Microglia in Neuroregeneration

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ABSTRACT Microglia has the potential to produce and release a range of factors that directly and/or indirectly promote regeneration in the injured nervous system. The overwhelming evidence indicates, however, that this potential is generally not expressed in vivo. Activated microglia may enhance neuronal degeneration following axotomy, thereby counteracting functional recovery. Microglia does not seem to contribute significantly to axonal outgrowth after peripheral nerve injury, since this process proceeds uneventful even if perineuronal microglia is eliminated. The phagocytic phenotype of microglia is highly suppressed during Wallerian degeneration in the central nervous system. Therefore, microglia is incapable of rapid and efficient removal of myelin debris and its putative growth inhibitory components. In this way, microglia may contribute to regeneration failure in the central nervous system. Structural and temporal correlations are compatible with participation by perineuronal microglia in axotomy-induced shedding of presynaptic terminals, but direct evidence for such participation is lacking. Currently, the most promising case for a promoting effect on neural repair by activated microglia appears to be as a mediator of collateral sprouting, at least in certain brain areas. However, final proof for a critical role of microglia in these instances is still lacking. Results from in vitro studies demonstrate that microglia can develop a regeneration supportive phenotype. Altering the microglial involvement following neural injury from a typically passive or even counterproductive state and into a condition where these cells are actively supporting regeneration and plasticity is, therefore, an exciting challenge and probably a realistic goal. *Microsc. Res. Tech. 54:40–46, 2001.*

INTRODUCTION

Microglia respond to any kind of pathological event that directly or indirectly affects the central nervous system (Kreutzberg, 1996). Extensive descriptive information exists on the reaction of microglia to neural injury, but there is little understanding of its role in neural regeneration. Among the reasons behind our present lack of a conceptual framework on the significance of microglia in this process are the divergent and often contradictory reactions reported in the literature. These contradictions are reflected in epithets such as microglia being ”friend or foe?” or having a ”Janus face.” These statements reflect logical interpretations of the data obtained under the particular circumstances of the experiment, and illustrate the elusive nature of the role played by activated microglia in neural injury and disease. Here, some pertinent features of microglial responses to axon injury in vivo will be reviewed, and placed in the context of neural repair. This review will focus on experimental situations where microglia is known to be the sole, or at least the predominant, participant of the mononuclear phagocyte system in the non-neuronal injury response.

REVIEW OF RELEVANT DATA
Microglial Responses to Peripheral Nerve Injury

Injury to Motor Axons. Lesion of peripheral motor axons is a “classical” way of inducing a prompt microglial response in the central nervous system (reviewed in, e.g., Aldskogius and Kozlova, 1998; Raivich et al., 1999). Already within 24 hours, the first signs of activation can be observed: beginning hypertrophy, withdrawal of processes, proliferation, up-regulation of the complement 3 receptor (CR3/C11d), and thrombospondin. Within the next few days, these signs become more prominent, expression of the receptor for colony stimulating factor (CSF) 1 is up-regulated, and microglial cells migrate towards the injured motorneuron cell bodies, separated from the neuronal membranes only by thin astroglial lamellae (Reisert et al., 1984; Svensson and Aldskogius, 1993a). In about one week, all characteristics associated with peripheral nerve injury–induced microglial activation are present (Figs. 1, 2). The period of process withdrawal, proliferation, and migration is over. There is strong expression of CR3, induction of major histocompatibility antigen class I and II, complement components C1, C1q, C3, and C9, as well as immunoglobulin G. In the event of successful muscle reinnervation by the injured motor axons, the microglial response rapidly subsides. This gradually occurs even in the case of a permanent disconnection of the motorneurons from their target (Sumner, 1979; Aldskogius, unpublished observations).

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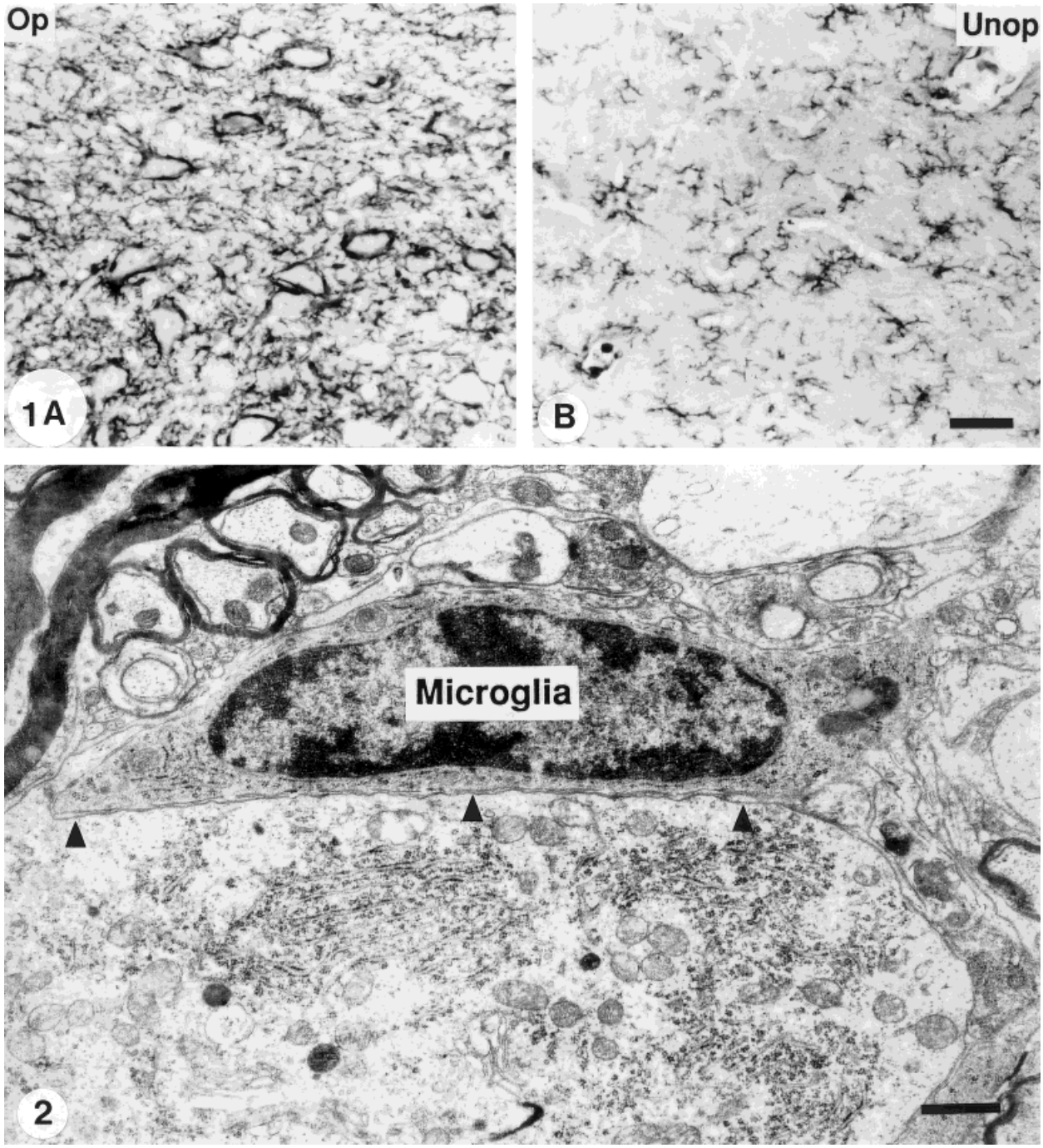

Fig. 1. The response of microglia 7 days after section of the motor axons in the hypoglossal nerve of the rat. Microglia are labeled with antibodies OX-42, which recognize a component of the complement 3 receptor. Note the large amounts of OX-42 immunoreactive profiles, many of which surround the motorneuron cell bodies, on the side of operation (op; A) compared to the unoperated side (unop; B). The primary immune complex was visualized with the ABC-method. Bar = 50 μ.

Fig. 2. Electron micrograph showing a microglial cell close to the cell body of a hypoglossal motorneuron 7 days after section of the hypoglossal nerve in the adult rat. Note the thin, electron-lucent profile intervening between the microglial and nerve cell membranes (arrowheads). This profile fulfills the criteria of an astroglial lamella. Bar = 1.5 μm. Reproduced from Svensson and Aldskogius (1993c) with permission of the publisher.

The microglial response following peripheral motor axon injury is not limited to the area of the motor nucleus. Proliferation of microglia is evident along the central nervous course of the affected motor axons (Svensson et al., 1994). These microglial cells appear to be located at the nodes of Ranvier, indicating that signals to microglial proliferation from the injured neurons are released at these non-myelinated axonal segments.

Injury to Sensory Axons. The response of microglia to peripheral sensory nerve injury shows striking similarities to the one following motor axon injury (reviewed in, e.g., Aldskogius et al., 1999). A limited proliferation of microglia occurs in the dorsal root transitional zone of lumbar dorsal roots as well as in the dorsal funiculus during a few days after sciatic nerve section (Liu et al., 2000). However, the predominant areas of microglial proliferation are in the dorsal horn and dorsal column nuclei, specifically in the projection territories of the central terminals of the peripherally injured sensory neurons (Persson et al., 1995). A response by microglia to peripheral nerve injury has also been demonstrated in the trigeminal nucleus following infraorbital nerve section (Eriksson et al., 1993; Melzer et al., 1997), and in the nuclei of the eighth nerve following labyrinthectomy (Campos-Torres et al., 1999). The molecular characteristics of microglia appear to be identical after peripheral motor or sensory axon injury (Liu et al., 1995; see Fig. 3B,C). The structural relationship between activated microglia in the dorsal horn and the neuronal elements there have not been clearly worked out, however. Large numbers of sensory axon terminals disappear from the most dorsal parts of the spinal cord, without any signs of being phagocytosed by microglia (Castro-Lopez et al., 1990). With time, microglia in more ventral areas contain debris of degenerating myelinating nerve fibers, but these situations are only occasionally observed (see Arvidsson, 1986; Bjelke et al., 1996).

Microglial Responses to Injury of Central Axons

Perineuronal Microglial Responses. Microglia respond with proliferation and increased OX-42 immunoreactivity around axotomized nerve cell bodies in the CNS (Barron et al., 1990; Tseng et al., 1996). This response appears to be markedly attenuated compared to the situation after peripheral nerve injury (Liu et al., 1998), with the exception of the visual system (Barron et al., 1986; reviewed in Moore and Thanos, 1996). The marked microglial response occurring in the retina after optic nerve injury is likely to reflect the rapid and extensive degeneration of retinal ganglion cells under these experimental conditions (Thanos, 1991).

Microglial Response to Wallerian Degeneration in the CNS. There is a distinct microglial response to disintegration of myelinated axons in the CNS. Microglia proliferate within a few days, and gradually begin to express markers for phagocytosis (Lawson et al., 1994; Liu et al., 1998). Other microglial expressions in these circumstances are induction of MHC classes I and II (Rao and Lund, 1989, 1993). However, considering the fact that a large amount of nerve fiber debris is accumulating in the degenerating white matter, the microglial reaction is strikingly subdued. Thus, the complement 3 receptor is only moderately upregulated (Fig. 3), and the reactive microglia fail to show other feature of full-blown phagocytes as well, such as induction of complement components (Liu et al., 1998; Fig. 4). Reactive microglia associated with degenerating central axons display characteristics of a generally down-regulated phenotype, which contrasts sharply with the macrophage response in degenerating peripheral nerve (Avellino et al., 1995; Reichert and Rotshenker, 1996; Zeev-Brann et al., 1998).

DISCUSSION

Microglia can support axon regeneration directly by contributing to one or more of the following aspects: (1) enhanced survival of injured neurons, (2) promotion of sprouting and/or elongation of injured axons, and (3) stimulation of collateral sprouting. Microglia could also stimulate these processes by influencing the production and/or release of survival- and/or growth-promoting factors from neighboring non-neuronal cells (see Svensson et al., 1993; Smith and Hale, 1997). Finally, microglia may help neural regeneration by removing or counteracting growth-inhibitory factors.

Microglia and Neuronal Survival Following Injury

Current evidence indicates that activated microglia is a potential hazard for neuronal survival. This is borne out, e.g., by experiments on axotomized retinal ganglion cells (Moore, and Thanos, 1996; Thanos et al., 1993). By administrating macrophage inhibitory factor (MIF), survival of injured retinal ganglion cells was enhanced. The opposite effect was obtained when the macrophage potentiation agent tuftsin was injected into the eye. This difference in neuronal survival was paralleled by the capacity of the injured optic nerve axons to enter and elongate within a peripheral nerve graft. Although similarly distinct data do not appear to exist from other systems, the neurotoxic potential of activated microglia is well documented in vitro. A substantial body of evidence also indicates that microglia contribute to the progression of neurodegenerative disease, such as Alzheimer's disease (reviewed in, e.g., Kalaria, 1999). Importantly as well, there appears to be no support from in vivo studies for the opposite situation, i.e., that activated microglia enhance survival of injured neurons.

Microglia and Peripheral Nerve Regeneration

The foregoing discussion leads to the inference that no distinct evidence exists for a survival-promoting effect of reactive perineuronal microglia after axon injury. Nevertheless, these cells could support—directly or indirectly—growth of peripherally injured axons. The circumstantial evidence that has prompted this notion is (1) the temporal correlation between microglial response and the process of axon regeneration, and (2) the observations that microglia has the potential to produce growth-promoting factors. How these factors would mediate improved axon regeneration is not clear, however. Only one study appears to have attempted to experimentally explore the role of microglia in motor axon regeneration. Elimination of proliferating microglia by intrathecal infusion of the mitotic inhibitor cytosine arabinoside C (ARA-C) did not retard the rate of motor axon outgrowth or muscle reinnervation following motor nerve crush (Svensson and Aldskogius, 1993b). This finding suggests that microglia does not play an essential role in peripheral nerve regeneration.

Microglia and Synaptic Plasticity
Microglial and Synaptic "Stripping" of Axotomized Motorneurons

Microglia is "homing" in on axotomized motorneuron cell bodies, concomitantly with the displacement of pre-

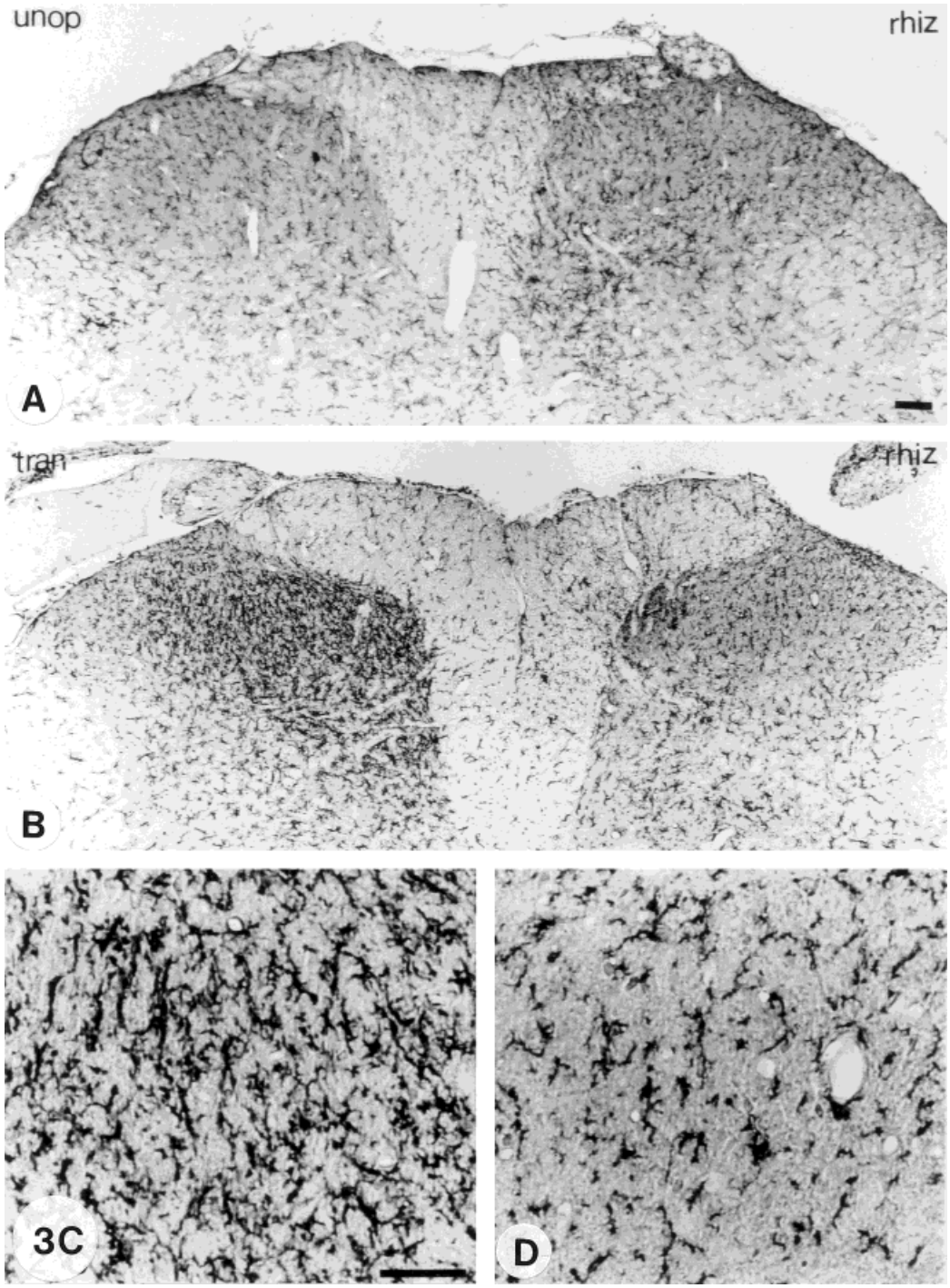

Fig. 3. Immunolabeling of microglia with antibody OX-42 in the spinal cord dorsal horn. **A:** Moderate increase in immunoreactivity 7 days after unilateral section of dorsal roots L4 and L5 (rhiz) compared to the contralateral, unoperated side (unop). **B:** Level of immunoreactivity after section of the sciatic nerve on one side (tran), and the dorsal roots L4 and L5 on the other side in the adult rat. **C,D:** Higher magnifications of the respective sides in B. Note that the OX-42 immunolabeling is stronger following peripheral nerve injury. The primary immune complex was visualized with the ABC-method. Bar = 100 μm. Reproduced from Liu et al. (1998) with permission of the publisher.

synaptic terminals from these motorneurons (see Figs. 1, 2). These structural correlations have prompted the suggestion that microglia actively participate in this synaptic displacement. An effect of this displacement is probably that the amount of synaptic impact on the axotomized neuron is reduced, which intuitively seems

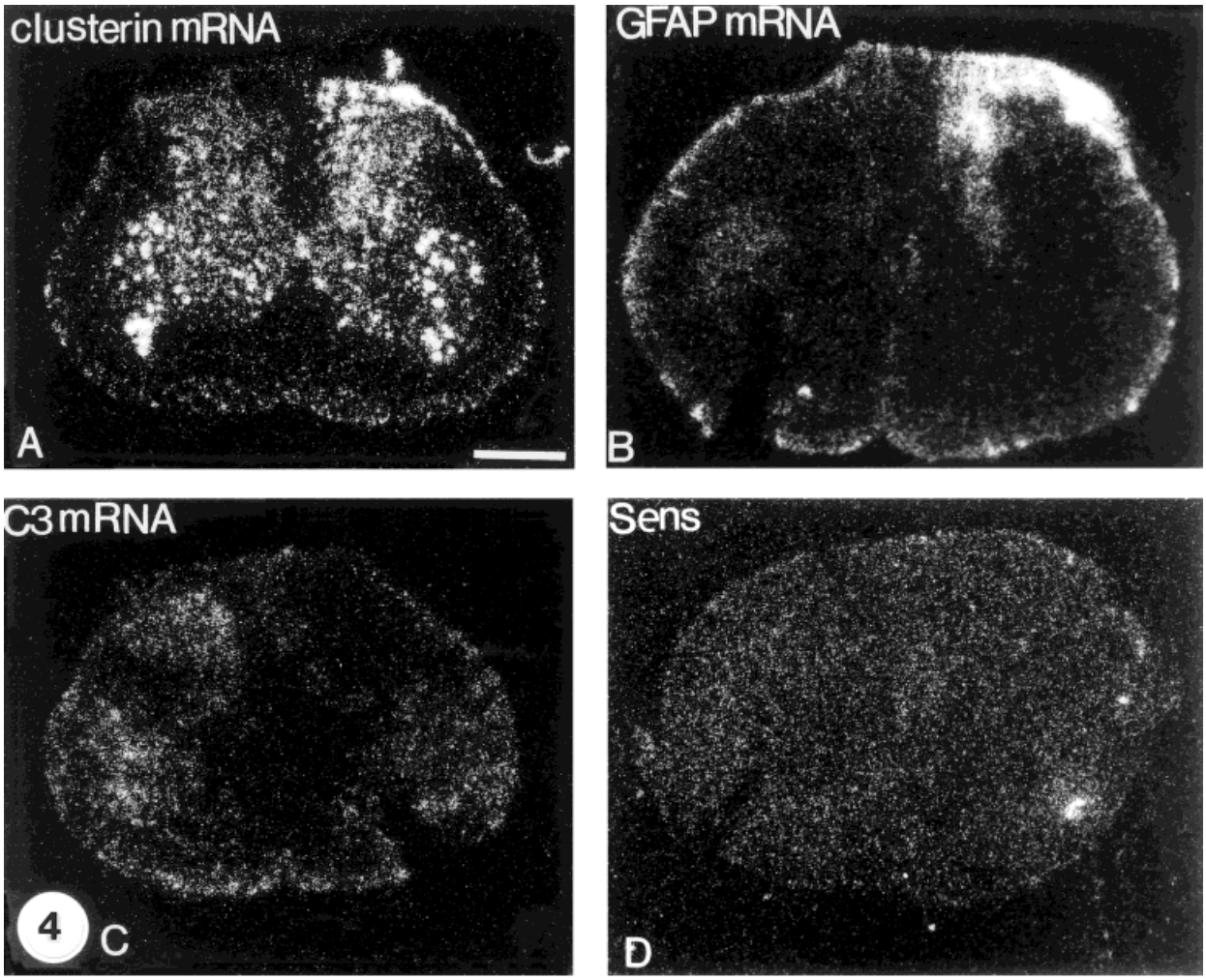

Fig. 4. In situ hybridization autoradiograms from the L4 spinal cord segment after sciatic nerve section (left sides), and section of dorsal roots L4 and L5 (right sides). The mRNA for two astrocytic markers, clusterin (**A**) and glial fibrillary acidic protein (GFAP; **B**) are strongly upregulated in the dorsal horn after dorsal root section. However, mRNA for complement component 3 (C3; **C**) is induced only after section of the sciatic nerve. **D:** Absence of labeling above background with a sense probe to mRNA for microtubule-associated protein (MAP) 2. A,B: 5 weeks after injury. C,D: 7 days after injury. Probes (48-mer) for rat clusterin, rat GFAP, and human C3 were labelled with (^{35}S)-dATP. Bar = 1 mm. Reproduced from Liu et al. (1998) with permission of the publisher.

to be favorable for recovery of the injured neuron. The mechanism(s) by which microglia would carry out this "stripping" have not been clarified, however. In fact, there are several observations that are difficult to reconcile with the interpretation that microglia are mediators of this process.

First, microglia appear to be separated from axotomized motorneuron cell bodies by thin astrocytic processes (Reisert et al., 1984; see Fig. 2). Astrocytes are in continuous membrane-to-membrane contact with nonsynaptic areas of neuronal membranes. It is, therefore, easy to envision that these cells play a predominant role in synaptic shedding, either as effectors or, secondarily, to rapidly cover denuded neuronal membrane segments. Second, the trigger to synaptic shedding is likely to arise in the nerve cell itself, e.g., as downregulation of anchoring molecules at the synaptic site. Third, when the axotomy-induced microglial response is blocked, loss of presynaptic terminals still occurs to the same extent as in axotomized, non-treated controls (Svensson and Aldskogius, 1993a). Fourth, extensive loss of synaptic terminals from axotomized motorneuron somata occurs also in the "natural" absence of a microglial response (Cova and Aldskogius, 1985; Svensson et al., 1991). Fifth, the predominant loss of synapses takes place at the dendrites (Brännström and Kellerth, 1998). There are no reports demonstrating a structural relationship between microglia and terminals on dendrites, which could support a role of microglia in this process.

In the absence of more than correlative evidence, it appears premature to assign a mechanistic role to microglia in the process of synaptic "stripping" from axotomized motorneurons.

Microglia and Deafferentation Induced Synaptic Plasticity. Degenerating axon terminal are largely removed through phagocytosis by microglia. This clearing process may expose denervated nerve cell mem-

brane, which may serve as a target for collateral sprouts. Microglia may also produce growth factors, which promote neurite formation (Bruce-Keller, 1999; Woods et al., 1998). Observations from several studies provide correlative data between microglial activation and terminal sprouting. Denervation of the dentate gyrus is accompanied by concomitant microglial activation and collateral sprouting by adjacent intact systems. This sprouting may be influenced by microglia-derived insulin-like growth factor-1 (Guthrie et al., 1995; Woods et al., 1998). Removal of the serotoninergic input to the striatum results in a substantial hyperinnervation of the ventral mesencephalon, a process that temporally and spatially coincides with reactive microglia (Revuelta et al., 1999). Striatial injury is accompanied by sprouting of dopaminergic fibers along the site of injury. This sprouting correlates with the expression of glial cell line-derived and brain-derived neurotrophic factor mRNA in microglia (Batchelor et al., 1999).

Microglia respond promptly to injury of peripheral sensory nerve fibers, with a particularly profound effect on the microglial response in the most dorsal parts of the spinal cord dorsal horn (Eriksson et al., 1993). This area normally receives terminals from non-myelinated sensory axons. After peripheral sensory nerve lesion, branches of myelinated fibers from more ventral areas of the spinal cord dorsal horn extend dorsally into the microglial-rich territory (reviewed in Aldskogius, 2000). Possibly, reactive microglia play a conducive part in this sprouting process. In this context, it is interesting to point out the intriguing observations that reactive microglia may be involved in hyperalgesic conditions by releasing mediators that influence the functional state of central nervous circuitry (Watkins et al., 1997).

Microglia and Regeneration Failure in the Central Nervous System

The microglial response to Wallerian degeneration presents several intriguing and contradictory features. On the one hand, microglia express a number of immune markers and develop into phagocytic cells that are able to ingest considerable amounts of debris from degenerating myelinated nerve fibers. On the other hand, the expression of, e.g., opsonizing complement components (Liu et al., 1998) are down-regulated in the same cells, thereby significantly decreasing the efficacy of the phagocytic process (see Dailey et al., 1998). As a result, products of disintegrating myelinated nerve fibers remain for long periods of time (Franson and Ronnevi, 1985; George and Griffin, 1994). This inefficient myelin removal by microglia presumably implies that myelin-associated axonal growth inhibitors (see Bandtlow and Schwab, 2000) will also remain for extended periods of time, in effect counteracting attempts by the injured axons to sprout and elongate.

Concluding Comments

Although circumstantial evidence is compatible with a role of activated microglia in neural plasticity, the overall impression is that microglia in the course of its natural responses are not involved in supporting neuronal survival, regeneration, or plasticity. Several pieces of information indicate, however, that microglia have the potential to be a constructive player in one or more of these processes. Microglial cells that have been introduced to the injured central nervous system support growth of injured axons (Prewitt et al., 1997; Rabchevsky and Streit, 1997). Third, injection into the spinal cord of agents, which stimulate microglia, such as lipopolysaccharide, enhances axonal growth (Lazar et al., 1999). By elucidating the mechanisms that underlie activation and de-activation of microglia, it should, therefore, be possible to transform the down-regulated microglial phenotype into one that plays an active role in restoring function to the injured central nervous system.

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