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Histochemical evidence for microglia-like macrophages in the rat trigeminal ganglion

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ABSTRACT

Of the 4 major cell types in CNS parenchyma, microglia appear to serve the unique functional role of tissue macrophages. The distribution of equivalent cells in the PNS is unclear. Recently, the B_4 isolectin of *Griffonia simplicifolia* was shown to bind selectively to microglia as well as to other macrophages under specific conditions. In the present study, this lectin was used to assess the existence of macrophages in the rat trigeminal ganglion. Vibratome sections of fixed ganglia were incubated with horseradish peroxidase (HRP)-conjugated isolectin, an HRP reaction subsequently performed, and sections processed for histology and viewed by light microscopy. Staining activity was found to be localised to a population of cells throughout the ganglion. These cells possessed small oval somata and several thin crenated processes, an appearance typical of ramified microglia. Stained cells also exhibited a regular, evenly spaced tissue distribution similar to CNS microglia. Finally, similar cells were also labelled by thiamine pyrophosphatase histochemistry, a cellular marker for CNS microglia/macrophages. It was concluded that there are microglia-like macrophages in the trigeminal ganglion and that these cells may function in immune reactions.

INTRODUCTION

As a unique component of neural tissue, microglial cells have been supported by recent studies as intrinsic macrophages and thus may function in immune reactions in this tissue. This is obviously significant since the nature of immune responses in such tissue has long been unclear. Microglia in normal adult CNS are characterised by a distinctive morphological appearance, including a small oval soma (5–10 μm) with scanty cytoplasm as the nucleus fills most of this structure, and long thin processes which branch and often exhibit a spiny or prickly texture. Cells with this morphology have been termed ramified microglia and clearly this appearance is markedly different from macrophages in other tissues. Consistent with the concept of 'immune privilege' as well as a unique nature of immune responses in neural tissue, ramified microglia have been proposed to be downregulated or inactive macrophages (Jordan & Thomas, 1988). It is suggested that these cells upregulate or convert into active macrophages on tissue injury or infection and that this transformation is accompanied by corresponding alterations in cellular morphology (Thomas, 1992).

In possibly the best studied example of microglial function as macrophages, involving the facial nucleus of the CNS, it has been demonstrated that these cells can be activated by neuronal injury (Graeber et al. 1988*b*). In this system, conversion of the ramified microglia into active macrophages has been shown to occur in 2 steps or stages (Streit et al. 1988). In response to axotomy or regenerative injury, microglia upregulate and express type 3 complement receptors (CR3) (Graeber et al. 1988*a*) and major histocompatibility complex (MHC) antigens (Streit et al. 1989*a*, *b*), but apparently still lack significant phagocytic activity. This partially active cell form is considered an intermediate between ramified cells and completely active macrophages; the partially active form has been termed 'activated microglia'. Activated microglia exhibit a morphology in which the cell body is enlarged in comparison to ramified cells and cellular processes are shorter and stouter. Lethal or degenerative neuronal injury, on the other hand, appears to induce complete conversion to active macrophages.

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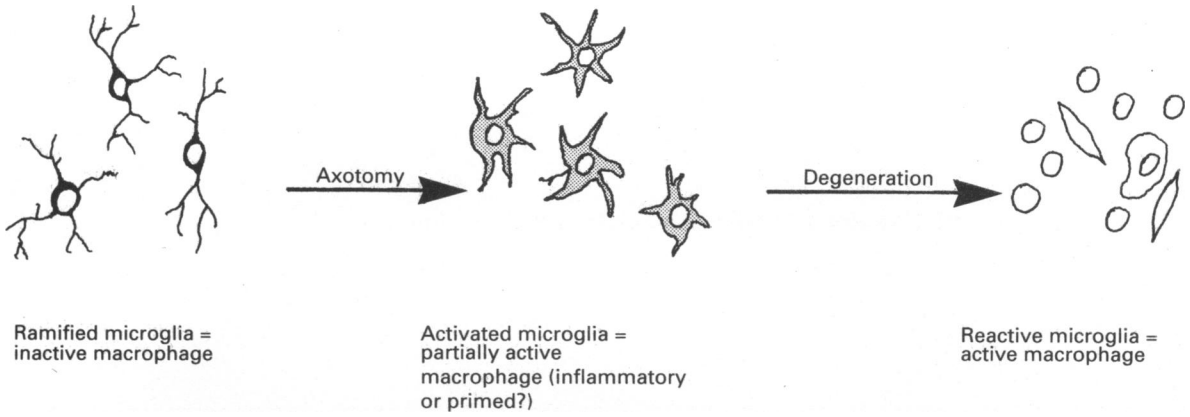

Fig. 1. Schematic diagram depicting the 2-step conversion of microglia to macrophages in the facial nucleus in response to neuronal injury. The type of injury is indicated above each arrow. Ramified microglial cells are the normal constituent of healthy adult brain tissue. Under conditions of axotomy, regenerative neuronal injury, it appears that these cells upregulate and express partial macrophage properties (activated microglia). Further, it is suggested that microglia transform into fully active macrophages (reactive microglia) when neurons undergo death and degeneration. Thus ramified microglia are thought to be inactive cells capable of conversion to active macrophages.

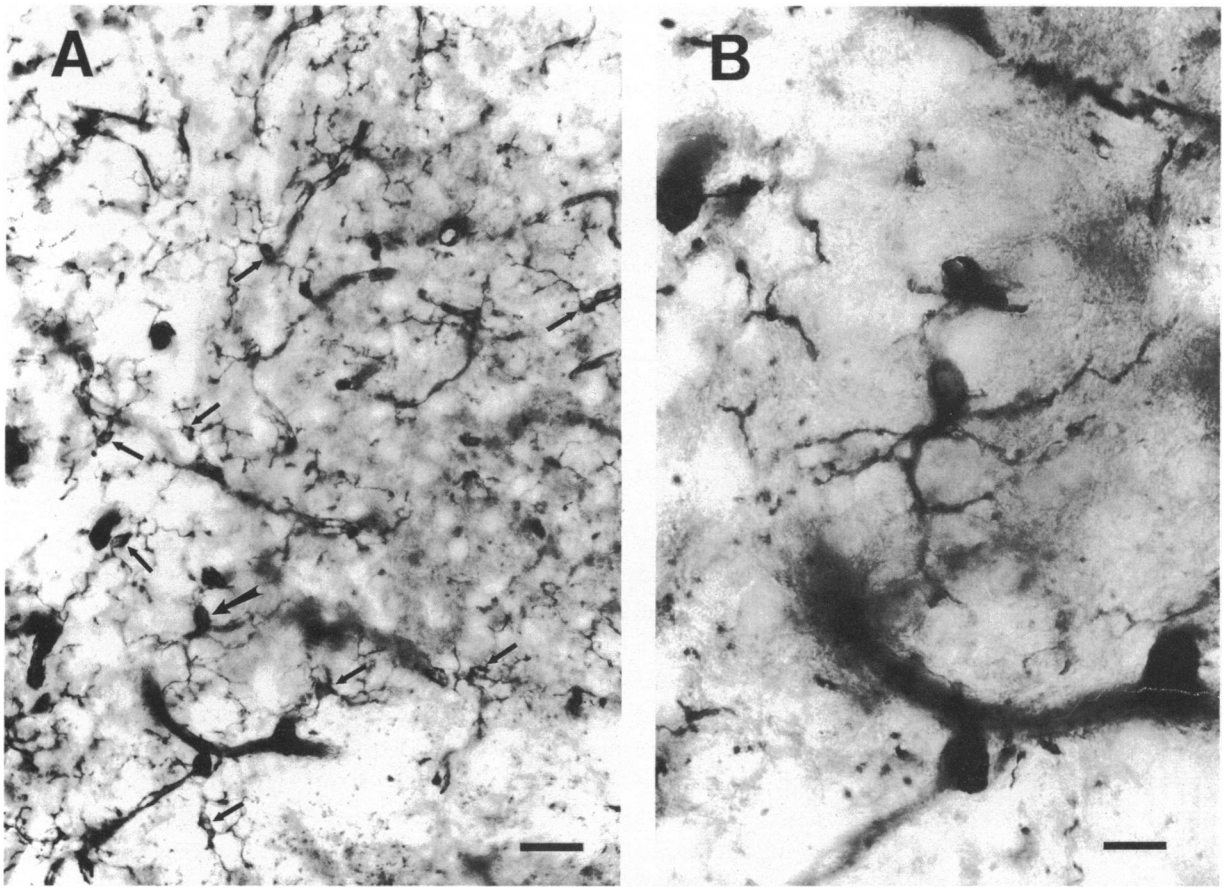

Fig. 2. Lectin staining of ramified microglia in CNS. (*A*) Low-magnification view from a vibratome section of normal rat cerebral cortical tissue stained with HRP-B_4. The staining reveals a dense and regular population of typical ramified microglia distributed throughout the tissue; several microglia with cell bodies in the plane of focus are present (arrows). Labelling of some vascular elements is also seen. (*B*) Individual stained ramified cell from the field in *A* (larger arrow) depicted at higher magnification. Bars: *A*, 20 μm; *B*, 13 μm.

These cells, termed 'reactive microglia', show a further increase in CR3 and MHC antigens (Streit et al. 1989*b*; Graeber et al. 1990) and possess full phagocytic capability (Streit & Kreutzberg, 1988). The reactive microglial cells display a round to rod-shaped morphology with little or no remnant of processes. This 2-step conversion sequence for ramified microglia to macrophages derived from studies of the facial nucleus is represented diagrammatically in Figure 1.

Activation of microglia as macrophages thus appears to occur in a stepwise fashion similar to that

described for activation in other macrophages (Adams & Hamilton, 1984). While microglia are known to be somewhat evenly distributed throughout the brain and spinal cord, their presence or distribution, or that of equivalent cells, in ganglia and neural structures outside the CNS has not been extensively investigated. As the trigeminal ganglion serves as a major site for the relay and processing of sensory and motor activity for the oral cavity, the presence of microglia/macrophages in this peripheral structure was investigated in the rat using a macrophage-specific staining technique. Some of the findings have been presented in a preliminary form (Sonceau et al. 1992).

MATERIALS AND METHODS

Young adult Sprague–Dawley rats (Harlan Farms, Indianapolis, IN, USA) of either sex were anaesthetised and perfused transcardially with fixative. The fixative solution contained 4% paraformaldehyde in 0.1 M phosphate-buffered saline, pH 7.2. A total of 10 animals were used. Both trigeminal ganglia were dissected from perfused animals and immersed overnight (12–24 h) in the same fixative. Fixed ganglia were then sectioned at 40–60 μm with a vibratome. The resulting sections were incubated with horseradish peroxidase-conjugated B_4 isolectin (HRP-B_4) from *Griffonia simplicifolia* according to the procedure described by Streit (1990). After incubation, a routine HRP reaction was performed using diaminobenzidine as substrate. Sections were affixed to gelatinised glass slides, dehydrated, cleared and mounted in Permount. Stained sections were viewed and photographed on a Zeiss Standard 16 microscope. In some cases, sections were counterstained with cresyl violet before dehydration.

The B_4 isolectin selectively labels macrophages under these conditions and has been shown by several laboratories to reveal ramified microglia in CNS (Streit & Kreutzberg, 1987; Streit, 1990; Boya et al. 1991*a*; Thomas, 1992); the HRP-B_4 used in the present study was obtained from Sigma Chemical Co. (St Louis, MO, USA). It should be noted that the recently reported staining of neurons in trigeminal ganglion (Ambalavanar & Morris, 1992) with other labelled forms of the same lectin involved different staining techniques. Histochemical staining for thiamine pyrophosphatase (TPP), which is also selective for CNS microglial cells, was employed to further confirm potential macrophage/microglia identification; this staining was performed essentially as previously described by this laboratory (Glenn et al. 1989, 1992).

RESULTS

The lectin staining of microglia was initially assessed in cerebral cortical tissue from the same animal and HRP reaction product selectively marked a subpopulation of cells. These cells corresponded to microglia as they displayed the regular or even distribution, size and morphology of ramified cells (Fig. 2). While there was also some staining of vascular structures, ramified microglia were the only intrinsic cells of the tissue exhibiting significant labelling and could readily be distinguished from stained vessels. When the staining procedure was applied to the trigeminal ganglion, a specific labelling pattern was observed. A subpopulation of cells regularly distributed within the ganglion was selectively revealed (Fig. 3*A–D*). These stained cells clearly possessed a complex morphology and on cresyl violet counterstaining it was possible to estimate that they corresponded to ~ 5–15% of the total cellular population. At higher magnification, the stained cells were seen to possess small elongated oval somata and numerous processes (Fig. 3*E, F*). The soma was usually 5–10 μm in diameter and cellular processes were long (frequently measuring 20–40 μm), thin (2–3 μm or less), usually nontapered and had a crenated appearance. While these general features were common to all cells, variations in these aspects gave each individual cell a relatively different appearance.

To assess further the overall morphology of stained trigeminal cells, camera lucida drawings of individual cells were undertaken. Such drawings emphasised a rich 3-dimensional structure with cellular processes radiating in all directions (Fig. 4). When ganglia sections were stained by the TPP histochemical technique, a population of cells similar to those stained by the lectin was detected (Fig. 5). The TPP reaction product was surface membrane-associated and noticeably more punctuate than lectin staining. This resulted in a less clear picture of cellular morphology and necessitated efforts to enhance the amount of reaction product; however, as the yield of enzyme product increased, there was a concomitant loss of localisation which compromised resolution. Thus the staining reaction involved balancing intensity against localisation and different cells in the same field reflected variations in this balance. Nevertheless, it was possible to discern the morphology of most TPP-

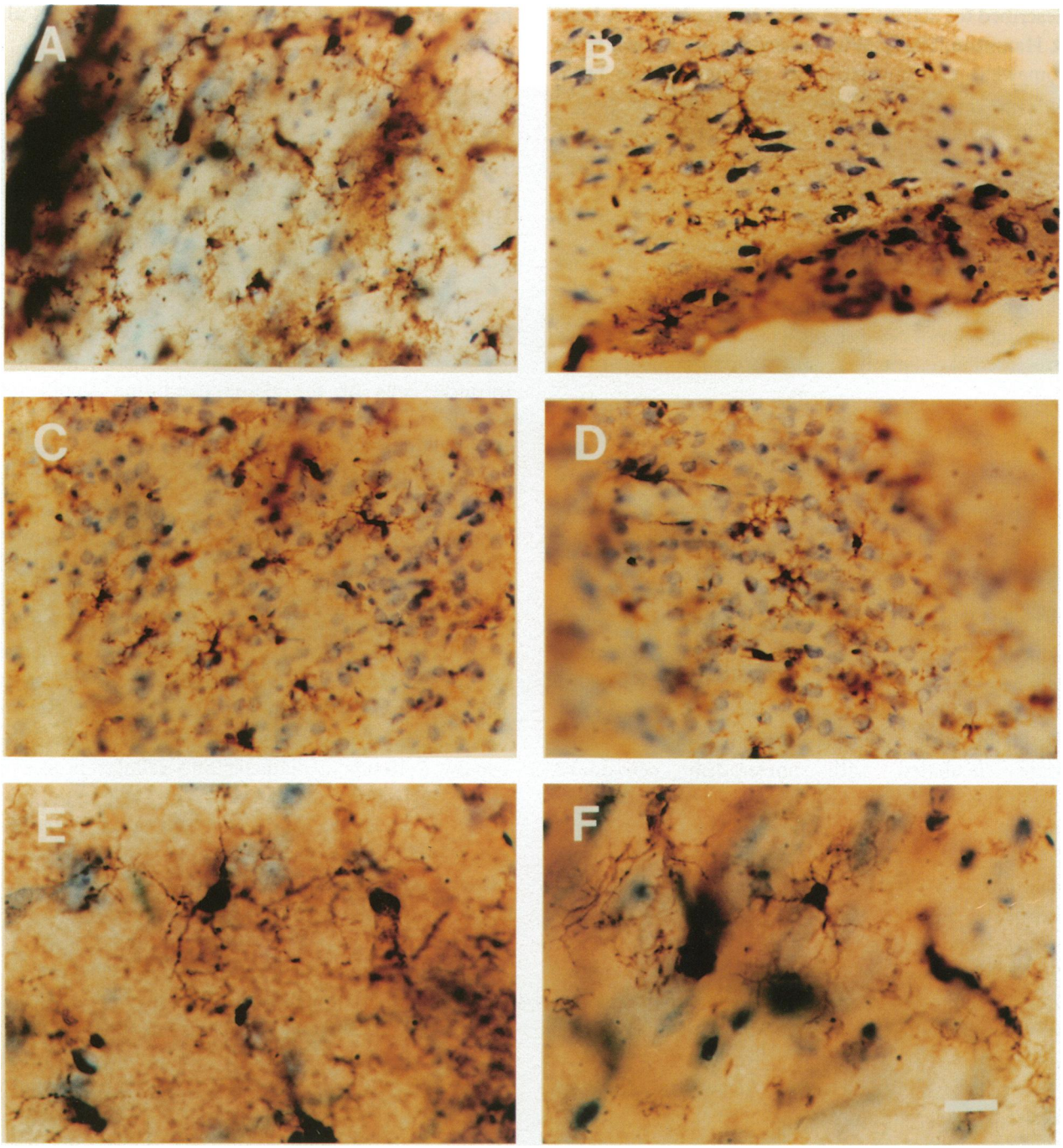

Fig. 3. Lectin staining of cells in trigeminal ganglion. The dark brown HRP reaction product denotes the sites of B_4 isolectin binding; somata of other ganglion cells are visualised through the purple colour from the counterstaining. (*A–D*) Four low-magnification fields from counterstained sections containing labelled cells. Note the overall shape and distribution for cells exhibiting labelling. (*E*, *F*) At higher magnification, labelled cells are seen to possess the distinctive morphology of microglia. Bar: *A–D*, 33 μm; *E*, *F*, 13 μm.

stained cells; soma size and shape, tissue distribution, and the features of cellular processes, were consistent with the lectin-stained cells.

DISCUSSION

The purpose of the present study was to assess the presence of microglia/macrophages specifically in the orally-relevant trigeminal ganglion. These cells are distributed throughout the CNS (brain, spinal cord and retina) (Perry et al. 1985; Perry & Gordon, 1988; Streit, 1990); however, their existence in peripheral ganglia is less clear. The overall result here is the identification of a cell population in rat trigeminal ganglia, apparently equivalent to ramified microglia. Several features of the identified cells support this

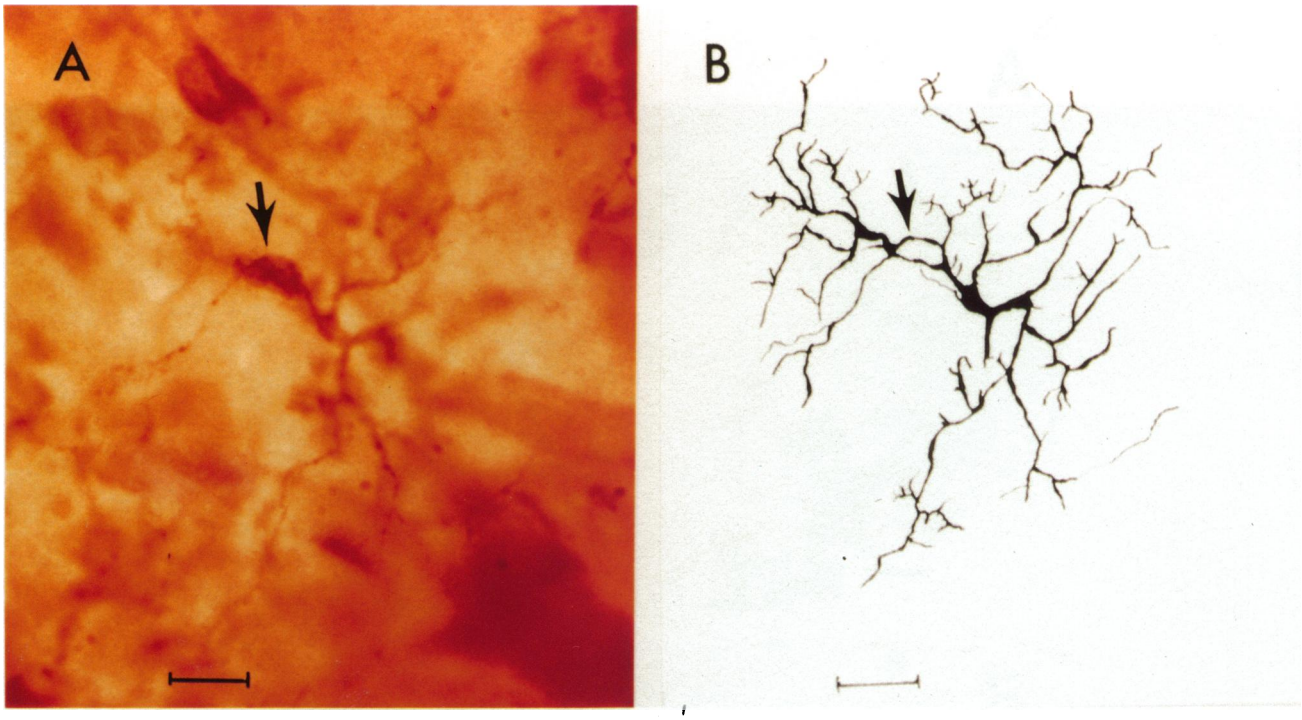

Fig. 4. Detailed morphology of a B_4 lectin-stained cell in the trigeminal ganglion. (*A*) Photomicrograph of an individual cell in a stained section. (*B*) Camera lucida drawing of the same cell. Note the small soma (arrow) with scanty cytoplasm (the nucleus, depicted as clear, almost fills the soma) and numerous thin processes, all characteristic for microglia. Bar, 10 μm.

conclusion, including the correlation of their tissue distribution, size and cellular morphology with those of CNS microglia. All of these properties are relatively unique or distinctive for microglial cells. Also, the binding and associated staining by the B_4 isolectin itself is supportive of this identity, since this agent has been shown to label microglia and macrophages selectively in nervous system tissue (Streit, 1990; Boya et al. 1991*b*). Finally, TPP enzyme, which also serves as a microglial cell marker (Murabe & Sano, 1981), was used to corroborate lectin staining. This activity exhibited a different type of staining but revealed highly similar cells. It is concluded that lectin and TPP staining label an identical cell population and that these cells correspond to ramified microglia.

A limited number of other studies have indicated resident macrophages in peripheral nervous tissue (Oldfors, 1980; Stoll et al. 1989) and the existence of macrophages in human dorsal root ganglia has been supported by several laboratories (Graus et al. 1990; Scaravilli et al. 1991). In studies on various human ganglia, Esiri & Reading (1989) used immunohistochemical techniques to demonstrate the consistent presence of macrophages. They also showed in the trigeminal ganglion a macrophage population very similar to that described in this study. Thus our results are supported by and confirm these previous findings. In addition, a possible correlation with CNS microglia is established here. With regard to peripheral nerves, Beuche & Friede (1984) reported that there were essentially no resident macrophages, or at least that no functional activity attributable to these cells had been found under the conditions that they had used. However, a recent study (Monaco et al. 1992) provides clear evidence for their existence. Particularly relevant is their finding of a strong morphological and functional resemblance between peripheral nerve resident macrophages and CNS microglia.

Several different monoclonal antibodies have been used with limited success to label microglia in CNS and 2 of these antibodies, OX-42 and ED3 (Streit et al. 1988), were employed in the course of this study. Neither provided significant or reliable staining in the trigeminal ganglion, although an occasional well stained cell was observed. The reason for the limitation on the successful use of these antibodies in the CNS is presumed to be a combination of the fact that they recognise macrophage-specific antigens and that the microglia are highly downregulated with regard to macrophage properties. Thus very infrequent staining in the trigeminal ganglion may reflect an enhanced degree of microglial inactivation. In any event, the lectin and TPP staining, both of

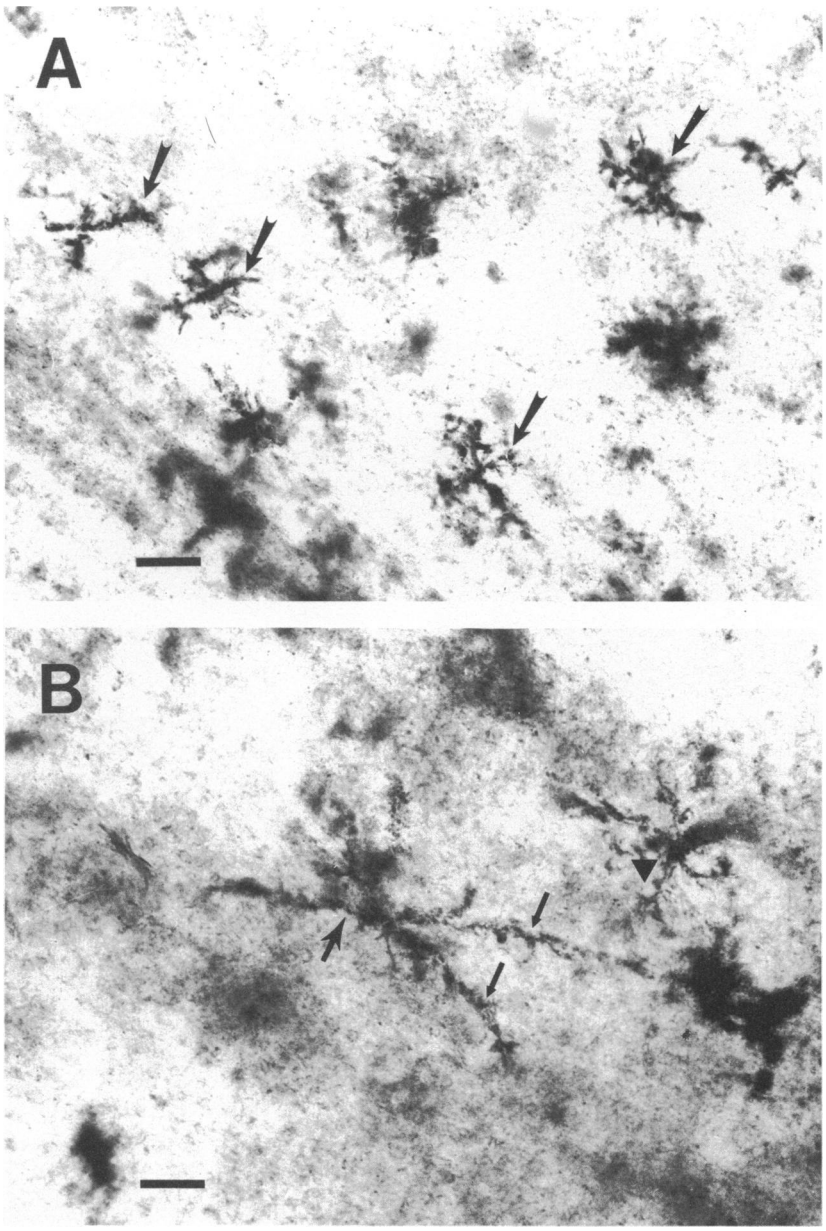

Fig. 5. Thiamine pyrophosphatase staining of cells in the trigeminal ganglion. (*A*) Although this staining was more punctate and often tended to obscure cellular morphology, cells with similar size, distribution and morphology as those labelled by the lectin were revealed. Four individual cells are indicated by the arrows. (*B*) At higher magnification, a small cell body (larger arrow; note the nonstained oval nucleus) with thin processes (2 are indicated by the small arrows), as well as the punctate nature of staining, can be discerned. The soma of an adjacent stained cell to the right is indicated by an arrowhead. Bars: *A* 25 μm, *B* 16 μm.

which label inactive ramified cells in CNS more intensely and consistently than the monoclonal antibodies, appeared to confirm the presence of ramified microglia-like cells in the trigeminal ganglion. Hence microglia may have a much wider distribution than just in the CNS, which has traditionally been the focus of their study. Further investigation of other peripheral ganglia now seems warranted and necessary.

The mechanism(s) of immune responses in neural tissue is currently unclear; in particular, the identity of antigen-presenting cells is an unresolved issue. Such cells can serve several roles in an immune response, including the pivotal function of antigen processing and presentation for lymphocyte activation. In most tissues, macrophages serve as the major component of antigen-presenting activity. The proposed identity of microglia as tissue macrophages suggests they may represent an intrinsic source of antigen-presenting activity in the nervous system and thus may be critical for immune function. The location and distribution of microglia is therefore significant.

Based on a presumed role as macrophages,

microglia may function in inflammation and regulation of immunological processes through cytokine secretion; also, these cells may regulate astrocyte proliferation, contribute to developmental histogenesis and inactivate diffusable neurotransmitters in adult tissue (see Thomas, 1992, for review). While all of these functions have been proposed for microglia in CNS, they obviously have not been investigated in PNS. This study provides direct evidence for the identification and presence of ramified microglia-like cells in the trigeminal ganglion; however, possession of the aforementioned functional properties as well as the ability to upregulate and convert into active macrophages have not been demonstrated. Assessment of these functional properties will be the subject of future studies.

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