Mechanisms and consequences of microglial responses to peripheral axotomy

Hakan Aldskogius

Department of Neuroscience, Uppsala University Biomedical Center, P.O. Box 593, 751 24 Uppsala, Sweden

TABLE OF CONTENTS

1. ABSTRACT

Microglia respond rapidly to injury of peripheral nerve axons (axotomy). This response is integrated into the responses of the injured neurons, *i.e.* processes for neuron survival, axon regeneration and restoration of target contact. The microglial response is also integrated in changes in presynaptic terminals on axotomized motor or autonomic neurons and in changes in the central terminals of peripherally axotomized sensory neurons. Microglia also has an established role in interacting with astrocytes to shape their response to peripheral axotomy. Axotomy models in mice have demonstrated a role for microglia in regulating the entry of lymphocytes into motor nuclei or sensory areas following peripheral axotomy. Whether this is a universal component of peripheral nerve injury remains to be determined. Under certain circumstances, microglia activated by axotomy are major contributors to CNS pathology, *e.g.* in models of neuropathic pain. However, the general roles played by microglia after peripheral nerve injury are still incompletely understood. Early proposals that the microglial reaction to peripheral nerve injury is preparatory for the eventuality of neuron degeneration may still have relevance.

2. INTRODUCTION

The aim of this review is to describe and discuss the response of microglia and its functional implications after peripheral axons are interrupted by crush or transection (axotomy) of peripheral nerves in adult mammals. Following these injuries the affected neurons undergo a marked shift in gene expression (reviewed in 1, 2). In simple terms, this shift brings the neurons from a "transmitting" to a "growing" mode, a shift which provides the neuron-intrinsic structural and molecular basis for regeneration of the injured axon and restoration of peripheral target contact. This process is associated with alterations in the synaptic network of the axotomized neurons, as well as with prominent changes in adjacent astrocytes and microglial cells, and under certain circumstances, an infiltration of hematogenous cells.

Based on experimental and clinical research during the recent two decades microglia is now considered to have a central and dual role in most neurodegenerative disorders, including traumatic brain injury, stroke, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Dual in the way that microglia may help

tissue repair through targeted phagocytosis and release of growth factors, whereas other aspects of microglia directly or indirectly may drive the neurodegenerative process (3). Compared to the disorders mentioned the microglial responses to peripheral nerve injury are unique in the sense that they occur at a considerable distance from the injury.

Historically, responses by microglia to peripheral nerve injury were first recognized in the vicinity of axotomized motor neurons. Later studies showed that that injury to peripheral sensory axons also led to a prompt microglial response in the central projection territories of peripherally axotomized sensory ganglion cells. Motor axon injuries in experimental animals have served and still serve as important models for exploring general mechanisms and functional implications of interactions between neurons and non-neuronal cells. Understanding the role of microglia is obviously relevant in the context of achieving efficient and optimal functional recovery following peripheral nerve injury. This objective has recently received particular attention in relation to sensory nerve injury, since there is strong evidence that microglia play a pivotal role in the emergence and maintenance of injury-induced neuropathic pain. These aspects have been discussed in a number of recent reviews (see *e.g.* ref 4-8) and will not be in the focus here.

In order to understand the implications of microglial responses following peripheral nerve injury the overall context of these responses will first be briefly described.

3. PERIPHERAL NERVE AXOTOMY

3.1. The neuron-glia network and the dynamic functions of brain and spinal cord

In the intact CNS, nerve cell bodies and dendrites are covered by synaptic terminals and intervening thin astroglial lamellae, a structural arrangement which serves to regulate local chemical homeostasis and secure optimal synaptic function. Activity in neuronal circuits is also modified by signals carried through local networks of astrocytes that communicate via gap junctions or through extracellular mediators (9). Microglia in the intact CNS are highly ramified and continuously extend and retract their processes (10, 11), sweeping them close to synapses in a manner which appears to be activity dependent (12) and then most likely also along astroglial cell surfaces. In view of these close interrelationships in the intact CNS, it should be anticipated that interruption of normal impulse propagation from motor neurons to muscle and sensory end organ to the CNS, respectively, will have a major impact on glial cells surrounding axotomized neurons.

3.2. Neuronal responses to peripheral axon injury – implications for glial activation

The intrinsic neuronal responses to peripheral axotomy are initiated and maintained through several independent mechanisms, which are likely to be fundamentally the same in motor and sensory neurons (2, 13, 14). The early changes in gene expression appear to be induced by the electrical discharge caused by the injury ("injury potential"). These are followed by i) influx at the injury site of extracellular mediators and their retrograde transport to the nerve cell body, ii) depletion of trophic factors produced in the target tissue, which are normally conveyed by retrograde transport to the nerve cell body, and iii) retrograde transport of post-translationally modified molecules from the end of the proximal axon stump.

Peripheral axotomy will have markedly different consequences on motor and sensory neurons, which relate to their distinct differences in morphology, location and function. Furthermore, motor and sensory neurons at the spinal and brainstem level have different developmental origin, and at both sites there are multiple morphologically and functionally different subtypes. Thus, there may be details in the neuronal response to axotomy among subpopulations of motor and sensory neurons that will have an impact on the associated microglial response.

3.3. Central responses to peripheral axotomy – general aspects

The microglial response to peripheral axotomy has an early phase which includes immediate changes in their motility, shortly followed by their proliferation, migration and activation. From a functional point of view, the activation phase is clearly the most important one, and the one which most strongly correlates with the severity of the injury: crushing the nerve results in a less intense and more time-limited microglial response compared to transection. In the activation phase, microglia will also be engaged in complex interactions with astrocytes, and, at least under certain circumstances, with infiltrating hematogenous cells that will influence the outcome in terms of neuronal survival and functional repair.

Our current information on neuronal and glial cell responses to peripheral axotomy, particularly as it comes to their molecular aspects and outcome, emanate almost exclusively from studies on rat and mouse. The basic morphological features from these studies appear to be reproducible in larger animal species, including humans (15), but important differences occur. E.g., observations in the cat indicate that motor nerve axotomy and synaptic stripping are not necessarily associated with microglial proliferation (16-19). Furthermore, extensive infiltration of T-cells following motor nerve axotomy is limited or absent in the rat, but a characteristic and functionally important feature in the mouse (20). In the absence of detailed comparative studies at the molecular level some caution is warranted in translating these findings to humans.

4. MOTOR NEURON AXOTOMY

4.1. Anatomical aspects on models of motor neuron axotomy

The predominating experimental models for studies on microglial response around motor neurons are the facial motor nucleus following injury to the facial nerve, and the lateral motor column at the lumbar spinal level following sciatic nerve or ventral root injury. To a lesser extent, the hypoglossal or the preganglionic parasympathetic dorsal motor nucleus of the vagus nerve

has been used. Facial nerve, hypoglossal nerve and ventral root injuries are pure motor axotomies, still different in the sense that the facial nerve is injured outside, the ventral root within the subarachnoid space, thereby influencing e.g. the local properties of the cerebrospinal fluid.

Sciatic nerve and vagus nerve injury affect not only the motor components of these nerves, but also sensory input to the motor neurons indirectly via interneurons in the spinal cord dorsal horn and nucleus of the solitary tract, respectively. Sciatic nerve injury also directly affects a small fraction of the synaptic input to motor neurons by injuring muscle spindle afferent axons with monosynaptic motor neuron connections. A distinct feature of the spinal motor neuron circuitry in contrast to the brainstem motor nuclei mentioned above is the presence of γ-motor neurons and the recurrent inhibition by Renshaw cells, which are driven by the activity of the α-motor neurons themselves.

Given these anatomical and functional differences, some caution is necessary in extrapolating information between different peripheral motor systems.

4.2. Cellular and molecular changes in axotomized motor neurons

In parallel with up-regulation of the expression of a set of growth-associated proteins, prominent molecular and structural modifications occur at the motor neuron interface with surrounding glia. Components of neurotransmitter receptors for e.g. glutamate and glycine are down-regulated (21, 22). Down-regulation of microtubule-associated protein (MAP)-2, a major structural protein of dendritic microtubules (23) is accompanied by extensive shrinkage of the dendritic tree (24, 25). Down-regulation of drebrin (26), a spine-associated protein, is likely to further impair normal dendritic functionality. In the other pole of the axotomized neuron, down-regulation of neurofilament proteins, the major structural proteins of myelinated axons is accompanied by extensive reduction of axon caliber, myelin sheath thickness and conduction velocity proximal to the injury (27). Thus, not only is the normal communication between motor neuron and target muscles interrupted, the conditions for local motor neuron network operations are also markedly altered.

These intrinsic events in the injured neurons are accompanied by the removal of a large number of synaptic terminals located on soma and dendrites, a process often referred to as "synaptic stripping" (28-30), an increased perineuronal occupation by fine lamellar processes from neighboring astrocytes, and proliferation, migration and molecular phenotypic transformation of microglia (31, 32).

4.3. Microglial responses to motor neuron axotomy

The mechanisms that initiate the microglial response after motor neuron axotomy are incompletely understood. Critical roles are probably played by the massive efflux of potassium ions from the injury discharge, closely followed or accompanied by the release of ATP from the microvasculature and perineuronal astrocytes. This notion is strengthened by previous findings

implicating potassium channels (33-36) and purine receptors (37) in various stages of microglial activation. The sensitivity of microglia to alterations in CNS homeostasis is evidenced by their response along waves of spreading depression, a phenomenon of neuronal depolarization, which can be induced in intact brain regions as a result of distant functional pathology (38-40). *In vivo* imaging studies have shown that cortical injury induces rapid and targeted extensions of microglial processes towards the lesion site (10, 11). Likewise, hypoxia prolongs the time periods of contact between sweeping microglial processes and synapses (12).

Following this initial response, microglia withdraw their processes, proliferate, and migrate towards the axotomized motor neuron cell bodies This activation is easily recognized by labeling for the complement receptor 3 (CD11b) (Figure 1). Concomitantly, microglia begin to express a range of immune system related molecules, including complement components (41, 42), trombospondin (43), interleukin (IL)-1beta, IL-6, tumor necrosis factor (TNF)-alpha, interferon-gamma, the co-stimulatory factor B7.2, as well as major histocompatibility complex glycoproteins (44-46). Activated microglia has the potential also to release and respond to a wide range of other cytokines and chemokines, depending on the state of activation (47, 48).

In rodents and mice microglial cells begin to proliferate within one-two days after peripheral nerve injury with a peak around four days and reaching baseline levels around seven to ten days after injury (49, 50). The signal(s) initiating this response is not yet precisely identified. Results from studies *in vitro* indicate that microglial proliferation can occur by autocrine factors (50). One of these factors is likely to be colony-stimulating factor (CSF)-1. CSF-1 can be produced by microglia and mice with a mutation in the gene for macrophage colony-stimulating factor (CSF)-1 (*op/op* mice) show a markedly reduced proliferation (52).

Microglia migrate towards axotomized motor neurons while still proliferating. An interesting feature of this migration is that although dendrites provide the overwhelming part of the motor neuron surface, the nerve cell body appears to be the main target for this process (cf. Figure 2). The precise structural interrelationship between microglia and axotomized motor neurons has been the subject of some controversy. Although microglial cells and their processes are in the immediate vicinity of these motor neurons, electron microscopic images often show the presence of fine astroglial processes intervening between microglia and the neuronal membrane (53, 54). Proliferating microglia occur also outside the motor neuron nucleus proper. Thus, proliferating microglia were observed along the intramedullary portion of the hypoglossal nerve, presumably in the vicinity of nodes of Ranvier (50).

The possibility for microglia to move into a close relationship with the neurons may depend on microglia mediated down-regulation of tenascin-R in the perineuronal
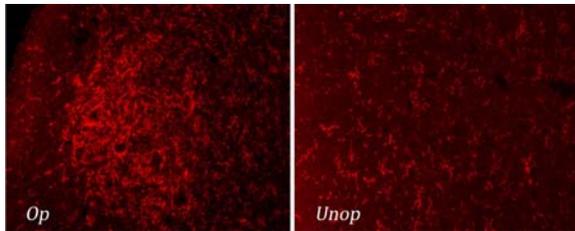
Figure 1. Microglia labeled with antibodies to CD11b in the ventral horn seven days following unilateral transection of the sciatic nerve in the rat. Op = operated side; Unop = unoperated side.
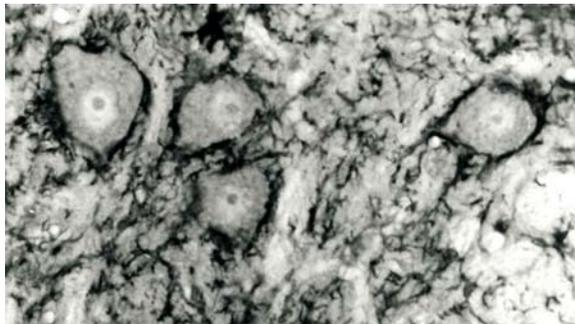
Figure 2. Microglia labeled with antibodies to CD11b strongly associate with motor neuron cell bodies in the spinal cord ventral horn seven days following transection of the sciatic nerve in the rat.

nets (55, 56). Deletion of cathepsin-S, a lysosomal/endosomal protease in cells of the mononuclear lineage, reduces migratory capacity of microglia towards axotomized facial motor neurons (56). Migration of microglia is associated with induction of Annexin III, which translocates to the actin cytoskeleton at the microglial cell membrane (57). These intracellular modifications of microglia cytoskeleton are accompanied by changes in the expression of integrin family cell adhesion molecules (58) and in the expression level of the non-integrin laminin receptor 37-LRP (59). Recent *in vitro* findings indicate that glutamate exerts a chemotaxic effect on microglia (60). Thus, migration of microglia after peripheral nerve injury is likely to be mediated by the combination of cell-cell interactions and diffusible molecules. Among the latter, synaptic signaling molecules may require more interest than has hitherto been the case.

The rate, extent and persistence of the early microglial responses are influenced by chemokines. Already six hours after facial or hypoglossal nerve injury in the mouse, affected motor neurons increase their synthesis of chemokine monocyte chemoattractant protein (MCP)-1 (CCL2) (61). Its corresponding receptor CCR2 is expressed by microglia and other cells of the monocyte lineage, and MCP-1 is critical for recruiting these cells to sites of injury. Mice with deletion of MCP-1 display a delayed microglial response in the lateral geniculate nucleus following visual cortex lesion (62). Surprisingly, mice with deletion of CCR2 showed similar microglia response as wildtype mice.

The chemokine fractalkine (CX3CL3) is produced and secreted by or shed from the surface of axotomized neurons, binds to its receptor CX3CR1 on microglia and induces cytokine production (63). However, fractalkine appears to be dispensable for axotomy-induced proliferation, migration and phenotypic alteration of microglia (64). The chemokine CCL21 is neuronally transported released from injured neurons and appears to have a critical role in several brain pathologies (65), but the possible implication of CCL21 in the context of peripheral axotomy is presently unknown.

4.4. Functional consequences of microglial responses to motor neuron axotomy

The microglial responses to motor neuron axotomy have since long been viewed in the context of motor neuron survival, motor axon regeneration, and displacement of presynaptic terminals. More recently, microglia has also been associated with influencing the properties of astrocytes and with promoting infiltration of T-cells into the axotomized motor nucleus.

4.4.1. Role of microglia for survival of axotomized motor neurons

It is now well established that activated microglia can take on a predominantly cytoprotective or cytotoxic role. In both situations, microglia are able to clear the tissue from degenerating elements, but in the first instance microglia produce and secrete growth supporting factors, whereas in the second instance agents that contribute to cell death are released. The complex mechanisms underlying these distinctive consequences of microglial activation are demonstrated by experiments on one hand showing that the presence of activated microglia is deleterious for neuron survival, on the other hand showing that elimination of microglial activation promotes neuron survival (3).

The extent of motor neuron degeneration after axotomy is highly dependent on the type of injury. Injuries with favorable conditions for rapid regeneration show no or minimal loss of motor neurons, whereas injuries in which contact between proximal and distal nerve stumps is prevented in general are associated with significant motor neuron degeneration (66, 67). The latter situation is typically associated with a greater and more prolonged microglial response. Studies on the influence of activated microglia in brain disorder have reported divergent results. E.g., selective ablation of proliferating microglia was found to exacerbate ischemic brain injury (68), whereas interfering with receptor-mediated microglial activation improved neuron survival (69, 70).

Earlier studies in the rat indicated that elimination of microglia from the axotomized rat hypoglossal nucleus did not reduce motor neuron survival (71). Thus, communication between the injured neuron and the environment in the distal stump of the injured peripheral nerve appears to be the main determinant for the extent of motor neuron degeneration. In this context, the more prominent microglial response after nerve transection-resection may just be secondary to the more extensive neurodegeneration. Such an interpretation would

be in line with previous observations of a correlation between the microglial response and neuron phagocytosis (72) and the extensive neuron loss and concomitant microglia response, including their transformation to cells positive for the phagocytosis marker ED1 following motor neuron axotomy in neonatal compared to adult animals (73). Moreover, modifying the microglial immune properties did not influence the extent of motor neuron degeneration in the axotomized facial motor nucleus (74)

However, results from recent studies in genetically manipulated mice have shown that interfering with microglial response after motor neuron axotomy may compromise motor neuron survival. Deletion of cathepsin-S limits microglial migration and spreading on axotomized facial motor neurons and reduces motor neuron survival (56). The chemokine receptor CCR5 is up-regulated in microglia, and its ligands regulated on activation normal T-cell-expressed and secreted (RANTES/CCL5), macrophage inflammatory protein (MIP)-1 in motor neurons after axotomy. CCR5-/- mice show increased motor neuron death after hypoglossal nerve injury (75). Microglia-mediated infiltration of Th2 cells into the facial nucleus protects loss of axotomized facial motor neuron in the mouse (76, 77). Taken together, these findings may reflect important species differences or a situation where functionally deficient microglial cells provide a more unfavorable environment for axotomized motor neurons than a complete absence of microglia. This interpretation may be in line with genetic analysis of rats and mice with diffent extent of motor neuron loss following ventral root avulsion. Data from these studies indicate that variations in genes associated with inflammatory mechanisms determine susceptibility to neurodegeneration (78, 79).

4.4.2. Role of microglia for axon regeneration

A role for activated microglia in axon regeneration could be expected since they are a potential source of growth factors such as brain-derived neurotrophic factor, insulin-like growth factor and transforming growth factor-beta, all of which are known to have beneficial effects on motor neurons *in vitro* and *in vivo*. Furthermore, activated macrophages which could potentially be derived from microglia are able to promote growth of adjacent intraspinal axons, albeit at the expense of simultaneously increased neurotoxicity (80). Complete elimination of microglia from the axotomized hypoglossal nucleus did not affect the rate or extent of motor axon regeneration and tongue muscle re-innervation (81). However, growth of injured axons from intrinsic CNS neurons into a peripheral nerve graft were found to be correlated with a perineuronal microglial response (82), indicating that microglia are able to promote axon regeneration, although this may not be required when conditions for this process are anyway favorable, such as after peripheral nerve crush.

4.4.3. Role of microglia for synapse removal

Synaptic stripping was first described on axotomized rat facial motor neurons and found to occur in the vicinity of activated microglia (28). Synaptic stripping has since been shown to be a general feature after motor neuron axotomy, in some instances affecting as many as 70% of the presynaptic terminals, preferentially those located on motor neuron dendrites (29, 30) and predominantly those of excitatory synapses. After muscle reinnervation, only a partial restoration of presynaptic terminals takes place. Thus axotmy-induced synapse remodeling occurs on a large scale and most likely has significant consequences for post-injury recovery of motor function. In fact, recent findings indicate that reducing the amount of synaptic stripping on motor neurons promotes functional recovery after sciatic nerve injury (83).

Based on the morphological coincidence between synapse removal and the perineuronal location of microglia, a causal relationship between these two events was suggested (28). Observations in later studies showed that astrocytes rather than microglia displayed the closest structural relationship with axotomized motor neurons during the process of synapse elimination (53, 54, 84). Using infrared gradient contrast live microscopy of slices containing axotomized facial motor neurons, microglial cells were found to move closely along motor neuron dendrites (85). These and observations *in vivo* showing that processes of microglia are sweeping close to synapses in the intact CNS and increasing this activity after injury (12) are compatible with an active role for microglia in synapse removal after motor neuron axotomy. However, synapse elimination was unaffected in the rat axotomized hypoglossal nucleus after complete elimination of microglia (54), indicating that microglia at least is not necessary for this process.

Microglia has a well established role in neural development as effectors of targeted phagocytosis of apoptotic cells and clearance of inappropriate synapses (86). Recently, the MHC and complement systems emerged as important regulators of developmental synaptic plasticity and synaptic stripping of axotomized motor neurons. Mice with impaired surface expression of MHCI showed a significant and selective increased removal of presynaptic terminals from axotomized spinal motor neurons (87, 88). Complement C3 -/- mice showed a significantly reduced removal of presynaptic terminals, a reduction which preferentially included terminals of excitatory synapses (83). These remarkable effects were accompanied by evidence for a decreased and an increased rate of functional recovery in functionally MHCI deficient and C3 -/- mice, respectively.

To what extent if at all, microglia is operative in these situations are unknown. MHCI is expressed on neurons and astrocytes, and although an up-regulation of C3 has been shown in microglia in the axotomized hypoglossal nucleus of the rat (41), C3 expression in the mouse spinal cord ventral horn appeared to be largely associated with astrocytes (79). Results from other studies have also emphasized a correlation between astroglial reactivity and the extent of presynaptic terminal removal (84, 89). Furthermore, the pronounced axotomy-induced changes in postsynaptic receptor expression and function (21, 22, 90, 91), the extensive shrinkage of the dendritic tree (29, 30), changes in the expression and conformation of synaptic adhesion molecules (92, 93), and in the

architecture of postsynaptic complexes (94) are also likely to have a direct influence on the distribution of motor neuron presynaptic terminals.

4.4.4. Role of microglia for astroglial reactivity

Astroglial responses are commonly assessed by the level of glial fibrillary acidic protein (GFAP) expression, the unique astroglial intermediate filament in the CNS. However, GFAP is part of the cytoskeletal core of astrocytes, whereas the actual functions of astrocytes are carried out in the distal lamellar processes, from which GFAP is absent. The levels of GFAP expression may therefore not be a sensitive measure of the functional activity of astrocytes.

In the absence of microglia, up-regulation of GFAP mRNA and protein are attenuated in the axotomized hypoglossal nucleus (95). Similarly, in mice with a genetic deletion of interleukin-6 (IL-6), presumably derived from microglia, up-regulation of GFAP does not occur in the axotomized facial nucleus (96). Furthermore, the functional properties of astrocytes are modified by a number of other inflammatory cytokines (97). At the same time, astrocytes have a central role in buffering extracellular potassium, are highly sensitive to ATP and adenosine, are responsible for the main part of glutamate and GABA uptake, in addition to being directly responsive to various neurotransmitters. The way these factors contribute to altered astroglial reactivity and function after motor neuron axotomy is still largely unknown.

4.4.5. Role of microglia for lymphocyte infiltration

Although there is a report of T-lymphocytes entering the axotomized rat facial nucleus (98), this phenomenon appears not to be typical for axotomized motor neurons in this species. However, facial nerve axotomy in the mouse produces extensive infiltration of lymphocytes (20). Initially, this invasion was considered to correlate with the extensive delayed motor neuron loss that occurs in the mouse compared to the rat following axotomy. However, results from recent studies indicate that T-cell infiltration may benefit motor neuron survival (99). These findings may lead to identification of survival promoting factors that can be helpful in other conditions of neuron degeneration and in other species. It is important to explore lymphocyte infiltration into the CNS after motor neuron axotomy in other mammals, including humans, to evaluate the generality of this phenomenon.

5. SENSORY NEURON AXOTOMY

5.1. Anatomical aspects on sensory neuron axotomy

Sensory neurons in dorsal root and cranial nerve ganglia are heterogeneous in terms of morphology, molecular phenotype, modality, impulse propagation properties and central projections. On stimulation they probably all release glutamate from their central terminals. In addition, many sensory neurons contain one or more peptides, e.g. substance P (SP) and calcitonin gene-related peptide (CGRP) which are also released following stimulation.

Here, we will discuss the role of microglia in the same injury models as for motor neuron axotomy, crush or transection with or without permitted peripheral regeneration. These experiments have been made on spinal nerves or the trigeminal nerve, with the sciatic nerve as by far the most popular one. Microglial proliferation and activation in the dorsal horn following sensory nerve injury were first demonstrated in the spinal cord dorsal horn following spinal nerve injury (100-102). Later studies have revealed that this is a general response following peripheral sensory axon injury (see. e.g. 103). Microglial responses in sensory areas of the dorsal horn and trigeminal nucleus have been the subject of intense research within the context of injury-induced neuropathic pain. Peripheral nerve injuries such as partial chronic constriction (CCI), spinal nerve ligation (SNL), spared nerve injury (SNI) and toxin-induced diabetic neuropathy results in sensory hypersensitivity and/or allodynia in experimental animals, which are interpreted as reflecting aspects of injury-induced neuropathic pain in humans. The central role of microglia in these conditions has been extensively reviewed (4-8)).

5.2. Cellular and molecular changes in axotomized sensory neurons

The expression pattern of regeneration-associated genes in the cell bodies of peripherally axotomized sensory neurons in dorsal root or cranial nerve ganglia is analogous to those occurring in axotomized motor neurons. These changes are primarily directed towards rebuilding the peripheral axon and restoring target contact. Associated with these changes are changes in the molecular phenotype of many sensory neurons. E.g., substance P and CGRP are down-regulated in the axotomized dorsal root ganglion cells, whereas e.g. galanin, pituitary adenylase cyclase activating polypeptide (PACAP) and neuropeptide Y (NPY) are up-regulated (2).

Furthermore, central terminals of peripherally axotomized sensory neurons display signs of degeneration or are lost by an unknown process (104-106), leading to a substantial deafferentation of postsynaptic neurons. Concomitantly, existing connections are modified and novel connections may appear (107). The loss of terminals from peripherally injured sensory neurons has some analogy to the synaptic stripping from axotomized motor neurons; in both cases leading to altered input to the neuron which is postsynaptic to the lost/retracted terminals. At the same time, evidence of regenerative processes occurs, as evidenced by an increase in the growth-associated protein GAP-43 in central primary sensory terminals, and the formation of novel synaptic connections (108, 109). Thus, the functional properties of the circuitries in the dorsal horn and cranial nerve sensory nuclei are dramatically altered following peripheral sensory axotomy.

5.3. Microglial responses to sensory neuron axotomy and their functional consequences

The microglial response to sensory neuron axotomy clearly resembles that around motor neurons in terms of their proliferation and activation as determined by
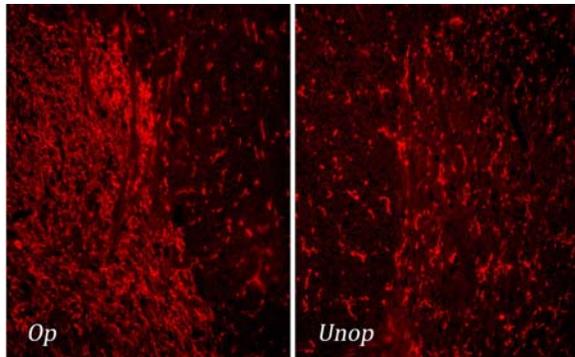
Figure 3. Microglia labeled with antibodies to CD11b in the dorsal horn seven days following unilateral transection of the sciatic nerve in the rat. Op = operated side; Unop = unoperated side.

their molecular expression pattern, including up-regulation of CD11b (Figure 3). As in motor neuron axotomy the immediate release of potassium, ATP and neurotransmitters are likely to underlie the initial activation of the local microglial population. In the subsequent enhancement and maintenance of the microglial response, chemokine CCL2 (MIP-1)/CCR2 and CXCL3 (fraktalkine)/CXCR3 signaling between neurons and microglia appear to be involved (4-8). However, it is unclear whether there is a distinct wave of migration after sensory neuron atotomy. Although, no detailed studies appear to exist on this issue, microglial cells appear to proliferate and become activated essentially at the same site. Electron microscopic images show these cells to be interspersed in the neuropil of the dorsal horn after spinal nerve injury and in the trigeminal sensory nuclei after trigeminal nerve injury without distinct relationship to nerve cell bodies (100, 110, 111).

Engulfment and presumed phagocytosis of degenerating axons or axon terminals were described after sensory neuron axotomy in the dorsal horn (99) and trigeminal nuclei (110, 111). However, the majority of microglia in sensory projection areas do not associate with neural degeneration. E.g., numerous markedly abnormal axons without any association with microglia are found in the gracile nucleus following sciatic nerve injury in the rat (112). Thus, apart from the reasonable assumption that an extended monitoring of the local CNS environment is called for, there is no defined physiological role for activated microglia after sensory neuron axotomy. The recent findings on the role of the MHC and complement systems for synapse elimination on axotomized motor neurons should prompt studies on whether related mechanisms are involved in synapse loss in sensory projection areas after sensory neuron axotomy.

Since the original reports of microglial proliferation and activation in the dorsal horn following sensory nerve injury microglial responses in sensory areas of the dorsal horn and trigeminal nucleus have been the subject of intense research within the context of injury-induced neuropathic pain. This research exploits somewhat different models than those commonly used for issues of

neuron-glial interactions in nerve regeneration or neuron degeneration. Partial chronic constriction (CCI), spinal nerve ligation (SNL), spared nerve injury (SNI) and various forms of inflammation or toxin-induced nerve injury results in sensory hypersensitivity and/or allodynia in experimental animals, which are interpreted as reflecting relevant aspects of injury-induced neuropathic pain in humans. The central role of microglia in these conditions has been extensively documented and the subject of numerous recent reviews (see e.g. 4-8).

Intuitively, one would like to view the microglial response to peripheral nerve injury, a presumably evolutionary well conserved response pattern, as a potentially beneficial response, or at least not harmful. An enigmatic issue in relation to apparently critical role of microglia in the development of neuropathic pain is why microglia takes on these properties in certain types of peripheral nerve injuries or after peripheral nerve injury in certain individuals. Genetic predisposition appears to play a major role, but its links to neuropathic pain behavior is still largely unclear (113).

6. SUMMARY AND PERSPECTIVES

Microglial proliferation, migration and phenotypic activation is a hallmark of peripheral nerve injury (axotomy), both in association with axotomized motor and autonomic neuron cell bodies and in association with central terminals of peripherally axotomized sensory neurons. Recent studies with live imaging and on transgenic mice have provided important and novel information on the mechanisms underlying microglial responses following injury. Studies using injury models of neuropathic pain have highlighted a central role for activated microglia in this condition. However, there are still substantial gaps in our understanding of the significance and implications of these responses for survival of axotomized neurons, and their ability to regenerate the axon and restore function. Studies using conditional gene regulation and *in vivo* RNA silencing should help to clarify these issues. Studies with an evolutionary perspective should be helpful in our understanding possible evolutionary modifications and their functional significance in the microglial response to peripheral nerve injury. The limited information available on this issue indicates that the prime role of microglia is to promote tissue repair and functional restoration (114). Finally, translational research to mammalian models closer to humans is necessary in order to verify the generality and validity of the data obtained in mouse and rat models.

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Send correspondence to: Hakan Aldskogius, Department of Neuroscience, Uppsala University Biomedical Center, 751 24 Uppsala, Sweden, Tel: 46-18-4714111, Fax: 46-18-511540, E-mail: Hakan.Aldskogius@neuro.uu.se