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SYMPOSIUM: Peripheral Neuropathies

Nerve Injury, Axonal Degeneration and Neural Regeneration: Basic Insights

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Axotomy or crush of a peripheral nerve leads to degeneration of the distal nerve stump referred to as Wallerian degeneration (WD). During WD a microenvironment is created that allows successful regrowth of nerve fibres from the proximal nerve segment. Schwann cells respond to loss of axons by extrusion of their myelin sheaths, downregulation of myelin genes, dedifferentiation and proliferation. They finally aline in tubes (Büngner bands) and express surface molecules that guide regenerating fibres. Hematogenous macrophages are rapidly recruited to the distal stump and remove the vast majority of myelin debris. Molecular changes in the distal stump include upregulation of neurotrophins, neural cell adhesion molecules, cytokines and other soluble factors and their corresponding receptors. Axonal injury not only induces muscle weakness and loss of sensation but also leads to adaptive responses and neuropathic pain. Regrowth of nerve fibres occurs with high specificity with formerly motor fibres preferentially reinnervating muscle. This involves recognition molecules of the L2/HNK-1 family. Nerve regeneration occurs at a rate of 3-4 mm/day after crush and 2-3 mm/day after sectioning a nerve. Nerve regeneration can be fostered pharmacologically. Upon reestablishment of axonal contact Schwann cells remyelinate nerve sprouts and downregulate surface molecules characteristic for precursor/premyelinating or nonmyelinating Schwann cells. At present it is unclear whether axonal regeneration after nerve injury is impeded in neuropathies.

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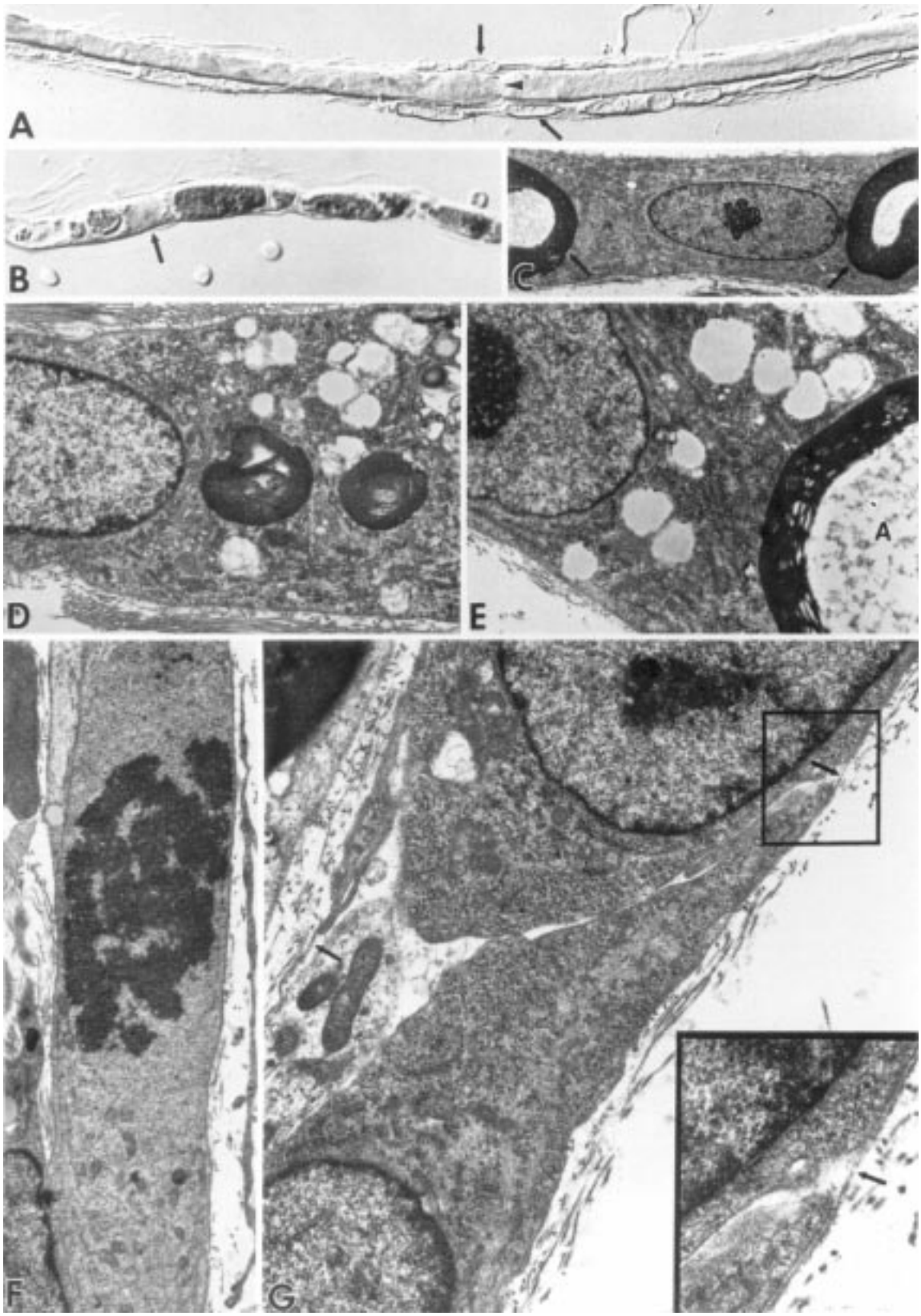
Introduction

There are two principal targets of peripheral nerve damage: the axon and the Schwann cells with their myelin sheaths. Attacks on myelin sheaths or myelinating Schwann cells as often seen in inflammatory neuropathies lead to focal demyelination with relative preservation of the axon. Repair mechanisms can fastly restore nerve conduction by remyelination. In contrast, axonal damage by crush, axotomy, ischemia, or inflammation leads to interruption of axonal integrity with ensuing degeneration of nerve fibres distal to the site of insult, a process named Wallerian degeneration (WD) (116). WD begins with prompt degradation of axoplasm and axolemma induced by the activation of axonal proteases and calcium influx (32, 93). To restore function, nerve fibres have to regrow from the site of axonal injury. While both nerve crush and axotomy induce WD, there is an important difference in the probability of successful regeneration. After a crush lesion the continuous basal lamina provides guidance for regenerating axons from the proximal nerve stump to their targets. After axotomy, however, separation of the proximal and distal stumps can impede reinnervation and often leads to the formation of neuroma.

WD sets in motion a machinery of molecular changes in the perikarya as well as in the distal degenerating stump of the injured motor and sensory neurons. Very rapidly nerve fibres from the proximal stump elongate growth cones into and through the distal segment and eventually reinnervate target tissues. The cellular and molecular mechanisms underlying WD and subsequent nerve regeneration are reviewed.

Cellular responses in the distal stump

As a prompt response to degenerating axons, Schwann cells within two days sequester small whorls of myelin debris and fragment their own myelin sheaths into ovoids (63, 102). Schwann cells phagocytose myelin debris to some extent and form lipid droplets before macrophages enter degenerating nerves (Fig. 1).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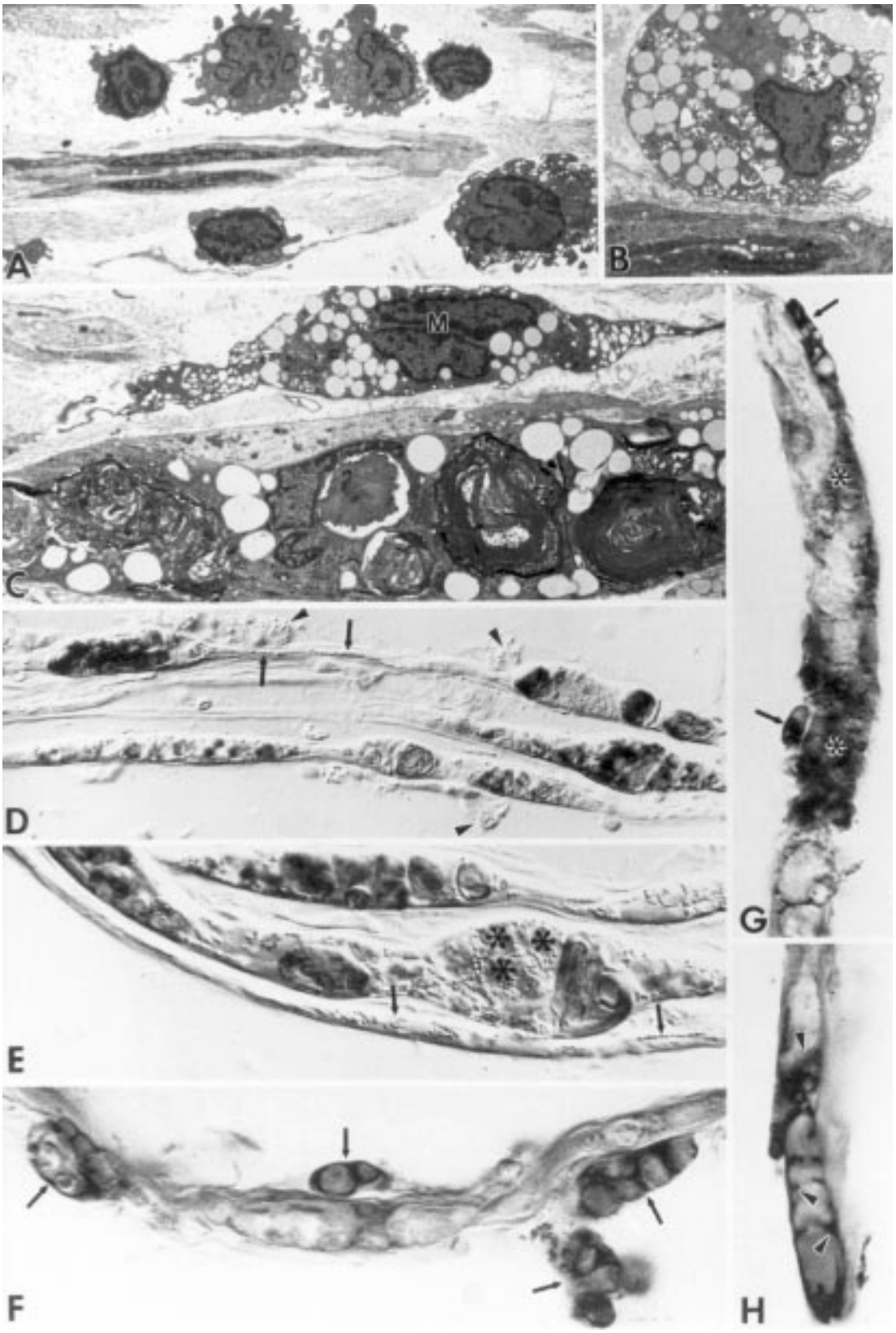
After the initial extrusion of myelin sheaths, Schwann cells divide with a maximum at day 3 (Fig. 1) (18, 102) and line up within the basal lamina tube to form bands of Büngner, that later provide guidance cues for regenerating nerve fibres. Beginning on day 2 and with a maximum between days 4 to 7, hematogenous macrophages enter the distal stump and migrate to the ovoids (Fig. 2). Within two weeks macrophages completely clear myelin debris (13, 63, 102). The molecular mechanisms involved in macrophage-mediated myelin clearance have recently been reviewed (13). Briefly, at least two surface molecules are involved in myelin recognition and uptake. Complement receptor type 3 (CR3) is constitutively expressed on macrophages. The corresponding complement component C3 is detectable at the surface of degenerating myelin sheaths by immunoelectronmicroscopy, indicating that myelin is opsonized by complement during WD (14). In support of a functional role of CR3, *in vivo*-application of the antibody Mac-1 against rat CR3 after nerve transection caused a significant reduction of myelin phagocytosis (15). The galactose-specific lectin MAC-2 is induced on both phagocytosing macrophages and Schwann cells during WD (86). Functionally, application of galactose and lactose which bind to MAC-2 also block myelin clearance during WD. Moreover, very slow progression of Wallerian degeneration in C57Bl/Ola mice (64) is associated with reduced upregulation of MAC-2 (86). MAC-2 expression on Schwann cells and macrophages can be induced by granulocyte macrophage colony stimulating factor (GM-CSF) (89). Fibroblasts but not Schwann cell and macrophages produce GM-CSF after nerve injury indicating a significant role of fibroblasts in the cascade of molecular events during WD.

There have been longstanding controversies to what extent Schwann cells versus macrophages and to what extent resident endoneurial (42) versus hematogenous macrophages are involved in myelin clearance (reviewed in 13). Brück and colleagues (16) depleted hematogenous macrophages by application of dichloromethylene diphosphonate containing liposomes. After nerve transection, the number of phagocytes within the degenerating nerves was significantly reduced in the macrophage depleted rats, but macrophage depletion did not completely abolish myelin degradation. This study further substantiated the predominant role of hematogenous macrophages in myelin clearance and, on the other hand, supported the notion that resident cells in the peripheral nerveous system participate in myelin removal and can partly replace hematogenous macrophages. Accordingly, Schwann cells in macrophage depleted animals contained more myelin debris than in sham treated controls after axotomy (16), and whole body irradiation moderately decreased myelin clearance after axotomy (81). Moreover, *in vitro* Schwann cells are capable to carry out degradation of short myelin segments without the assistance of macrophages (28). In summary, there is overwhelming evidence that under normal conditions hematogenous macrophages remove the vast majority of myelin debris.

The mechanism of recruitment of hematogenous macrophages in WD is still unclear. There is conflicting evidence on the putative role of cellular adhesion molecules. Tissue inflammation involves multiple cell adhesion steps between invading leukocytes and endothelial cells (reviewed in 100). Macrophages bear the CD11a/CD18 (LFA-1), the very late antigen-4 (VLA-4) and the CD11b/CD18 (Mac-1; complement type 3 receptor) complex on their surface. The corresponding endothelial counterreceptors are intercellular adhesion molecule-1 (ICAM-1) for LFA-1/Mac-1 and vascular cellular adhesion molecule-1 (VCAM-1) for VLA-4. Usually, ICAM-1 and VCAM-1 are expressed at low levels on endothelial cells, but are strongly upregulated in inflammatory diseases by cytokines. While one study described ICAM-1 and VCAM-1 expression during WD in mice (19), we did not find upregulation of ICAM-1 at

Figure 1. (Left) Teased fibres and electron micrographs illustrating early stages of Wallerian degeneration. (**A**) In this bundle of three teased fibres 24h after transection, the small fibres (arrows) have already undergone segmentation of myelin into early ovoids. The large myelinated fibre with its node (arrowhead) appears normal at this time. x 720. (**B**) By 72 h the Schwann cell perikaryon (arrow) occupies the whole cross-sectional area of the nerve fibre and myelin fragmentation into early ovoids is well underway. x 790. (**C**) Electron micrograph of a longitudinal section illustrating the Schwann cell perikaryon 48h after nerve transection; note the myelin sheath remnants at either side of the perikaryon (arrows). x 2930. (**D**) At 3 days, small whorls of myelin debris are sequestered within the Schwann cell cytoplasma and lipid droplets can be seen. x 6170. (**E**) Note numerous lipid droplets adjacent to the Schwann cell nucleus, and clearly within Schwann cell cytoplasm. Note also that residual myelin remains but the axoplasma (A) has been replaced by granular and amorphous debris. x 8250. (**F**) An example of frequent mitotic figures found in Schwann cells on day 3. x 6350. (**G**) Post-mitotic Schwann cells (day 3) separate longitudinally, leaving interdigitating processes. Note that both Schwann cells are covered by the original basal lamina (arrows), while apposing Schwann cell surfaces are not covered by basal lamina. x 13 500. Inset shows boxed region at higher power. x 27 800. Reprinted from Stoll et al. (1989) J Neurocytol 18:671-683 with permission.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the maximum of macrophage infiltration at day 4 in the rat (103). As shown by Brown *et al*. (10) application of antibodies against ICAM-1 had no influence on macrophage entry into the distal nerve segment. On the other hand, Vougioukas *et al*. (113) described significantly lower numbers of macrophages in transgenic ICAM-1 deficient mice after axotomy. Based on these conflicting data, the role of the LFA-1/Mac-1 complex and endothelial ICAM-1 expression in macrophage recruitment in WD awaits further clarification. The VLA-4 / VCAM-1 pathway appears not to be involved in cellular infiltration after nerve injury. We did not see VCAM-1 mRNA expression in the distal stump of degenerating nerves (50) and application of anti-VCAM-1 antibodies had no influence on macrophage infiltration (10). Interestingly, T cell inflammation is lacking in WD (33). In contrast to other nonimmune lesion paradigms of the nervous system such as cerebral ischemia (94), neutrophils are only transiently present during the first few hours after peripheral nerve injury.

Molecular responses in the distal stump

Transection or crush of a peripheral nerve sets in motion a dramatic change in the molecular composition of the distal nerve segments (25, 34, 36). Thereby a microenvironment develops that supports axonal regeneration in the PNS and, moreover, allows elongation of usually nonregenerating transected CNS fibre tracts into grafted PNS nerve segments (2). Upon loss of axonal contact myelinating Schwann cells downregulate steady state mRNA levels of the myelin components myelin basic protein (MBP), myelin associated glycoprotein (MAG), protein zero (P_0), peripheral myelin protein-22 (PMP22) and periaxin within two days after injury (61, 92, 98). Formerly myelinating Schwann cells dedifferentiate and acquire the phenotype of pre/nonmyelinating Schwann cells by expression of p75 low affinity nerve growth factor receptors (NGF-R), glial fibrillary acidic protein (GFAP), glial maturation factor-β, the cell adhesion molecule L1 and neural cell adhesion molecule (N-CAM) (8, 52, 68). Transcription factors Pax3, SCIP, c-jun and Krox-20 are involved in the regulation of Schwann cell de- and redifferentiation (54, 123). Denervated Schwann cells strongly reexpress Pax3 and c-jun, but downregulate Krox-20. Pax3, a paired-domain transcription factor, is expressed in embryonic Schwann cells, persists in non-myelinating Schwann cells in the adult, but is downregulated in myelin-forming Schwann cells (53). Functionally, Pax3 represses transcription of myelin genes (53). SCIP is a POU-domain transcription factor. SCIP is expressed in early myelinating Schwann cells for a short period and is downregulated in Schwann cells that maintain a myelin sheath (72, 91, 123). *in vitro*, SCIP acts as a repressor of the genes encoding P_0 and MBP (72). Transgenic mice lacking SCIP show a delay in Schwann cell differentiation, but myelinate normally (49). Krox-20 is an immediate early gene that belongs to a class of transcription factors with zinc-finger motifs. Krox-20 expression in the adult peripheral nerve is restricted to myelin forming Schwann cells (123). C-jun is expressed only by non-myelinating Schwann cells in normal nerve, but reexpressed by denervated formerly myelinating Schwann cells after axotomy (97). When axons regenerate into the distal stump, the expression of c-jun declines as Schwann cells remyelinate axons. Functionally, C-jun seems not to directly affect myelin-specific gene expression in Schwann cells (97).

As a response to axonal loss Schwann cells divide during WD. Glial growth factors (GGF) are potent Schwann cell mitogens *in vitro* and have been implicated in Schwann cell proliferation *in vivo*. Induction of mRNAs encoding the GGF subfamily of neuregulins occurs in nerves beginning 3 days postaxotomy and thus coincides with the onset of Schwann cell DNA synthesis and proliferation (18). GGF expression is accompanied by upregulation of GGF-receptors, the erbB membrane tyrosine kinases 2 and 3. Further studies showed

Figure 2. (Left) Electron micrographs and teased fibres illustrating macrophages in nerves during mid-stages of Wallerian degeneration. (**A**) A collection of mononuclear cells within the endoneurial space 4 days after transection. x 2520. (**B**) Foamy macrophage near a blood vessel 14 days after transection. x 3990. (**C**), Electron micrograph of longitudinal section of a nerve illustrating an ovoid with an overlying lipid-filled macrophage (M) outside the nerve fibre (14 days). x 4000. (**D,E**) Teased nerve fibres (14 days) visualized with Nomarski optics. (**D**) Note the segregation of myelin debris into discrete ovoids connected by attenuated Schwann cell bands containing lipid droplets (arrows). Note also that cells containing lipid droplets are present outside the nerve fibres (arrowheads); as demonstrated below, these cells are macrophages. x 610. (**E**) In the region of the myelin masses there are numerous nuclei (asterisks). Note also the widely distributed lipid droplets, including lipid droplets within attenuated Schwann cells bands (arrows). x 1100. (**F**) Teased fibre (14 days) stained with anti-ED1 antibody, an immunocytochemical marker for macrophages. Note that around these ovoids there are multiple ED1-positive macrophages (arrows). x 1370. (**G**) In this 14-day teased fibre, ED1-positive macrophages are present outside (arrows) and inside the fibre (Asterisks). x 1050. (**H**) In these ovoids, ED1 immunoreactivity surrounds the myelin masses within the nerve fibre (arrowheads). x 940. Reprinted from Stoll et al. (1989) J Neurocytol 18:671-683 with permission.

that phosphorylation of erbB coincides with Schwann cells proliferation (59). Although Schwann cell proliferation rapidly ceases GGF expression persists into late stages of WD.

Nerve injury leads to upregulation of neurotrophic factors and their receptors in the degenerating nerve segment (6, 31, 52). The functional role of neurotrophins in the peripheral nervous system will be described in detail in an accompanying chapter in this issue. Briefly, within hours after axonal damage bioactivity as well as mRNA levels of nerve growth factor (NGF) increase dramatically and show a second peak of expression 2-3 days after injury (31, 46). Moroever, there is a continous slow increase of brain derived neurotrophic factor (BDNF) mRNA starting after day 3 post-lesion and reaching maximal levels 3-4 weeks later (71). In contrast to the above mentioned neurotrophins, expression of ciliary neurotrophic factor (CNTF) requires intact axon-Schwann cell interactions and is reduced both on the mRNA and protein level in WD (29, 96). Schwann cells produce NGF and BDNF after nerve injury. Changes in neurotrophin expression are accompanied by the upregulation of the corresponding receptors (31). Insulin-like growth factor-I (IGF-I) is another neurotrophic factor produced by Schwann cells in the early stages of WD. Schwann cells also express the corresponding receptor. Interestingly, after postlesion day 7 infiltrating macrophages become the predominant source of IGF-I in the distal stump (22) suggesting that inflammatory cells also provide neurotrophic support.

In WD transcripts and protein levels for pro- and antiinflammatory cytokines are strongly upregulated (7, 9, 24, 35, 85, 88). Cytokine induction occurs in the absence of T cell inflammation. Cytokines play an important role in the pathogenesis of T cell-mediated autoimmune diseases of the nervous system where they orchestrate immune responses (reviewed in 3, 44). The fundamental role of cytokines in neural development (reviewed in 70) and nerve repair after injury have only recently widely been appreciated (35, 62, 108). Within 24 hours after nerve crush IL1β-mRNA levels are increased and remain at high levels throughout the first week (35). IL1 protein could be extracted from distal nerve segments of transected peripheral nerves (88). It has been shown that IL1 induces synthesis of NGF in Schwann cells (62). In support of an important role of IL1 in nerve regeneration, application of interleukin-1 receptor antagonist, which binds to and antagonizes IL1, impedes peripheral nerve regeneration (43). Simultaneously with the induction of IL1, increased levels of IL6- and IL10-mRNA and protein are detectable within 1 day after nerve crush (7, 9, 35, 85). Schwann cells express IL10-mRNA (51). IL6 protein has been localized in Schwann cells, fibroblasts and macrophages (7, 85). With a delay of few days significant mRNA induction for the proinflammatory cytokines IFNγ and IL12 has been found after nerve crush. IL12-mRNA expression peaks between days 7 and 14 (35), when myelin phagocytosis by macrophages is at maximum (102). The functional roles of IFNγ and IL12 in nerve degeneration and regeneration are unknown at present. Schwann cells express TNF-immunoreactivity in normal and injured sciatic nerves (115). In addition, after nerve injury infiltrating macrophages express strong TNF immunoreactivity (104). TNFα has been inflicted in neuropathic pain during WD (114; see below). In normal adult sciatic nerve TGF-beta 1, -beta 2, and beta-3 are expressed in the cytoplasm of Schwann cells (90). Axotomy leads to an increase in TGF-beta 1 mRNA levels, while TGF-beta 3 mRNA falls. Although the roles of cytokines in nerve regeneration have to be further elucidated, these studies show that a significant cytokine induction can occur in the peripheral nervous system despite the absence of a T cell response.

Leukemia inhibitory factor (LIF) is another important cytokine that shows an increased expression after axotomy (24, 58). The cellular source of LIF in WD is unknown. Embryonic Schwann cells express high levels of LIF mRNA in culture (58). When applied to the site of nerve transection LIF enhances the survival of sensory and motor neurons in neonatal rats (20, 21). LIF is retrogradly transported in sensory, motor and sympathetic neurons in adult animals (24, 45). Tham and colleagues (108) cut sciatic nerves of adult rats and bridged proximal and distal nerve stumps by a silicone cuff containing LIF. At 12 weeks after transection LIF treatment significantly increased the conduction velocity of the newly regenerated nerve, the diameter and number of regenerated myelinated axons, the force of contraction of the reinnervated muscle, and the muscle mass.

In Wallerian degeneration myelin-derived lipids are reutilized for regeneration and remyelination. Apolipoproteins D and E (ApoD, ApoE) are lipid binding proteins which accumulate in the distal stump after axotomy (74, 99). ApoE is produced by infiltrating macrophages (105) while ApoD is simultaneously expressed by endoneurial fibroblasts (99). Functional studies showed that lipoproteins are taken up by neuritic growth cones and Schwann cells (48, 87). However, nerve regeneration and cholesterol reutilization may also occur in the absence of apolipoproteins E and A-I as shown in transgenic mice (40).

Wallerian degeneration and hyperalgesia

Axotomy of sciatic nerve not only leads to muscle weakness and loss of sensation but also to adaptive responses with ensuing neuropathic pain. Chronic loose constriction of the sciatic nerve produces mechanoallodynia and thermal hyperalgesia in rats and mice during the time in which the nerve distal to the ligature site undergoes WD (79, 84). C57Bl/WLD mice, showing delayed Wallerian degeneration after axotomy, concomitantly exhibit reduced hyperalgesia temporally associated with reduced numbers of phagocytic cells in injured nerve. The pathomechanism of lesion-induced hyperalgesia still needs to be elucidated, although there is evidence that the cytokine TNFα and sprouting of sympathetic fibres are involved. As described above, infiltrating macrophages in Wallerian degeneration express TNFα immunoreactivity (104) and intraneural injection of TNFα into nerves induces neuropathic pain (114). Sympathetic axons invade the DRG following a peripheral nerve lesion and form baskets around large DRG neurons (84). This sympathetic sprouting is markedly delayed in C57Bl/WLD mice. Moreover, nerve injury produces a long-lasting rearrangement in the organisation of primary afferent central terminals (120). Usually low-threshold mechanoreceptors terminate in laminae III and IV and unmyelinated C fibres, most of which are nocireceptors, terminate predominately in lamina II in the dorsal horn of the spinal cord. After peripheral nerve injury the central terminals of axotomized myelinated afferents, including large A beta fibres, sprout into lamina II that normally receives only C-fibre input with the consequence of hyperalgesia. Interestingly, even intact myelinated primary afferents have the capacity for collateral sprouting (26). Two weeks after cutting the posterior cutaneous nerve and leaving the adjacent saphenous nerve intact, fibres from the saphenous nerve sprouted into an area of lamina II that is normally innervated exclusively by the adjacent posterior cutaneous nerve. This sprouting process could explain the observed sensory hypersensitivity at the edges of denervated skin. Taken together these studies provided strong evidence that a peripheral nerve lesion may induce structural reorganisation in the adult central nervous system.

Responses of dorsal root ganglia and motor neurons to peripheral nerve injury

The early response of the perikaryon to axonal injury includes chromatolysis (41) and upregulation of the transcription factor Jun which persists until regeneration of the peripheral nerve is completed (60). Growth-associated protein (GAP)-43/B50 and the intermediate filament protein peripherin were upregulated within the first day after an axonal lesion and have been inflicted in axon elongation (111, 119). The three neurofilament genes NF-L, NF-M, and NF-H are downregulated (76, 119) while class II and III β-tubulin mRNAs and proteins increase after axotomy (47,73).

In sensory neurons axonal injury leads to additional changes in neuropeptide and cytokine expression. IL6 mRNA and protein appear within 1 day in large and medium-sized ganglia after sciatic nerve transection with a maximal expression after 2 and 4 days and a decrease below threshold of detection within 1 week (78). Moreover, IL1β and TNFα mRNAs are upregulated, but the cellular sources have not yet been defined. There is evidence that the cytokine LIF is involved in triggering part of the cell body reaction in sensory neurons (23, 107). Following axonal damage the neuropeptide galanin which plays a tonic inhibitory role in the mediation of spinal cord excitability (118) is upregulated. In transgenic mice in which the gene for the cytokine LIF had been knocked out, galanin expression in DRG after sciatic nerve lesion was reduced (23, 107). Furthermore, axonal damage induces a marked upregulation of nitric oxide synthase in primary sensory neurons (112, 122).

Axotomized motoneurons respond by increased NGF-R mRNA and protein (55), as well as trkB mRNA expression, which is the receptor for BDNF and NT-4 (82). Increase is maximal at day 3 and returns to normal within three weeks. Additional changes include modification of glutamate receptor expression (83) and a transient increase in calcitonin gene-related peptide immunoreactivity (5).

Regeneration of nerve fibres

After axonal injury peripheral nerve fibres regenerate from the proximal stump. This process can be examined electrophysiologically. By using implanted electrodes in the cat, Fugleholm and colleagues (30) could follow the speed of axonal regrowth and found that the rate of elongation was 3-4mm/day after crushing a nerve, but only 2.5mm/d after sectioning. Regenerating axons preferentially grew inside internal structures of endoneurial Schwann cell tubes. Depletion of Schwann cells did not influence axonal elongation when the basal lamina remained in continuity suggesting a critical role of extracellular matrix proteins in axon regeneration (30).

In the distal stump a number of neurite outgrowth promoting molecules are upregulated (reviewed in 67).

From postlesional day 4 onwards increasing numbers of previously myelin forming Schwann cells expressed the cell surface molecules L1 and N-CAM (68). *In vitro*, L1 and to a lesser degree N-CAM are involved in Schwann cell-mediated neurite extension (95). Ninjurin (nerve injury-induced protein) is another homophilic adhesion molecule that is upregulated after axotomy (4). Ninjurin is located on the cellular surfaces of axons and Schwann cells and promotes neurite extension of dorsal root ganglion neurons *in vitro*. Among the extracellular matrix components, laminin is one of the most effective promotors of neurite extension *in vitro*. Surprisingly, laminin B chain mRNA steady state levels are decreased in the distal stump and gradually increase to baseline levels as regenerating nerves arrive at distal segments and reestablish normal axon-Schwann cell contact (27). At axon-Schwann cell contacts, however, laminin accumulates (57). To assess the functional role of laminin in nerve regeneration, Agius and Cochard (1) used a tissue section culture in which embryonic chick sensory neurons were grown on a denervated peripheral nerve substrate. Anti-laminin-2 (merosin) antibodies drastically reduced the percentage of growing neurons and the total length of neurites on the denervated nerve sections. Similarly, application of anti-laminin antibodies impeded axonal regeneration in Schwann cell depleted nerve implants *in vivo* (117).

In injured nerves axonal regeneration leads to Schwann cell redifferentiation which occurs in a proximal-to-distal direction relative to the site of injury. When Schwann cells are recontacted by regrowing axons, myelin-gene repressing transcription factor Pax3 and c-jun are downregulated, while SCIP is transiently upregulated indicating a premyelinating Schwann cell stage (54, 123). Downregulation of SCIP is followed by reinduction of myelin specific genes (61) and persistent expression the transcription factor Krox-20 (123). Krox-20 is critically involved in the completion of myelination (109). During early stages of myelination Schwann cell mesaxon membranes containing MAG are converted to compact myelin lamellae. This is accompanied by removal of MAG from and insertion of MBP, PMP22 and P_0 into mesaxon membranes (110). Transgenic Krox-20 -/- mice are able to produce MAG, but lack MBP and P_0 suggesting a functional role of Krox-20 in the induction of late myelin components (109). Upon remyelination of regenerated axons, Schwann cells loose dedifferentiation markers L1, N-CAM, GFAP and p75 NGF-R characteristic for a nonmyelinating stage (67, 91). The regeneration-induced molecular Schwann cell program leads to rapid remyelination of nerve sprouts and exactly recapitulates myelination during nerve development with respect to the sequence and timing of gene-induction.

Upon appropriate length of axonal regrowth target organs are finally reached and muscle and skin are reinnervated. This occurs with an extraordinary high specificity even from injured mixed sensory-motor nerves. Brushart (17) showed that motor axons preferentially reinnervated motor pathways. Subsequent studies revealed that guidance molecules of the L2/HNK-1 family were involved in this process (69). The L2/HNK-1 carbohydrate epitope was originally described as a cell surface component of human natural killer cells and is common in a large family of recognition molecules (reviewed in 67). L2/HNK-1 is detectable in association with Schwann cells in ventral spinal roots and motor axon-related Schwann cells of muscle nerves, but not in dorsal roots and sensory cutaneous nerves (69). After nerve transection myelinating Schwann cells previously associated with motor neurons differ from those Schwann cells that had myelinated sensory axons by their ability to express L2 when contacted by motor axons. L2/HNK-1 expression during critical stages of reinnervation provides an advantage for motor axons regenerating into the appropriate muscle pathways over those regenerating into the inappropriate sensory pathways (69). In regenerating femoral nerve of rat, motor axons seem to explore possible pathways by sending out collateral branches into both appropriate and inappropriate nerve branches. Pathway specificity is subsequently gained by pruning off those collaterals which have grown into the inappropriate nerve branch (17). Recently, anatomical evidence for specificity was also provided during regeneration of sensory afferent projections to muscle (66). The accuracy of sensory afferent regeneration was highly correlated with the accuracy of motor regeneration suggesting that two distinct neuronal populations that project to muscle respond in parallel to specific guidance factors during the regeneration process. The accuracy of pathway finding of sensory fibres to skin and the signal molecules involved have not yet been addressed directly. Zelena and Zacharova (121), however, could show that pacinian corpuscles are reinnervated after sciatic nerve crush.

Successful regeneration seems to depend tightly on the appropriate timing of the cellular and molecular degeneration program. In C57Bl/Ola mice Wallerian degeneration following a nerve lesion is very slow due to an axonal defect (37, 64). Concomitantly, recruitment of macrophages is delayed and the dramatic increase in mRNA levels for both NGF and low affinity NGF recep-

tor (p75) as observed in wild type mice is lacking. These changes lead to impairment of sensory axon regeneration (11, 12). In contrast, motor nerves regenerate at normal velocity even through uncleared myelin debris (11, 12, 75). At present it is unclear whether the rate of nerve regeneration is altered in neuropathies. In a chronic inflammatory neuropathy model in rabbits, antibodies against the myelin component galactocerebroside had no influence on the speed and efficiency of axonal regeneration and remyelination (106).

There have been several attempts to increase the rate of regeneration pharmacologically. Gold and colleagues (39) showed that subcutaneous application of the immunosuppressant FK506 increases regeneration of sensory fibres in the rat by 16% after nerve crush. *In vitro* FK506 promotes neurite outgrowth in PC12 cells and sensory ganglia (101). FK506 is inactive by itself and requires binding to an FK506-binding protein-12 (FKBP-12), a member of the immunophilin family (38). Following sciatic nerve crush FKBP-12 mRNA strongly increases in lumbar motor neurons and dorsal root ganglia neurons (65). The FK506/FKBP-12 complex inhibits the activity of the calcium activated phosphatase, calcineurin, which itself activates GAP-43 and nitric oxide synthase. It turned out, however, that the neurotrophic effects of FK506 are independent of calcineurin inactivation and not related to its immunosuppressive properties (38, 101). Prosaposin and the above described cytokine LIF are further compounds that significantly increase the number of regenerating nerve fibres *in vivo* (56, 108). Prosaposin is the precursor of saposins which activate sphingolipid hydrolases. Functionally, prosaposin stimulates neuritogenesis in neuroblastoma cells (80). It is significantly upregulated after peripheral nerve injury (34). When added to collagen-filled nerve guides after nerve transection prosaposin dramatically increased the number of regenerating nerve fibres within the guide (56). The potential role of exogenous application of neurotrophins in facilitating nerve regeneration is reviewed in an accompanying chapter in this volume. Recent evidence suggests that neurotrophins may reverse axotomy-induced changes in adult motor and sensory neurons (77). The physiologically lower nerve conduction velocity in the proximal part of transected nerve fibres could be increased by local application of neurotrophins 3 and 4/5.

Limitations and Perspectives

During the past decade enormous progress has been made in the understanding of the cellular events and molecular changes during degeneration and regeneration of peripheral nerves. However, our knowledge of the regulatory mechanisms and signaling cascades underlying the complex molecular regeneration program is still very limited. This is not surprising. Systematic differential hybridization screening approaches using cDNA libraries of normal and regenerating rat sciatic nerve revealed that the majority of the cloned sequences encode novel genes or known genes that were not previously shown to be expressed in the nervous system (25, 34). Thus, the majority of the molecular players involved in nerve regeneration await identification. Moreover, with respect to neuropathies it is unclear whether peripheral nerve disorders can interfere with this regeneration program and impede regenerative responses. This is of particular interest in chronic disease stages such as diabetic, chronic inflammatory and hereditary neuropathies with axonal loss. Isolation and characterization of further regulatory genes that trigger and control the genetic program is probably the most critical step towards a better understanding of the physiology and pathophysiology of nerve regeneration.

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