

THE MACROPHAGE RESPONSE TO CENTRAL AND  
PERIPHERAL NERVE INJURY

A Possible Role for Macrophages in Regeneration

BY V. H. PERRY\*, M. C. BROWN‡, AND S. GORDON§

*From the \*Department of Experimental Psychology, the †University Laboratory of Physiology,  
and the §Sir William Dunn School of Pathology, University of Oxford,  
Oxford, OX1 3UD United Kingdom*

Injuries to axons in both the central and peripheral nervous systems (CNS and PNS) of mammals result in axonal degeneration distal to the site of the lesion (Wallerian degeneration) and removal of their surrounding myelin sheaths. In the PNS, myelin is rapidly degraded over a few days or weeks, and the axons regenerate but in the CNS the myelin may persist for months (1, 2), and the axons do not regenerate. We have no explanation for this difference, and there is controversy as to which cells are responsible for the rapid removal of myelin in the PNS (3). Recent evidence suggests that macrophages ( $M\phi$ ) may be important in the removal of myelin from the degenerating peripheral nerve (4). Using the mAb F4/80 (5), specific for mouse  $M\phi$ , we have examined the contribution of  $M\phi$  to Wallerian degeneration in the CNS and PNS and considered the possible role they may play in subsequent repair.

Materials and Methods

We anesthetized adult mice (CBA/J, BALB/c) with chloral hydrate (0.2 ml per 10 g of 3.5% chloral hydrate); rats (Long-Evans) with chlornembutal (0.3 ml per 100 g of 4.2% chloral hydrate plus 1% sodium pentobarbital). Using jewellers forceps, we crushed one sciatic nerve in the upper thigh or one optic nerve a few millimeters behind the optic disk. Animals were killed after 0.5, 1, 3, 4, 5, 7, 10, 14, and 21 d. We removed the nerves unfixed or after perfusion fixation with periodate/lysine/paraformaldehyde (1.5% paraformaldehyde (6)). We rapid-froze the nerves and cut them on a cryostat at 10  $\mu$ m in the transverse or longitudinal plane. We saved sections at 0.25–1.0-mm intervals along the length of the nerves both distal and proximal to the crush site. Sections of mouse tissue were processed for the localization of  $M\phi$  using the mouse  $M\phi$ -specific mAb F/80 (4); rat tissue was stained for leukocytes using the mAbs OX1 and OX30 directed against the leukocyte common antigen (LCA) (7). Endogenous peroxidase was eliminated using the glucose oxidase method (8). We incubated the sections with mAbs for 1 h. Binding was revealed by the avidin/biotin complex immunoperoxidase method (9) using reagents supplied by Sera-Laboratories, West Sussex, United Kingdom. We used diaminobenzidine tetrahydrochloride as the chromogen. Some unfixed sections were reacted in diaminobenzidine with hydrogen peroxide to reveal cells with myeloperoxidase. Control sections showed that mAb staining did not result from nonspecific binding. Sections were lightly counterstained with cresyl violet or exposed to 1% osmium tetroxide to intensify the

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reaction product and reveal the myelin sheaths. Other sections were counterstained with oil red O to show lipid accumulation resulting from the myelin breakdown.

### Results

In the sciatic nerve of the normal mouse a small number of cells were F4/80<sup>+</sup>. These cells had small nuclei, many short branches, and were irregularly scattered throughout the nerve (Fig. 1, *A* and *B*). After a crush injury to the sciatic nerve myeloperoxidase-positive cells rapidly invaded the site of injury and distal segment (Fig. 1 *C*); these cells appeared to be polymorphonuclear cells. They had increased to an appreciable number within 12 h, further increased over the next few days, and then remained stable over the rest of the period examined. The number of F4/80<sup>+</sup> cells was not noticeably different from normal at 1 d after the injury, but by 3 d the density had increased. The recruited F4/80<sup>+</sup> cells were either monocytic or stellate, with processes extending between the axons (Fig. 1 *D*). The number of these cells was further increased at 5 d after injury, and they were restricted to the degenerating portion of the distal segment after a partial crush (Fig. 1, *E-H*). At  $\geq 7$  d it was more difficult to localize F4/80<sup>+</sup> cells in transverse sections, and the staining appeared to be diffusely distributed on processes with no well-defined form. However, in longitudinal sections we could see that this resulted from a dramatic change in their morphology; they appeared bloated and swollen, and in material stained with oil red O the cytoplasm was filled with liquid droplets, presumably as a result of phagocytosed myelin debris. These swollen cells persisted for the remainder of the time examined. In the portion of the nerve containing degenerating axons we found that the background staining was greater than in normal nerves, so we also examined nerves from rats, where this was less of a problem.

The rat sciatic nerve was very similar, although in these animals the mAbs used did not discriminate between M $\phi$  and other leukocytes, and there were thus more LCA<sup>+</sup> cells in the rat than F4/80<sup>+</sup> cells in the mouse at corresponding stages. In longitudinal sections of nerve at 3 and 5 d after injury we observed a number of mitotic figures, and estimated that ~40% of these mitotic figures were LCA<sup>+</sup>, the remainder presumably being Schwann cells. As in the mouse, the increased density of LCA<sup>+</sup> cells was restricted to the portion of the nerve containing degenerating myelin and, in sections stained with oil red O, neutral lipid was visible within the cytoplasm of LCA<sup>+</sup> cells.

To compare the effects of injury in peripheral and central nerves we have studied the optic nerve. In the normal mouse optic nerve, F4/80<sup>+</sup> cells were typical microglia, with a small nucleus and long thin crenelated processes that branched (10) (Fig. 2*A*). The microglia were more numerous than the F4/80<sup>+</sup> cells in normal peripheral nerve. The resident LCA<sup>+</sup> cells in the normal rat optic nerve were only weakly stained by these mAbs but there was sufficient detail to see that these were microglia.

A crush injury to the mouse optic nerve produced very different results from those seen in sciatic nerves. First, myeloperoxidase-positive cells were almost entirely restricted to the site of injury and did not invade the distal segment of the nerve in appreciable numbers. Second, for the first 5 d after injury the number of F4/80<sup>+</sup> cells in the optic nerve was unchanged, except at the site of

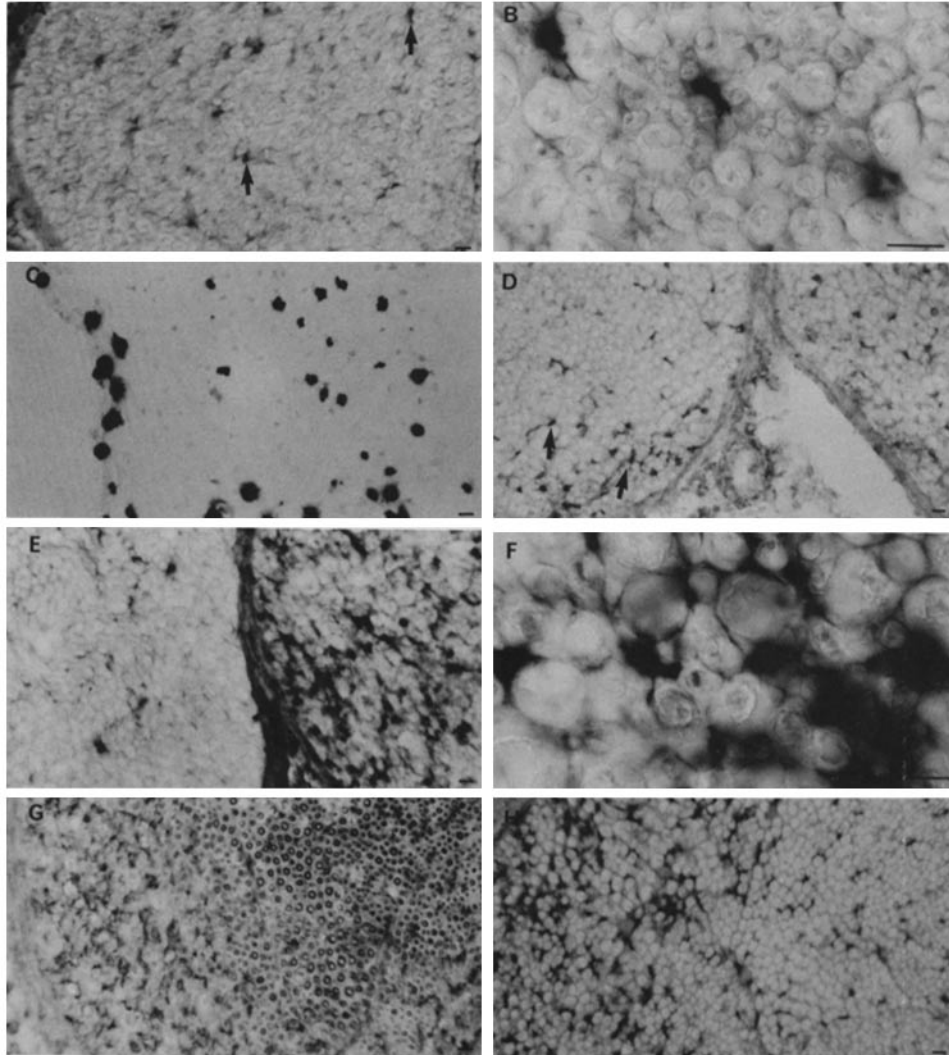


FIGURE 1. Photomicrographs to illustrate the distribution of  $M\phi$  in the distal segment of the mouse sciatic nerve, at least 1.5 mm distal to the crush. The pictures were taken with a dark blue filter and the diaminobenzidine reaction product appears black. (A and B)  $F4/80^+$   $M\phi$  (arrows) in the normal nerve. (C) Myeloperoxidase-positive cells 3 d after injury. (D)  $F4/80^+$  cells 3 d after injury. (E and F)  $F4/80^+$  cells 4 d after partial injury; note the cells are restricted to a portion of the nerve. (G)  $F4/80^+$  cells 5 d after injury. The section has been exposed to osmium tetroxide to reveal the myelin sheaths. Note that the  $F4/80^+$  cells are restricted to the region devoid of myelin sheaths. (H)  $F4/80^+$  cells 5 d after a partial crush of the sciatic nerve. Again the  $M\phi$  are localized to the degenerating region. Scale bars are 10  $\mu\text{m}$ .

injury, where there were many  $F4/80^+$  cells, often large and rounded. Distal to the crush the  $F4/80^+$  cells were microglial in form and although there was no obvious change in their numbers or distribution the cells in this region were somewhat more intensely stained than in the normal nerve (Fig. 2B). By 10 d the number of  $F4/80^+$  cells had increased slightly. Most of these were microglia but there were also some more rounded cells. In the rat the effect of optic nerve

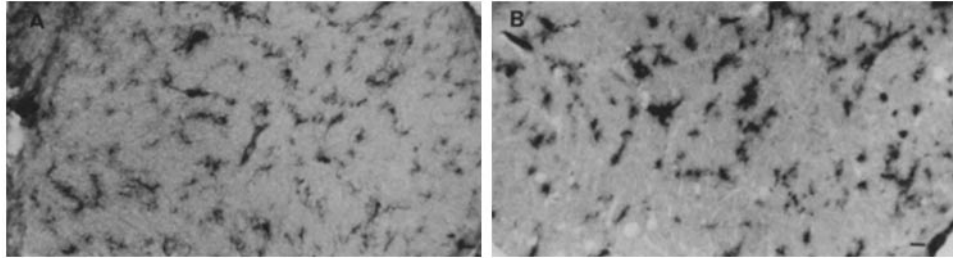


FIGURE 2. (A) F4/80<sup>+</sup> microglia in the normal optic nerve. (B) F4/80<sup>+</sup> cells in the optic nerve 5 d after crush, the section is 2 mm distal to the crush. Scale bars are 10  $\mu$ m.

damage was similar except that the increase in intensity of the LCA staining was more marked than seen with F4/80 mAb in the mouse because the resident rat microglia in control nerves were only weakly stained. After 5 d, as in the mouse, rat nerve showed only a small increase in the number of monocytic cells. It was not possible in this material to be certain whether they were transformed resident cells or had been recruited. Even 3 wk after the injury the neutral lipid seen with the oil red O was largely confined to the site of injury.

### Discussion

These results draw attention to a number of interesting differences in the response of recruited inflammatory cells, especially M $\phi$ , after injury in the PNS and CNS. We have established, using a definitive marker, that M $\phi$  form an important part of the cellular response to peripheral nerve injury. They are recruited in significant numbers in the first 3–5 d, restricted to the region containing degenerating axons, and they phagocytose myelin, thus filling with neutral lipid. When pieces of peripheral nerve are placed in chambers within the peritoneal cavity (4), or when teased fibers are placed in culture (11), the Schwann cells extrude their myelin through the basement membrane surrounding the fiber and thus allow M $\phi$  to phagocytose myelin. Our results support the idea that M $\phi$  phagocytose myelin *in vivo*. Extracellular myelin degradation is also likely, because M $\phi$  recruited outside the nervous system are known to secrete potent myelinolytic neutral protease activities, including plasminogen activator (12).

The fact that Schwann cells do not phagocytose myelin and are unable to proliferate when pieces of peripheral nerve are placed in the peritoneal cavity in chambers that exclude M $\phi$  suggests that M $\phi$  may also be required for Schwann cell proliferation (4). M $\phi$  are known to secrete a variety of proteins that initiate or enhance proliferation in nonneuronal cells (13, 14). The large increase that we have observed in the M $\phi$  population of the degenerating nerve occurs just before and during the period of Schwann cell proliferation (15). Furthermore, M $\phi$  and other mononuclear cells may secrete factors to promote neuronal elongation, and further studies are required to examine this possibility.

The slow rate of Wallerian degeneration in the CNS has been well documented (1, 2), but there is little evidence to show why this might be the case. Our results suggest that limited recruitment of M $\phi$  after injury might be important because it is these cells that play a major role in myelin removal in the PNS. It is not clear why there should be such limited recruitment in the optic nerve. An obvious

possibility is the existence of the blood-brain barrier in the CNS. However this is not necessarily the only factor, because after optic and sciatic nerve damage the blood vessels are rendered permeable to a comparable extent and mainly at the site of injury (16, 17). We do not know whether, after injury, the distal portion of the optic nerve where the blood-brain barrier is intact fails to generate an appropriate chemotactic signal, the signal does not cross the blood-brain barrier, or whether the leukocytes fail to emigrate.

### Summary

Using mAbs and immunocytochemistry we have examined the response of macrophages ( $M\phi$ ) after crush injury to the sciatic or optic nerve in the mouse and rat. We have established that large numbers of  $M\phi$  enter peripheral nerves containing degenerating axons; the  $M\phi$  are localized to the portion containing damaged axons, and they phagocytose myelin. The period of recruitment of the  $M\phi$  in the peripheral nerve is before and during the period of maximal proliferation of the Schwann cells. In contrast, the degenerating optic nerve attracts few  $M\phi$ , and the removal of myelin is much slower. These results show the clearly different responses of  $M\phi$  to damage in the central and peripheral nervous systems, and suggest that  $M\phi$  may be an important component of subsequent repair as well as myelin degradation.

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