertain. They are not professional phagocytes, but are capable of substantial degradation of myelin if the numbers of macrophages are depleted.^{69,81,98}

Three to four days after injury, Schwann cells throughout the distal stump and at the tip of the proximal stump start to divide, probably responding to mitogens exposed during the processing of myelin and axonal debris by macrophages. Some of the regulatory steps in the mitotic pathway are known. They include upregulation of the expression of erythropoietin, which may potentiate division of Schwann cells through an autocrine pathway involving phosphorylation of JAK2,⁹⁹ and of ERK (extracellular signal-regulated kinase)^{100,101} and downregulation of a matrix metallo-

proteinase (MMP-3)¹⁰² which normally acts on fibronectin to generate a fragment that inhibits division of Schwann cells.¹⁰³ If the proliferative burst is blocked experimentally, axonolysis continues, but other major events, such as the recruitment of macrophages, myelinolysis and axonal outgrowth from the proximal stump, are delayed until the onset of division of Schwann cells.⁸³ The de-differentiated daughter cells upregulate expression of numerous regeneration-associated genes, whose protein products (some of which are listed in Table I), either facilitate axonal elongation or maintain the Schwann cells in an axon-responsive state (but see below).

Denervated Schwann cells which are contacted by regenerating axons uncouple.^{92,93} They frequently go through another division, probably responding to a contact-dependent, neuregulin-containing axonal mitogen,¹⁰⁴ and then leave the cell cycle and differentiate.¹⁰⁵⁻¹⁰⁹ Driven by axonal signals, some of the differentiating cells will upregulate expression of myelin-related mRNAs^{91,110} and start to secrete myelin sheaths, while others remain unmyelinated. **Chronically-denervated Schwann cells.** With time, many of the Schwann cells which have not been re-innervated apoptose and disappear.^{111,112} Apoptosis is driven by either a Bcl-2-blockable pathway, initiated by loss of trophic support, or by a Bcl-2-independent pathway, initiated by endogenously produced NGF.^{113,114} Cells which have been denervated for more than six months are morphologically and functionally different from their acutely denervated counterparts.^{115,116} They remain S-100- and laminin-positive, but downregulate expression of c-erbB¹¹⁷ and p75^{NTR}¹¹⁸ receptors. This change is significant because pathways for c-erbB-neuregulin and p75^{NTR}-neurotrophin normally play important roles in Schwann-cell axon signalling. Loss of these receptors may therefore be the reason why denervated Schwann cells become progressively less able to support regenerating axons.¹¹⁹ From a therapeutic perspective, it is encouraging to know that this transformation is reversible, at least in rats, and that chronically-denervated Schwann cells revert to an axon-responsive phenotype when explanted *in vitro*.^{120,121} They could presumably be persuaded, with appropriate post-operative intervention, to retain this phenotype *in vivo* e.g., at the distal end of a long graft and/or distal stump.

The weight of experimental evidence strongly suggests that there is a relatively narrow window of opportunity when transected neurones are in survival mode and denervated Schwann cells are axon-responsive. This reinforces the view that nerve repair should not be delayed when there is unequivocal evidence of the separation of nerve stumps.¹²²⁻¹²⁴

Axonal regeneration

Cellular outgrowth from the proximal nerve stump. At the interface between the normal and 'reactive' regions of a proximal stump, sprouts start to bud from the damaged axons at their sealed tips and at hemi-nodes of Ranvier. The sprouts grow towards the lesion site inside the basal lamina

tubes which enveloped their parent axons, in association with the de-differentiated progeny of the original Schwann cells and a transient population of invading macrophages. Although sprouting usually starts within hours of injury, it may be several days before a cellular outgrowth emerges from the proximal stump, and at least four weeks before all regrowing axons have negotiated the interface between directly-coated proximal and distal stumps, a process which has been termed 'regeneration stagger'.^{125,126}

Axon sprouts and Schwann cells in the proximal stump are 'compartmentalised' by fibroblasts and perineurial cells derived from the inner layers of the disrupted perineurium. The process coincides with an increased endoneurial expression of s-laminin, which is the isoform of laminin that characterises the basal laminae of perineurial cells but not of Schwann cells and of *desert hedgehog* (*dhh*), a member of the *hedgehog* family of signalling molecules involved in normal Schwann-cell-perineurial cell signalling.¹²⁷

In the absence of a distal stump or nerve graft, the cellular outgrowth from a proximal nerve stump consists of small bundles of axons and Schwann cells. Each bundle is surrounded by perineurial cells and fibroblasts and is usually called a minifascicle. The cellular outgrowth from a distal nerve stump is not similarly organised, suggesting that the formation of minifascicles may be neuronally driven, and is perhaps a means of protecting the osmotic milieu of outgrowing axons in the absence of a competent perineurium. This would be consistent with findings of a minifascicular neuropathy in the *dhh*-null mutant mouse where the perineurium is dysfunctional,¹²⁸ and in a nerve biopsy from a patient with a homozygous mis-sense mutation of the *dhh* gene.¹²⁹ Compartmentalisation and the formation of minifascicles presumably affect patterns of axonal dispersion across an interstump gap.

A disinhibited microenvironment. The microenvironment of an intact peripheral nerve is hostile to regrowing axons¹³⁰⁻¹³⁴ and that of a chronically-denervated distal stump supplies very limited support. The conventional wisdom that the peripheral nervous system is 'regeneration-friendly', in marked contrast to the CNS, is therefore true only when speaking of an acutely-denervated distal stump or nerve graft. Proliferation of Schwann cells and axonal sprouting and elongation are all inhibited in normal nerve, but are permitted in repairing nerve.

Axons do not sprout in an intact nerve. The fact that they do so in Wallerian degeneration and also in experimental demyelination, in which axonal continuity is maintained but contact with Schwann cells is transiently lost, suggests that axonal sprouting may normally be inhibited by signals derived from endsheathing Schwann cells.^{135,136}

Depending upon the type of repair, most axon sprouts either negotiate the site of a suture and enter a distal stump or nerve graft, or grow in minifascicles within a wound bed or conduit lumen. Those that enter an endoneurium penetrate the open ends of the Schwann tubes which they encounter and their growth cones palpate the laminin-rich

inner surface of the basal laminae surrounding each tube. This response is routinely exploited in tissue-culture experiments, when explanted neurones are grown on laminin-coated cover slips. It was also the reason for using acellular muscle grafts as an alternative to nerve grafts, on the grounds that laminin-rich muscle sarcolemmae would provide a supportive substrate for regenerating axons, which they did, but only in the presence of Schwann cells.¹³⁷ Laminins are the most abundant non-collagenous structural glycoproteins in basement membranes, and have numerous functions in different tissues,¹³⁸ including supporting axonal growth. The neurite-promoting activity of the laminin isoform found in Schwann cell basal laminae is inactivated in the intact nerve by CSPG (chondroitin sulphate proteoglycan),^{139,140} but during Wallerian degeneration, matrix metalloproteinases expressed by Schwann cells and macrophages degrade CSPG, revealing the neurite-promoting activity of laminin to the ingrowing axon sprouts. Experimental local application of chondroitinase ABC, which removes CSPG glycosaminoglycans, improved axonal regrowth across the interface of coapted sciatic nerve stumps,¹⁴¹ and also enhanced axonal regrowth into acellular nerve grafts,¹⁴² suggesting that a similar protocol may have a therapeutic application. Downregulation of another matrix metalloproteinase, MMP-3, facilitates proliferation of Schwann cells.

Pathway selection. One of the most important determinants of a satisfactory functional outcome is the accuracy of target re-innervation. If axons degenerate without rupture of the basal laminae which surround each Schwann tube, e.g. in an ischaemic or compressive lesion, then the axon sprouts are unlikely to be misrouted upon resolution of the underlying pathology. That is not the case after traumatic injuries in which a nerve is physically disrupted. Whether the resulting proximal and distal nerve stumps are sutured without tension,¹⁴³⁻¹⁴⁵ or are bridged by an intervening graft, the axon sprouts which emerge from the proximal stump are bound to encounter unfamiliar Schwann tubes. Most sprouts, once they have negotiated the site of the suture, will remain within the endoneurium of the distal stump or graft. Those nearest to the periphery of the proximal stump may either escape with their Schwann cells into the epineurium through breaches in the damaged perineurium, or they may grow ectopically between the layers of the perineurium. In both situations their behaviour may produce a painful neuroma.

The mechanism by which axonal growth cones select Schwann tubes has yet to be established. In a mixed nerve, the 'specificity' of re-innervation of motor and sensory tubes appears to be produced by the selective pruning of axon collaterals which initially elongate within inappropriate tubes,^{146,147} and may be influenced by the relative levels of target-derived trophic support¹⁴⁸ and/or by molecular cues on Schwann cells, e.g. the carbohydrate epitope L2/HNK-1, expressed on Schwann cells, may specify motor tubes.^{149,150}

Do motor and sensory nerves supply different molecular cues to axon sprouts? A recent demonstration that motor axons regenerate better across motor or mixed nerve grafts than across sensory nerve grafts,¹⁵¹ is consistent with an earlier suggestion that motor axons may respond to as yet unidentified motor-specific factors within a mixed nerve that are not present in a pure sensory pathway.¹⁵² The ability to manipulate axonal outgrowth to maximise the occupation of modality-specific tubes would be a significant clinical advance. Motor autografts are not a practical clinical option, but allografts or biosynthetic conduits seeded with suitably manipulated Schwann cells, if indeed they are the source of the cues, are attractive alternatives.

Axonal elongation may be confirmed by the proximodistal progression of a positive Tinel's sign. Since axons grow slowly (1 mm/day), distance may ultimately defeat successful reinnervation even when Schwann tubes have not been disrupted by injury, i.e. even when axons grow within appropriate Schwann tubes they may not reach their targets before the latter have succumbed to the effects of chronic denervation.

Fibrosis

Little is known about the way in which different structural elements of a peripheral nerve normally respond to mechanical loading. In the context of the surgical repair of an injured nerve, changes in these structural components, e.g. in terms of their capacity to respond to stretch and deformation, are likely to influence outcome.¹⁵³ Thus extraneural fibrosis and wound-bed adhesions may tether the suture site(s) and adjacent nerve or graft in the wound bed, not only impairing local sliding of the site in response to movement of the limb or digit,¹⁵⁴ but also possibly generating painful dysaesthesiae. They may cause increases in intraneural tension capable of compromising the clinical outcome^{155,156} (but see¹⁵⁷). Internal endoneurial fibrosis at the site of a suture will inevitably divert, and may also compress, regrowing axons, particularly if they are growing within a rigid conduit. Any scarring, whether extraneural or intraneural, which compromises the microvascular bed of a nerve may potentially provoke secondary axonal degeneration.

Alternatives to autografts

Nerve autografts remain the 'gold standard' against which all other protocols for bridging an interstump gap are judged. Since they contain acutely-denervated Schwann cells within a scaffold of longitudinally aligned Schwann tubes, it has always been assumed that they offer the best microenvironment to support regrowing axons. However, experience has shown that they are not ideal, particularly when defects are extensive, or when autologous sensory nerve grafts are used to repair deficits in motor nerves.¹⁵¹

Alternative protocols include allografting in combination with low-grade immunosuppression,¹⁵⁸⁻¹⁶⁰ terminolateral neurorrhaphy,¹⁶¹⁻¹⁶⁶ and entubulation, when the nerve

Fig. 3

The mean amplitude (μV) of compound sensory action potentials (CSAP) in control and in regenerated nerves obtained more than 882 days after nerve repair. The horizontal dashed line indicates the lower 95% confidence limits in the control nerves. The final outcome was lower than the control in all types of repair (ANOVA $p < 0.0005$). The amplitudes after implantation of 50 mm nerve guides were significantly lower ($p < 0.05^*$) than after other types of repair, and the amplitude after a 50 mm graft repair was significantly lower than after a 5 mm graft repair ($p < 0.05$, *). Error bars indicate the SEM (reproduced with permission from Krarup C, Archibald S, Madison R. Factors that influence peripheral nerve regeneration: an electrophysiological study of the monkey median nerve. *Ann Neurol* 2002;51:69-81).

Fig. 4

Diagram of the properties of an ideal nerve-guidance channel. The desired physical properties of a nerve conduit include (clockwise from top left): a biodegradable and porous channel wall; the ability to deliver bioactive factors such as growth factors; the incorporation of support cells, an internal oriented matrix to support cell migration; intraluminal channels to mimic the structure of nerve fascicles; and electrical activity (reproduced with permission from Hudson TW et al, *Clin Plast Surg* 1999;26:617-28).

stumps are enclosed (entubulated) within some type of non-neural conduit, either synthetic or biological.¹⁶⁷⁻¹⁷¹

Conduits. Much of the information about the cellular and molecular events which occur during Wallerian degeneration has been obtained from studies using empty or saline-filled silicone tubes to bridge short gaps (< 1 cm) in rodent nerves.⁵ This simple technique has transferred to the clinic with some success.¹⁶

Bridging a long (> 10 cm) interstump gap remains a major challenge, both experimentally and clinically (Fig. 3).^{122,172} An empty silicone tube is not fit for this purpose and no bio-engineered device has yet proved capable of supporting axonal regrowth across long gaps in laboratory models. There are undoubtedly many reasons, operating locally or at a distance, why axons fail to cross long interstump gaps within a conduit, e.g. poor vascularisation or

internal fibrosis. One of the most intensively researched ideas has been that axons stop growing because the pool of available co-migrating Schwann cells has become exhausted. To compensate for this perceived loss, exogenous Schwann cells have therefore been added to the lumen or walls of a conduit, e.g. by sandwiching lengths of nerve between segments of acellular muscle,¹⁷³ or by introducing a bolus of cultured cells into either a vein autograft¹⁷⁴⁻¹⁷⁶ or into conduits containing longitudinal polyglactin sutures.¹⁷⁷ These strategies have achieved modest increases, of the order of an additional 2 to 3 cm, in the distances which axons will regrow in experimental animals. Given that axonal regrowth depends upon the precisely regulated interactions of numerous cell types, and that Schwann cells, although essential, do not operate in a 'cellular vacuum', this is perhaps not surprising.

It is possible to speculate about the design specification of an ideal conduit, particularly one that is to be used either for bridging long interstump gaps or when taking an interventionist approach with shorter gaps. The data distilled from numerous preclinical experiments over the last 20 years suggest that a device needs to have walls that are bio-compatible and resorbable, sufficiently robust to resist collapse, and yet not so rigid that they compress the structures which they surround, and a lumen whose contents (e.g. Schwann cells, growth factor-releasing biodegradable beads, aligned collagen fibrils, micropatterned biodegradable films), facilitate orientated and sustained axonal elongation (Fig. 4). It goes without saying that it must also be easy to handle and to suture. It also goes without saying that such a device does not currently exist.

Stem cells. Obtaining sufficient numbers of autologous human Schwann cells is not a practical proposition in the clinic, on the grounds of technical difficulty and the time required to expand cultures. Stem-cell technology for the treatment of nerve injuries is in its infancy, but may ultimately provide an alternative source of Schwann cells.¹⁷⁸ Very recently, bone-marrow stromal cells have been induced to differentiate into cells with the characteristics of Schwann cells that appear to support regeneration when transplanted into degenerating rat sciatic nerves.^{179,180}

The future?

Therapeutic intervention, using some type of tissue-engineered device to manipulate endoneurial events at the molecular level, offers a potentially attractive alternative to the use of autografts.¹⁸¹ Collaboration between basic scientists and clinicians working in fields in which tissue engineering is now widely applied, such as orthopaedics and cardiovascular surgery, is likely to prove rewarding, and is to be encouraged.

I am indebted to Professor R. Birch for reading and commenting on drafts of this review, and to Professor G. Lundborg for permission to use his diagram of Wallerian degeneration (Fig. 2).

References

- Rosberg HE, Carlsson KS, Hojgard S, et al. Injury to the human median and ulnar nerves in the forearm: analysis of costs for treatment and rehabilitation of 69 patients in southern Sweden. *J Hand Surg [Br]* 2005;30:35-9.
- Lundborg G. Nerve injury and repair: a challenge to the plastic brain. *J Periph Nerv Syst* 2003;8:209-26.
- Ramer MS, Priestley JV, McMahon SB. Functional regeneration of sensory axons into the adult spinal cord. *Nature* 2000;403:312-16.
- Kaas JH, Florence SL. Mechanisms of reorganization in sensory systems of primates after peripheral nerve injury. *Adv Neurol* 1997;73:147-58.
- Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol* 1997;14:67-116.
- Ygge J. Neuronal loss in lumbar dorsal root ganglia after proximal compared to distal sciatic nerve resection: a quantitative study in the rat. *Brain Res* 1989;478:193-5.
- Hu P, McLachlan EM. Selective reactions of cutaneous and muscle afferent neurons to peripheral nerve transection in rats. *J Neurosci* 2003;23:10559-67.
- Costigan M, Befort K, Karchewski L, et al. Replicate high-density rat genome oligonucleotide microarrays reveal hundred of regulated genes in the dorsal root ganglion after peripheral nerve injury. *BMC Neurosci* 2002;3:16.
- Xiao HS, Hunag QH, Zhang FX, et al. Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. *Proc Natl Acad Sci USA* 2002;99:8360-5.
- Fernyhough P, Smith DR, Schapansky J, et al. Activation of nuclear factor-kappaB via endogenous tumor necrosis factor alpha regulates survival of axotomized adult sensory neurons. *J Neurosci* 2005;25:1682-90.
- Wall JT, Xu J, Wang X. Human brain plasticity: an emerging view of the multiple substrates and mechanisms that cause cortical changes and related sensory dysfunctions after injuries of sensory inputs from the body. *Brain Res Brain Res Rev* 2002;39:181-215.
- Lundborg G. Brain plasticity and hand surgery: an overview. *J Hand Surg [Br]* 2000;25:242-52.
- Rosen B, Lundborg G. Sensory re-education after nerve repair: aspects of timing. *Handchir Mikrochir Plast Chir* 2004;36:8-12.
- Taylor BK. Pathophysiologic mechanisms of neuropathic pain. *Curr Pain Headache Rep* 2001;5:151-61.
- Zimmerman M. Pathobiology of neuropathic pain. *Eur J Pharmacol* 2001;429:23-37.
- Lundborg G, Rosen B, Dahlin L, Holmberg J, Rosen I. Tubular repair of the median or ulnar nerve in the human forearm: a 5-year follow-up. *J Hand Surg [Br]* 2004;29:100-7.
- Waller AV. Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. *Philosophical Trans Royal Society of London, B. Biological Sciences* 1850;140:423-9.
- Pearce JM. Wallerian degeneration. *J Neurol Neurosurg Psychiatry* 2000;69:791.
- Koeppen AH. Wallerian degeneration: history and clinical significance. *J Neurol Sci* 2004;220:115-17.
- Eddleman CS, Ballinger ML, Smyers ME, Fishman HM, Bittner GD. Endocytotic formation of vessels and other membranous structures induced by Ca2+ and axolemmal injury. *J Neurosci* 1998;18:4028-41.
- Eddleman CS, Bittner GD, Fishman HM. Barrier permeability at cut axonal ends progressively decreases until an ionic seal is formed. *Biophys J* 2000;79:1883-90.
- Zochodne DW, Levy D, Zwiers H, et al. Evidence for nitric oxide and nitric oxide synthase activity in proximal stumps of transected peripheral nerves. *Neuroscience* 1999;91:1515-27.
- Glass JD, Culver DG, Levey AI, Nash NR. Very early activation of m-calpain in peripheral nerve during Wallerian degeneration. *J Neurol Sci* 2002;196:9-20.
- Schlaepfer WW, Bunge RP. Effects of calcium ion concentration on the degeneration of amputated axons in tissue culture. *J Cell Biol* 1973;59:456-70.
- Schlaepfer WW. Calcium-induced degeneration of axoplasm in isolated segments of rat peripheral nerve. *Brain Res* 1974;69:203-15.
- Schlaepfer WW. Structural alterations of peripheral nerve induced by the calcium ionophore A23187. *Brain Res* 1977;136:1-9.
- George EB, Glass JD, Griffin JW. Axotomy-induced axonal degeneration is mediated by calcium influx through ion-specific channels. *J Neurosci* 1995;15:6445-52.
- Smith KL, Kapoor R, Hall SM, Davies M. Electrically active axons degenerate when exposed to nitric oxide. *Ann Neurol* 2001;49:470-6.
- Tsao JW, George EB, Griffin JW. Temperature modulation reveals three distinct stages of Wallerian degeneration. *J Neurosci* 1999;19:4718-26.
- Gamble HJ, Jha BD. Some effects of temperature upon the rate and progress of wallerian degeneration in mammalian nerve fibres. *J Anat* 1958;92:171-7.
- Lunn ER, Perry VH, Brown MC, Rosen H, Gordon S. Absence of wallerian degeneration does not hinder regeneration in peripheral nerve. *Eur J Neurosci* 1989;1:27-33.
- Perry VH, Brown MC, Lunn ER, Tree P, Gordon S. Evidence that very slow wallerian degeneration in C57BL/6 mice is an intrinsic property of the peripheral nerve. *Eur J Neurosci* 1990;2:802-8.
- Perry VH, Lunn ER, Brown MC, Cahusac S, Gordon S. Evidence that the rate of wallerian degeneration is controlled by a single autosomal dominant gene. *Eur J Neurosci* 1990;2:408-13.
- Glass JD, Schryer BL, Griffin JW. Calcium-mediated degeneration of the axonal cytoskeleton in the OLA mouse. *J Neurochem* 1994;62:2472-5.
- Finn JT, Weil M, Archer F, et al. Evidence that wallerian degeneration and localized axon degeneration induced by local neurotrophin deprivation do not involve caspases. *J Neurosci* 2000;20:1333-41.
- Raff MC, Whitmore AV, Finn JT. Axonal self-destruction and neurodegeneration. *Science* 2002;296:868-71.
- Ehlers MD. Deconstructing the axon: wallerian degeneration and the ubiquitin-proteasome system. *Trends Neurosci* 2004;27:3-6.
- Chaudhry V, Cornblath DR. Wallerian degeneration in human nerves: serial electrophysiological studies. *Muscle Nerve* 1992;15:687-93.
- Birch R, Achan P. Peripheral nerve repairs and their results in children. *Hand Clin* 2000;16:579-95.
- Perry VH, Brown MC, Gordon S. The macrophage response to central and peripheral nerve injury: a possible role for macrophages in regeneration. *J Exp Med* 1987;165:1218-23.

41. **Carroll SL, Frohnert PW.** Expression of JE (monocyte chemoattractant protein-1) is induced by sciatic axotomy in wild type rodents but not in C57BL/6J mice. *J Neuropathol Exp Neurol* 1998;57:915-30.
42. **Toews AD, Barrett C, Morell P.** Monocyte chemoattractant protein 1 is responsible for macrophage recruitment following injury to sciatic nerve. *J Neurosci Res* 1998;53:260-7.
43. **Reichert F, Levitzky R, Rotshenker S.** Interleukin 6 in intact and injured mouse peripheral nerves. *Eur J Neurosci* 1996;8:530-5.
44. **Wagner R, Myers RR.** Schwann cells produce tumor necrosis factor alpha: expression in injured and non-injured nerves. *Neuroscience* 1996;73:625-9.
45. **La Fleur M, Underwood JL, Rappolee DA, Werb Z.** Basement membrane and repair of injury to peripheral nerve: defining a potential role for macrophages, matrix metalloproteinases, and tissue inhibitor of metalloproteinases-1. *J Exp Med* 1996;184:2311-26.
46. **Rotshenker S.** The cytokine network of wallerian degeneration. *Curr Top Neurochem* 1997;1:147-56.
47. **Be'eri H, Reichert D, Saada A, Rotshenker S.** The cytokine network of wallerian degeneration: IL-10 and GM-CSF. *Eur J Neurosci* 1998;10:2707-13.
48. **Menge T, Jander S, Stoll G.** Induction of the proinflammatory cytokine interleukin-18 by axonal injury. *J Neurosci Res* 2001;65:332-9.
49. **Taskinen HS, Olsson T, Bucht A, et al.** Peripheral nerve injury induces endoneurial expression of IFN-gamma, IL-10 and TNF-alpha mRNA. *J Neuroimmunol* 2000;102:17-25.
50. **Stoll G, Jander S, Myers RR.** Degeneration and regeneration of the peripheral nervous system: from Augustus Waller's observations to neuroinflammation. *J Peripher Nerv Syst* 2002;7:13-27.
51. **Shamash S, Reichert F, Rotshenker S.** The cytokine network of wallerian degeneration: tumor necrosis factor-alpha, interleukin-1alpha, and interleukin-1beta. *J Neurosci* 2002;22:3052-60.
52. **Lindholm D, Heumann R, Meyer M, Thoenen H.** Interleukin-1 regulated synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature* 1987;330:658-9.
53. **Levy D, Hoke A, Zochodne DW.** Local expression of inducible nitric oxide synthase in an animal model of neuropathic pain. *Neurosci Lett* 1999;260:207-9.
54. **Levy D, Tal M, Hoke A, Zochodne DW.** Transient action of the endothelial constitutive nitric oxide synthase (eNOS) mediates the development of thermal hypersensitivity following peripheral nerve injury. *Eur J Neurosci* 2000;12:2323-32.
55. **Levy D, Kubes P, Zochodne DW.** Delayed peripheral nerve degeneration, regeneration, and pain in mice lacking inducible nitric oxide synthase. *J Neuropathol Exp Neurol* 2001;60:411-21.
56. **Shubayev VI, Myers RR.** Upregulation and interaction of TNFalpha and gelatinases A and B in painful peripheral nerve injury. *Brain Res* 2000;855:83-9.
57. **Wagner R, Myers RR.** Endoneurial injection of TNF-alpha produces neuropathic pain behaviors. *Neuroreport* 1996;7:2897-901.
58. **Sampaio EP, Sarno EN, Galilly R, Cohn ZA, Kaplan G.** Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes: PG-699-703. *J Exp Med* 1991;173:699-703.
59. **Sommer C, Marziniak M, Myers RR.** The effect of thalidomide treatment on vascular pathology and hyperalgesia caused by chronic constriction injury of rat nerve. *Pain* 1998;74:83-91.
60. **Schafers M, Geis C, Brors D, Yaksh TL, Sommer C.** Anterograde transport of tumor necrosis factor-alpha in the intact and injured rat sciatic nerve. *J Neurosci* 2002;22:536-45.
61. **Durrenberger PF, Facer P, Gray RA, et al.** Cyclooxygenase-2 (Cox-2) in injured human nerve and a rat model of nerve injury. *J Peripher Nerve Syst* 2004;9:15-25.
62. **Mueller M, Wacker K, Ringelstein B, et al.** Rapid response of identified resident endoneurial macrophages to nerve injury. *Am J Pathol* 2001;159:2187-97.
63. **Jander S, Bussini S, Neuen-Jacob E, et al.** Osteopontin: a novel axon-regulated Schwann cell gene. *J Neurosci Res* 2002;67:156-66.
64. **Siebert H, Sachse A, Kuziel WA, Maeda N, Bruck W.** The chemokine receptor CCR2 is involved in macrophage recruitment to the injured peripheral nervous system. *J Neuroimmunol* 2000;110:177-85.
65. **Dailey AT, Avellino AM, Benthem L, Silver J, Kliot M.** Complement depletion reduces macrophage infiltration and activation during wallerian degeneration and axonal regeneration. *J Neurosci* 1998;18:6713-22.
66. **da Costa CC, van der Laan LJ, Dijkstra CD, Bruck W.** The role of the mouse macrophage scavenger receptor in myelin phagocytosis. *Eur J Neurosci* 1997;9:2650-7.
67. **Eta M, Yoshikawa H, Fujimura H, et al.** The role of CD36 in peripheral nerve remyelination after crush injury. *Eur J Neurosci* 2003;17:2659-66.
68. **Naba I, Yoshikawa H, Sakoda S, et al.** Successful generation of peripheral neuropathy with onion-bulb formation in the macrophage scavenger receptor class A knockout mouse treated with isoniazid. *Neurosci Lett* 2000;290:5-8.
69. **Fernandez-Valle C, Bunge RP, Bunge MB.** Schwann cells degrade myelin and proliferate in the absence of macrophages: evidence from in vitro studies of wallerian degeneration. *J Neurocytol* 1995;24:667-79.
70. **Salzer JL, Bunge RP.** Studies of Schwann cell proliferation. I: an analysis in tissue culture of proliferation during development, wallerian degeneration, and direct injury. *J Cell Biol* 1980;84:739-52.
71. **Salzer JL, Bunge RP, Glaser L.** Studies of Schwann cell proliferation. III: evidence for the surface localization of the neurite mitogen. *J Cell Biol* 1980;84:767-78.
72. **Salzer JL, Williams AK, Glaser L, Bunge RP.** Studies of Schwann cell proliferation: II: characterization of the stimulation and specificity of the response to a neurite membrane fraction. *J Cell Biol* 1980;84:753-66.
73. **Sobue G, Kreider B, Asbury A, Pleasure D.** Specific and potent mitogenic effect of axolemmal fraction on Schwann cells from rat sciatic nerves in serum-containing and defined media. *Brain Res* 1983;280:263-75.
74. **Sobue G, Pleasure D.** Adhesion of axolemmal fragments to Schwann cells: a signal-and target-specific process closely linked to axolemmal induction of Schwann cell mitosis. *J Neurosci* 1985;5:379-87.
75. **Ratner N, Bunge RP, Glaser L.** A neuronal cell surface heparan sulfate proteoglycan is required for dorsal root ganglion neuron stimulation of Schwann cell proliferation. *J Cell Biol* 1985;101:744-54.
76. **Ratner N, Bunge RP, Glaser L.** Schwann cell proliferation in vitro: an overview. *Ann N Y Acad Sci* 1986;486:170-81.
77. **Saunders RD, Brandon YW, DeVries GH.** Role of intracellular pH in the axolemma- and myelin-induced proliferation of Schwann cells. *J Neurochem* 1989;52:1576-81.
78. **Baichwal RR, Bigbee JW, DeVries GH.** Macrophage-mediated myelin-related mitogenic factor for cultured Schwann cells. *Proc Natl Acad Sci USA* 1988;85:1701-5.
79. **Tang S, Shen YJ, DeBellard ME, et al.** Myelin-associated glycoprotein interacts with neurons via a sialic acid binding site at ARG118 and a distinct neurite inhibition site. *J Cell Biol* 1997;138:1355-66.
80. **Mears S, Schachner M, Brushart TM.** Antibodies to myelin-associated glycoprotein accelerate preferential motor reinnervation. *J Peripher Nerv Syst* 2003;8:91-9.
81. **Vougioukas VI, Roeske S, Michel U, Bruck W.** Wallerian degeneration in ICAM-1-deficient mice. *Am J Pathol* 1998;152:241-9.
82. **Liefner M, Siebert H, Sachse T, et al.** The role of TNF-alpha during wallerian degeneration. *J Neuroimmunol* 2000;108:147-52.
83. **Hall SM, Gregson N.** The effects of mitomycin C on the process of regeneration in the mammalian peripheral nervous system. *Neuropathology App Neurobiology* 1977;3:65-78.
84. **Kuhlmann T, Bitsch A, Stadelmann C, Siebert H, Bruck W.** Macrophages are eliminated from the injured peripheral nerve via local apoptosis and circulation to regional lymph nodes and the spleen. *J Neurosci* 2001;21:3401-8.
85. **Omura K, Ohbayashi M, Sano M, et al.** The recovery of blood-nerve barrier in crush nerve injury: a quantitative analysis utilizing immunohistochemistry. *Brain Res* 2004;1001:13-21.
86. **Jessen KR, Mirsky R.** Origin and early development of Schwann cells. *Microsc Res Tech* 1998;41:393-402.
87. **Hall SM, Li H, Kent AP.** Schwann cells responding to primary demyelination in vivo express p75NTR and c-erbB receptors: a light and electron immunohistochemical study. *J Neurocytol* 1997;26:679-90.
88. **Trapp BD, Hauer P, Lemke G.** Axonal regulation of myelin protein mRNA levels in actively myelinating Schwann cells. *J Neurosci* 1988;8:3515-21.
89. **Gupta SK, Poduslo JF, Mezei C.** Temporal changes in PO and MBP gene expression after crush-injury of the adult peripheral nerve. *Brain Res* 1988;464:133-41.
90. **Willison HJ, Trapp BD, Bacher JD, Quarles RH.** The expression of myelin-associated glycoprotein in regenerating cat sciatic nerve. *Brain Res* 1988;444:10-16.
91. **Mitchell LS, Griffiths IR, Morrison S, et al.** Expression of myelin protein gene transcripts by Schwann cells of regenerating nerve. *J Neurosci Res* 1990;27:125-35.
92. **Chandross KJ, Kessler JA, Cohen RI, et al.** Altered connexin expression after peripheral nerve injury. *Mol Cell Neurosci* 1996;7:501-18.
93. **Chandross K.** Nerve injury and inflammatory cytokines modulate gap junctions in the peripheral nervous system. *Glia* 1998;24:21-31.
94. **Arroyo EJ, Scherer SS.** On the molecular architecture of myelinated fibres. *Histochem Cell Biol* 2000;113:1-18.
95. **Menichella DM, Baron PL, Scarpini E, et al.** Protein zero is necessary for E-cadherin-mediated adherens junction formation in Schwann cells. *Mol Cell Neurosci* 2001;18:606-18.
96. **Scherer SS, Arroyo EJ.** Recent progress on the molecular organization of myelinated axons. *J Peripher Nerv Syst* 2002;7:1-12.
97. **Mikol DD, Scherer SS, Duckett SJ, Hong HL, Feldman EL.** Schwann cell caveolin-1 expression increases during myelination and decreases after axotomy. *Glia* 2002;38:191-9.

98. Perry VH, Tsao JW, Fearn S, Brown MC. Radiation-induced reductions in macrophage recruitment have only slight effects on myelin degeneration in sectioned peripheral nerves of mice. *Eur J Neurosci* 1995;7:271-80.
99. Campana WM, Myers RR. Erythropoietin and erythropoietin receptors in the peripheral nervous system: changes after nerve injury. *FASEB J* 2001;15:1804-6.
100. Sheu JY, Kulhanek DJ, Eckenstein FP. Differential patterns of ERK and STAT3 phosphorylation after sciatic nerve transection in the rat. *Exp Neurol* 2000;166:392-402.
101. Harrisingh MC, Perez-Nadales E, Parkinson DB, et al. The Ras/Raf/ERK signalling pathway drives Schwann cell dedifferentiation. *EMBO J* 2004;23:3061-71.
102. Hughes PM, Wells GM, Perry VH, Brown MC, Miller KM. Comparison of matrix metalloproteinase expression during wallerian degeneration in the central and peripheral nervous systems. *Neuroscience* 2002;113:273-87.
103. Muir D, Manthorpe M. Stromelysin generates a fibronectin fragment that inhibits Schwann cell proliferation. *J Cell Biol* 1992;116:177-85.
104. Morrissey TK, Levi AD, Nuijens A, Sliwkowski MX, Bunge RP. Axon-induced mitogenesis of human Schwann cells involve heregulin and p185erbB2. *Proc Natl Acad Sci USA* 1995;92:1431-5.
105. Scherer SS, Wang DY, Kuhn R, et al. Axons regulate Schwann cell expression of the POU transcription factor SCIP. *J Neurosci* 1994;14:1930-42.
106. Topilko P, Schneider-Maunoury S, Levi G, et al. Krox-20 controls myelination in the peripheral nervous system. *Nature* 1994;371:796-9.
107. Kioussi C, Gross MK, Gruss P. Pax3: a paired domain gene as a regulator in PNS myelination. *Neuron* 1995;15:553-62.
108. Britsch S, Goerich DE, Riethmacher D, et al. The transcription factor Sox10 is a key regulator of peripheral glial development. *Genes Dev* 2001;15:66-78.
109. Jessen KR, Mirsky R. Signals that determine Schwann cell identity. *J Anat* 2002;200:367-76.
110. Gupta SK, Pringle J, Poduslo JF, Mezei C. Induction of myelin genes during peripheral nerve remyelination requires a continuous signal from the ingrowing axon. *J Neurosci Res* 1993;34:14-23.
111. Syroid DE, Maycox PR, Burrola PG, et al. Cell death in the Schwann cell lineage and its regulation by neuregulin. *Proc Natl Acad Sci USA* 1996;93:9229-34.
112. Grinspan JB, Marchionni MA, Reeves M, Coulaloglou M, Scherer SS. Axonal interactions regulate Schwann cell apoptosis in developing nerve: neuregulin receptors and the role of neuregulins. *J Neurosci* 1996;16:6107-18.
113. Soiliu-Hanninen M, Ekert P, Bucci T, et al. Nerve growth factor signalling through p75 induces apoptosis in Schwann cells via a Bcl-2-independent pathway. *J Neurosci* 1999;19:4828-38.
114. Petratos S, Butzkueven H, Shipham K, et al. Schwann cell apoptosis in the post-natal axotomized sciatic nerve is mediated via NGF through the low-affinity neurotrophin receptor. *J Neuropathol Exp Neurol* 2003;62:398-411.
115. Bradley JL, Abernethy DA, King RH, Muddle JR, Thomas PK. Neural architecture in transected rabbit sciatic nerve after prolonged nonreinnervation. *J Anat* 1998;192:529-38.
116. Terenghi G, Calder JS, Birch R, Hall SM. A morphological study of Schwann cells and axonal regeneration in chronically transected human peripheral nerves. *J Hand Surg [Br]* 1998;23:583-7.
117. Li H, Terenghi G, Hall SM. Effects of delayed re-innervation on the expression of c-erbB receptors by chronically denervated rat Schwann cells in vivo. *Glia* 1997;20:333-47.
118. You S, Petrov T, Chung PH, Gordon T. The expression of the low affinity nerve growth factor receptor in long-term denervated Schwann cells. *Glia* 1997;20:87-100.
119. Hall SM. The biology of chronically denervated Schwann cells. *Ann N Y Acad Sci* 1999;883:215-33.
120. Li H, Wigley C, Hall SM. Chronically denervated rat Schwann cells respond to GGF in vitro. *Glia* 1998;24:290-303.
121. Sulaiman OA, Gordon T. Transforming growth factor-beta and forskolin attenuate the adverse effects of long-term Schwann cell denervation on peripheral nerve regeneration in vivo. *Glia* 2002;37:206-18.
122. Hems TE, Glasby MA. The limit of graft length in the experimental use of muscle grafts of nerve repair. *J Hand Surg [Br]* 1993;18:165-70.
123. Calder JS, Norris RW. Repair of mixed peripheral nerves using muscle autografts: a preliminary communication. *Br J Plast Surg* 1993;46:557-64.
124. Robinson LR. Traumatic injury to peripheral nerves. *Muscle Nerve* 2000;23:863-73.
125. Al-Majed AA, Neumann CM, Brushart TM, Gordon T. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. *J Neurosci* 2000;20:2602-8.
126. Brushart TM, Hoffman PN, Royall RM, et al. Electrical stimulation promotes motoneuron regeneration without increasing its speed or conditioning the neuron. *J Neurosci* 2002;22:6631-8.
127. Berg A, Reilly JP, Drake E, et al. Upregulation of desert hedgehog signalling during peripheral nerve regeneration [abstract]. *Proc Society Neuroscience*, 2000.
128. Parmantier E, Lynn B, Lawson D, et al. Schwann cell-derived desert hedgehog control the development of peripheral nerve sheaths. *Neuron* 1999;23:713-24.
129. Umehara F, Tate G, Itoh K, et al. A novel mutation of desert hedgehog in a patient with 46,XY partial gonadal dysgenesis accompanied by minifascicular neuropathy. *Am J Hum Genet* 2000;67:1302-5.
130. Langley JN, Anderson HK. The union of different kinds of nerve fibres. *J Physiol (London)* 1904;31:365-91.
131. Hall SM. Observations on the progress of wallerian degeneration in transected peripheral nerves of C57BL/Wld mice in the presence of recruited macrophages. *J Neurocytol* 1993;22:480-90.
132. Brown MC, Perry VJH, Hunt SP, Lapper SR. Further studies on motor and sensory nerve regeneration in mice with delayed wallerian degeneration. *Eur J Neurosci* 1994;6:420-8.
133. Bedi KS, Winter J, Berry M, Cohen J. Adult rat dorsal root ganglion neurons extend neurites on predegenerated but not on normal peripheral nerves in vitro. *Eur J Neurosci* 1992;4:193-200.
134. Zuo J, Hernandez YJ, Muir D. Chondroitin sulfate proteoglycan with neurite-inhibiting activity is up-regulated following peripheral nerve injury. *J Neurobiol* 1998;34:41-54.
135. Tapia M, Inestrosa NC, Alvarez J. Early axonal regeneration: repression by Schwann cells and a protease? *Exp Neurol* 1995;131:124-32.
136. Alvarez J, Giuditta A, Koenig E. Protein synthesis in axons and terminals: significance for maintenance, plasticity and regulation of phenotype: with a critique of slow transport theory. *Prog Neurobiol* 2000;62:1-62.
137. Enver MK, Hall SM. Are Schwann cells essential for axonal degeneration into muscle autografts? *Neuropathol Appl Neurobiol* 1994;20:587-98.
138. Aumailley M, Smyth N. The role of laminins in basement membrane function. *J Anat* 1998;193:1-21.
139. Zuo J, Ferguson TA, Hernandez YJ, Stetler-Stevenson WG, Muir D. Neuronal matrix metalloproteinase-2 degrades and inactivates a neurite-inhibiting chondroitin sulfate proteoglycan. *J Neurosci* 1998;18:5203-11.
140. Ferguson TA, Muir D. MMP-2 and MMP-9 increase the neurite-promoting potential of schwann cell based laminae and are upregulated in degenerated nerve. *Mol Cell Neurosci* 2000;16:157-67.
141. Zuo J, Neubauer D, Graham J, et al. Regeneration of axons after nerve transection repair is enhanced by degradation of chondroitin sulfate proteoglycan. *Exp Neurol* 2002;176:221-8.
142. Krekoski CA, Neubauer D, Zuo J, Muir D. Axonal regeneration into acellular nerve grafts is enhanced by degradation of chondroitin sulfate proteoglycan. *J Neurosci* 2001;21:6206-13.
143. Terzis J, Faibisoff B, Williams B. The nerve gap: suture under tension vs. graft. *Plast Reconstr Surg* 1975;56:166-70.
144. Maeda T, Hori S, Sasaki S, Maruo S. Effects of tension at the site of coaptation on recovery of sciatic nerve function after neurorrhaphy: evaluation by walking-track measurement, electrophysiology, histomorphometry, and electron probe x-ray microanalysis. *Microsurgery* 1999;19:200-7.
145. Sunderland IR, Brenner MJ, Singham J, et al. Effect of tension on nerve regeneration in rat sciatic nerve transection model. *Ann Plast Surg* 2004;53:382-7.
146. Brushart TM. Preferential reinnervation of motor nerves by regenerating motor axons. *J Neurosci* 1988;8:1026-31.
147. Madison RD, Archibald SJ, Brushart TM. Reinnervation accuracy of the rat femoral nerve by motor and sensory neurons. *J Neurosci* 1996;16:5698-703.
148. Robinson GA, Madison RD. Motor neurons can preferentially reinnervate cutaneous pathways. *Exp Neurol* 2004;190:407-13.
149. Martini R, Xin Y, Schmitz B, Schachner M. The L2/HNK-1 carbohydrate epitope is involved in the preferential outgrowth of motor neurons on ventral roots and motor nerves. *Eur J Neurosci* 1992;4:628-39.
150. Martini R, Schachner M, Brushart TM. The L2/HNK-1 carbohydrate is preferentially expressed by previously motor axon-associated schwann cells in reinnervated peripheral nerves. *J Neurosci* 1994;14:7180-91.
151. Nichols CM, Brenner MJ, Fox IK, et al. Effects of motor versus sensory nerve grafts on peripheral nerve regeneration. *Exp Neurol* 2004;190:347-55.
152. Ghaliab N, Houstava L, Haninec P, Dubovy P. Morphometric analysis of early regeneration of motor axons through motor and cutaneous nerve grafts. *Ann Anat* 2001;183:363-8.
153. Tillett RL, Afoke A, Hall SM, Brown RA, Phillips JB. Investigating mechanical behaviour at a core-sheath interface in peripheral nerve. *J Peripher Nerve Syst* 2004;9:255-62.
154. Dilley A, Lynn B, Greening J, DeLeon N. Quantitative in vivo studies of median nerve sliding in response to wrist, elbow, shoulder and neck movements. *Clin Biomech (Bristol, Avon)* 2003;18:899-907.
155. Millesi H, Zoch G, Rath T. The gliding apparatus of peripheral nerve and its clinical significance. *Ann Chir Main Memb Super* 1990;9:87-97.

156. Millesi H, Zoch G, Reihsner R. Mechanical properties of peripheral nerves. *Clin Orthop* 1995;314:76-83.
157. Smith DH, Wolf JA, Lusardi TA, Lee VM, Meaney DF. High tolerance and delayed elastic response of cultured axons to dynamic stretch injury. *J Neurosci* 1999;19:4263-9.
158. Grand AG, Myckatyn TM, Mackinnon SE, Hunter DA. Axonal regeneration after cold preservation of nerve allografts and immunosuppression with tacrolimus in mice. *J Neurosurg* 2002;96:924-32.
159. Udina E, Gold BG, Navarro X. Comparison of continuous and discontinuous FK506 administration on autograft or allograft repair of sciatic nerve resection. *Muscle Nerve* 2004;29:812-22.
160. Jensen JN, Tung TH, Mackinnon SE, Brenner MJ, Hunter DA. Use of anti-CD40 ligand monoclonal antibody as antirejection therapy in a murine peripheral nerve allograft model. *Microsurgery* 2004;24:309-15.
161. Viterbo F, Palhares A, Franciosi LF. Restoration of sensitivity after removal of the sural nerve: a new application of latero-terminal neurotaphy. *Rev Paul Med* 1994;112:658-60.
162. Noah EM, Williams A, Fortes W, Terzis JK. A new animal model to investigate axonal sprouting after end-to-side neurotaphy. *J Reconstr Microsurg* 1997;13:317-25.
163. Mennen U. End-to-side nerve suture: a technique to repair peripheral nerve injury. *S Afr Med J* 1999;89:1188-94.
164. Rovak JM, Cederna PS, Macionis V, et al. Termino-lateral neurotaphy: the functional axonal anatomy. *Microsurgery* 2000;20:6-14.
165. Yan JG, Matloub HS, Sanger JR, et al. A modified end-to-side method for peripheral nerve repair: large epineurial window helicoid technique versus small epineurial window standard end-to-side technique. *J Hand Surg [Am]* 2002;27:484-92.
166. Walker JC, Brenner MJ, Mackinnon SE, Winograd JM, Hunter DA. Effect of perineurial window size on nerve regeneration, blood-nerve barrier integrity, and functional recovery. *J Neurotrauma* 2004;21:217-27.
167. Doolabh VB, Hertl MC, Mackinnon SE. The role of conduits in nerve repair: a review. *Rev Neurosci* 1996;7:47-84.
168. Heath CA, Rutkowski GE. The development of bioartificial nerve grafts for peripheral-nerve regeneration. *Trends Biotechnol* 1998;16:163-8.
169. Sundback C, Hadlock T, Cheney M, Vacanti J. Manufacture of porous polymer nerve conduits by a novel low-pressure injection moulding process. *Biomaterials* 2003;24:819-30.
170. Schmidt CE, Leach JB. Neural tissue engineering: strategies for repair and regeneration. *Annu Rev Biomed Eng* 2003;5:293-347.
171. McDonald DS, Zochodne DW. An injectable nerve regeneration chamber for studies of unstable soluble growth factors. *J Neurosci Methods* 2003;122:171-8.
172. Krarup C, Archibald SJ, Madison RD. Factors that influence peripheral nerve regeneration: an electrophysiological study of the monkey median nerve. *Ann Neurol* 2002;51:69-81.
173. Calder JS, Green CJ. Nerve-muscle sandwich grafts: the importance of schwann cells in peripheral nerve regeneration through muscle basal lamina conduits. *J Hand Surg [Br]* 1995;20:423-8.
174. Tang JB, Gu YQ, Song YS. Repair of digital nerve defect with autogenous vein graft during flexor tendon surgery in zone 2. *J Hand Surg [Br]* 1993;18:449-53.
175. Foidart-Dessalle M, Dubuisson A, Lejeune A, et al. Sciatic nerve regeneration through venous or nervous grafts in the rat. *Exp Neurol* 1997;148:236-46.
176. Battiston B, Tos P, Geuna S, Giacobini-Robecchi MG, Guglielmo R. Nerve repair by means of vein filled with muscle grafts. II: morphological analysis of regeneration. *Microsurgery* 2000;20:37-41.
177. Scherman P, Lundborg G, Kanje M, Dahlin LB. Neural regeneration along longitudinal polyglactin sutures across short and extended defects in the rat sciatic nerve. *J Neurosurg* 2001;95:316-23.
178. Tohill MP, Terenghi G. Stem cell plasticity and therapy for injuries of the peripheral nervous system. *Biotechnol Appl Biochem* 2004;40:17-24.
179. Cuevas P, Carceller F, Dujovny M, et al. Peripheral nerve regeneration by bone marrow stromal cells. *Neural Res* 2002;24:634-8.
180. Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H. Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. *Eur J Neurosci* 2001;14:1771-6.
181. Constans A. Neural tissue engineering. *The Scientist* 2004;18:40-3.
182. Taniuchi M, Clark HB, Johnson EM Jr. Induction of nerve growth factor receptor in schwann cells after axotomy. *Proc Natl Acad Sci USA* 1986;83:4094-8.
183. Heumann R, Korsching S, Bandtlow C, Thoenen H. Changes of nerve growth factor synthesis in non-neuronal cells in response to sciatic nerve transection. *J Cell Biol* 1987;104:1623-31.
184. Matsuoka I, Meyer M, Thoenen H. Cell-type-specific regulation of nerve growth factor (NGF) synthesis in non-neuronal cells: comparison of schwann cells with other cell types. *J Neurosci* 1991;11:3165-77.
185. Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J Cell Biol* 1992;119:45-54.
186. Korsching S. The neurotrophic factor concept: a reexamination. *J Neurosci* 1993;13:2739-48.
187. Marcinkiewicz M, Savaria D, Marcinkiewicz J. The pro-protein convertase PC1 is induced with PC5, furin and PC7, implication in pro-BDNF processing. *Brain Res Mol Brain Res* 1998;59:229-46.
188. Cohen JA, Yachnis AT, Arai M, Davis JG, Scherer SS. Expression of the neu proto-oncogene by Schwann cells during peripheral nerve development and wallerian degeneration. *J Neurosci Res* 1992;31:622-34.
189. Garratt AN, Britsch S, Birchmeier C. Neuregulin, a factor with many functions in the life of a schwann cell. *Bioessays* 2000;22:987-96.
190. Weiner JA, Fukushima N, Contos JJ, Scherer SS, Chun J. Regulation of Schwann cell morphology and adhesion by receptor-mediated lysophosphatidic acid signaling. *J Neurosci* 2001;21:7069-78.
191. Jessen KR, Mirsky R, Morgan L. Myelinated, but not unmyelinated axons, reversibly down-regulate N-CAM in Schwann cells. *J Neurocytol* 1987;16:681-8.
192. Martini R, Scachner M. Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and myelin-associated glycoprotein) in regenerating adult mouse sciatic nerve. *J Cell Biol* 1988;106:1735-46.
193. Araki T, Milbrandt J. Ninjurin2, a novel homophilic adhesion molecule, is expressed in mature sensory and enteric neurons and promotes neurite outgrowth. *J Neurosci* 2000;20:187-95.
194. Hughes RC. Galectins as modulators of cell adhesion. *Biochimie* 2001;83:667-76.
195. Horie H, Inagaki Y, Sohma Y, et al. Galectin-1 regulates initial axonal growth in peripheral nerves after axotomy. *J Neurosci* 1999;19:9964-74.
196. Kuecherer-Ehret A, Graeber MB, Edgar D, Thoenen H, Kreutzberg GW. Immunoelectron microscopic localization of laminin in normal and regenerating mouse sciatic nerve. *J Neurocytol* 1990;19:101-9.
197. Martini R, Schachner M. Complex expression pattern of tenascin during innervation of the posterior limb buds of the developing chicken. *J Neurosci Res* 1991;28:261-79.
198. Lefcort F, Venstrom K, McDonald JA, Reichardt LF. Regulation of expression of fibronectin and its receptor, alpha 5 beta 1, during development and regeneration of peripheral nerve. *Development* 1992;116:767-82.
199. Feltri ML, Scherer SS, Nemni R, et al. Beta 4 integrin expression in myelinating schwann cells is polarized, developmentally regulated and axonally dependent. *Development* 1994;120:1287-301.
200. Chernousov MA, Scherer SS, Stahl RC, Carey DJ. p200, a collagen secreted by schwann cells, is expressed in developing nerves and in adult nerves following axotomy. *J Neurosci Res* 1999;56:284-94.
201. Madison RD, Zomorodi A, Robinson GA. Netrin-1 and peripheral nerve regeneration in the adult rat. *Exp Neurol* 2000;161:563-70.
202. Curtis R, Stewart HJ, Hall SM, et al. GAP-43 is expressed by nonmyelin-forming schwann cells of the peripheral nervous system. *J Cell Biol* 1992;116:1455-64.
203. Plantinga LC, Verhaagen J, Edwards PM, et al. The expression of B-50/GAP-43 in schwann cells is upregulated in degenerating peripheral nerve stumps following nerve injury. *Brain Res* 1993;602:69-76.
204. Kurek JB, Austin L, Cheema SS, Bartlett PF, Murphy M. Up-regulation of leukaemia inhibitory factor and interleukin-6 in transected sciatic nerve and muscle following denervation. *Neuromuscular Disord* 1996;6:105-14.
205. Subang MC, Richardson PM. Synthesis of leukemia inhibitory factor in injured peripheral nerves and their cells. *Brain Res* 2001;900:329-31.
206. Shubayev VI, Myers RR. Endoneurial remodeling by TNFalpha- and TNFalpha-releasing proteases: a spatial and temporal co-localization study in painful neuropathy. *J Peripher Nerve Syst* 2002;7:28-36.
207. Scherer SS, Kamholz J, Jakowlew SB. Axons modulate the expression of transforming growth factor-betas in schwann cells. *Glia* 1993;8:265-76.
208. Einheber S, Hannocks MJ, Metz CN, Rifkin DB, Salzer JL. Transforming growth factor-beta 1 regulates axon/schwann cell interactions. *J Cell Biol* 1995;129:443-58.
209. Matsuoka I, Nakane A, Kurihara K. Induction of LIF-mRNA by TGF-beta 1 in Schwann cells. *Brain Res* 1997;776:170-80.
210. Digicaylioglu M, Garden G, Timberlake S, Fletcher L, Lipton SA. Acute neuroprotective synergy of erythropoietin and insulin-like growth factor I. *Proc Natl Acad Sci USA* 2004;101:9855-60.
211. Keswani SC, Buldanlioglu U, Fischer A, et al. A novel endogenous erythropoietin mediated pathway prevents axonal degeneration. *Ann Neurol* 2004;56:815-26.
212. Camborieux L, Bertrand N, Swerts JP. Changes in expression and localization of hemopexin and its transcripts in injured nervous system: a comparison of central and peripheral tissues. *Neuroscience* 1998;82:1039-52.