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Expression of major histocompatibility complex and leukocyte common antigens in amoeboid microglia in postnatal rats

E. A. LING, C. KAUR AND W. C. WONG

Department of Anatomy, Faculty of Medicine, National University of Singapore, Singapore 0511

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INTRODUCTION

Recent studies have indicated that besides their primary role in phagocytosis (Penfield, 1932; for review, see Ling, 1981), microglial cells in the central nervous system (CNS) may also be involved in immunological processes in the brain (for review, see Streit, Graeber & Kreutzberg, 1988). In this connection, a remarkable observation was the detection of the antigens of the major histocompatibility complex (MHC) in these cells in neurodegenerative disorders such as multiple sclerosis (Hayes, Woodroofe & Cuzner, 1987; Woodroofe *et al.* 1986), Alzheimer's disease (McGeer, Itagaki, Tago & McGeer, 1987) and experimentally induced neurodegeneration (Akiyama, Itagaki & McGeer, 1988; Akiyama & McGeer, 1989; Poltorak & Freed, 1989; Streit, Graeber & Kreutzberg, 1989; Weinstein, Walker, Akiyama & McGeer, 1990). These findings suggest that microglial cells in the brain may play a role in self-recognition and antigen presentation. In adult normal brain, however, the expression of MHC antigens on microglia is extremely low (Matsumoto & Fujiwara, 1986, 1987) or virtually absent (Hickey & Kimura, 1987; Streit *et al.* 1988). It would seem therefore that the MHC encoded antigens are elevated in activated microglia in various neuropathological conditions. In this study, we decided to establish whether the amoeboid microglial cells which are considered to be an activated form of microglia (for review, see Ling, 1981; Innocenti, Clarke & Koppel, 1983) in the developing brain would show similar surface antigens.

MATERIALS AND METHODS

Male Wistar rats aged 2, 5, 9, 15, 21 and 30 days were used in this study. Under ether or 3·5% chloral hydrate anaesthesia, they were perfused with Ringer's solution. The Ringer's prewash lasted for a few minutes until the liver and lungs were clear of blood. This was followed by the aldehyde fixative composed of a mixture of periodate lysine–paraformaldehyde according to the method of McLean & Nakane (1974), with a concentration of 4% paraformaldehyde. The total perfusion time was 10–15 min. After perfusion, the brain was removed and was fixed in a similar fixative for another 2 h. The brain was then kept in 0·1 M phosphate buffer containing 10% sucrose overnight at 4 °C. Frozen sections (40 μm) were cut and rinsed in phosphate buffered saline (PBS). The sections were then incubated with the monoclonal antibodies OX-18 (Sera-Lab MAS 101b), OX-6 (Sera-Lab MAS 043b), OX-3 (Sera-Lab MAS 028b) and OX-1 (Sera-Lab MAS 026b) diluted 1:100 with PBS. Incubation time was between 18 and 20 h at 4 °C. Subsequent antibody detection was carried out by using

the Vectastain ABC-Kit (PK-4002, Vector Laboratories, Burlingame, CA) against mouse IgG with 3,3′-diaminobenzidine (DAB, Sigma-5637) as a peroxidase substrate and intensified with ammonium nickel sulphate. For electron microscopy, 50 μm vibratome sections were prepared. Only tissue samples incubated in OX-18 were processed for ultrastructural study. Following immunocytochemical treatment, the vibratome sections were postosmicated for 30 min in 1% osmium tetroxide in 0·1 M phosphate buffer. The sections were then dehydrated in a graded series of alcohol and embedded in Araldite. Ultrathin sections were stained with lead citrate only, and were examined in a JEOL 1200 EX electron microscope.

OBSERVATIONS

On light microscopy the amoeboid microglia in the corpus callosum above the lateral ventricles (Fig. 1) as well as in the cavum septum pellucidum were selectively labelled with OX-18. Between the 2nd and 5th postnatal days the stained cells in the corpus callosum were mostly round with an intense reaction at the cell membrane (Figs 2, 3). Some of the labelled cells showed stout processes (Fig. 3). From day 15 onwards, the stained cells assumed an oval or ramified appearance typical of microglial cells (Figs 4, 5). By the time of weaning, i.e. 21 days postnatally, the immunoreaction on microglial cells was extremely faint. This continued to diminish so that by 30 days of age the immunoreactivity was barely visible on some branched cells (Fig. 6).

The present electron microscopic study confirmed the OX-18 immunoreactivity in amoeboid microglial cells in postnatal rat brain. Intense reaction was confined to their plasma membrane (Fig. 7) as well as its tubular invaginations (Fig. 8). Many positively stained vesicular profiles which were probably profiles of the plasmalemmal invaginations were seen near the nucleus (Fig. 8). Cytoplasmic vacuoles and lysosomal granules were negative (Fig. 8). Some of the OX-18 positive amoeboid microglia showed heterogenous inclusions of phagosomes (Fig. 9). Whilst most of the OX-18 positive amoeboid microglia were round and contained abundant cytoplasm, some elongated cells were observed in regions where the callosal fibres were more closely packed, particularly in the region above the cavum septum pellucidum (Fig. 10). Plasmalemmal invaginations which were an obvious feature in the round cells were generally not seen in the elongated amoeboid microglia. The latter cells were common in rats older than 15 days of age.

The results with OX-1 paralleled those with OX-18 in terms of the spatial distribution and temporal pattern of the labelled amoeboid microglial cells (Figs 11, 12). Most if not all of the amoeboid microglia in the corpus callosum were labelled

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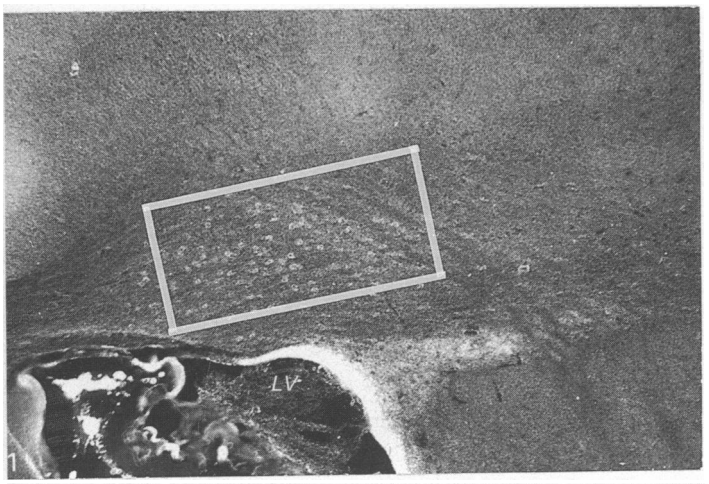
Fig. 1. Dark field photomicrograph showing the staining of amoeboid microglial cells in the corpus callosum (area outlined) above the lateral ventricle (*LV*). Ox-18. 2-day-old rat. × 194.

Fig. 2. OX-18 positive amoeboid microglial cells in the corpus callosum in a 2-day-old rat. The majority of the stained cells are round. The immunoreactivity is confined to the plasma membrane (arrows). × 270.

Fig. 3. OX-18 positive amoeboid microglial cells in the corpus callosum in a 5-day-old rat. Note that some of the stained cells show stout processes (arrows). × 270.

Figs 4, 5. OX-18 positive amoeboid microglial cells in the corpus callosum in a 15-day-old rat. Among the stained cells some are elongated (Fig. 4), others are branched (Fig. 5). Fig. 4 × 270; Fig. 5 × 170.

Fig. 6. Arrows indicate a few extremely branched microglial cells in the corpus callosum in a 30-day-old rat. × 190.



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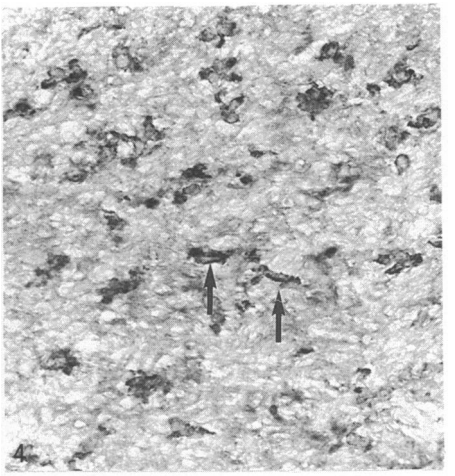

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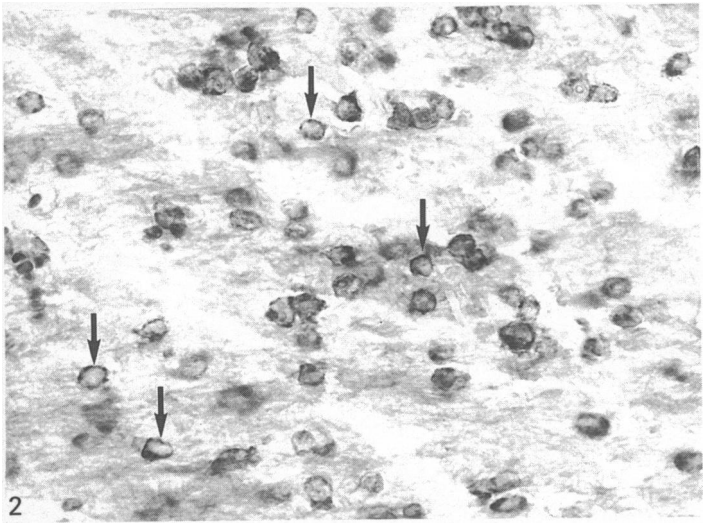

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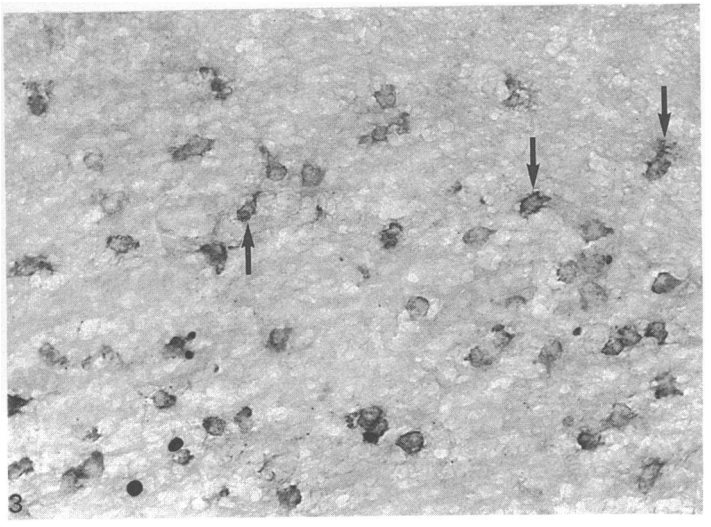

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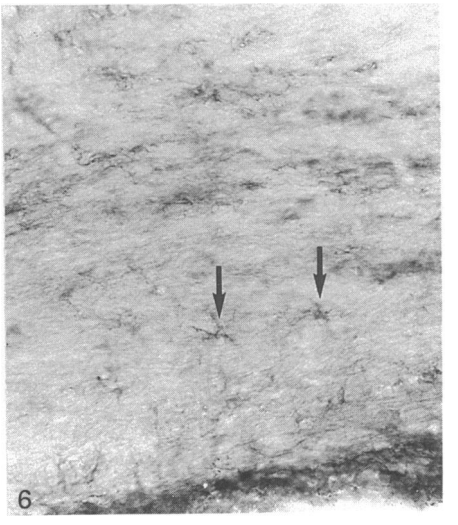

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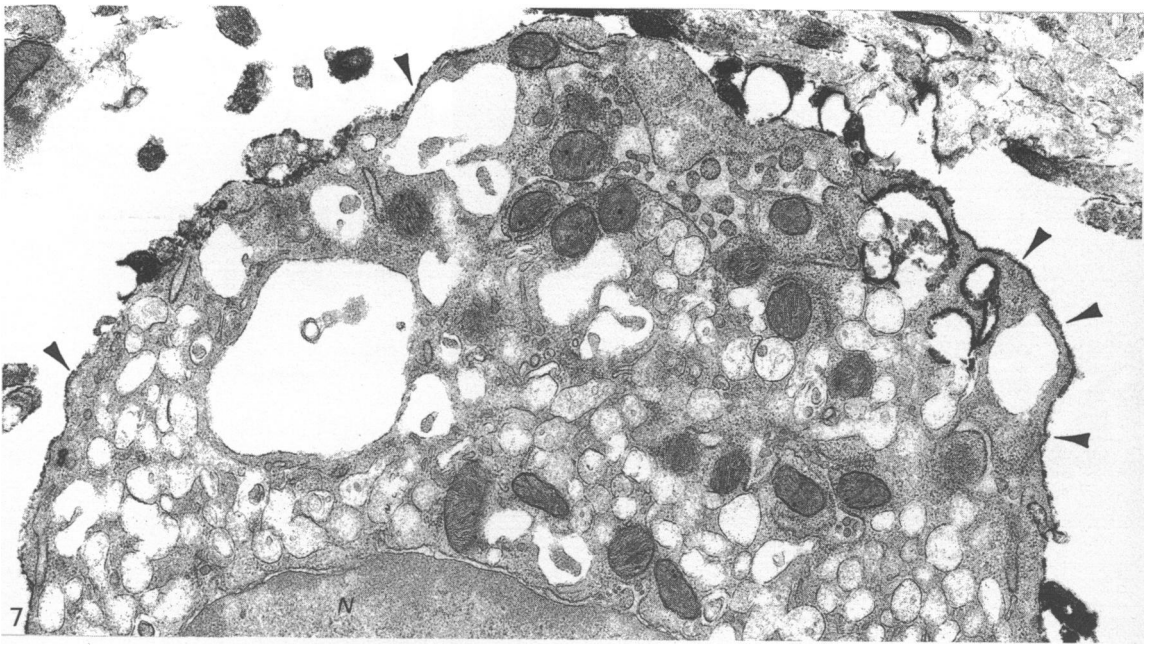
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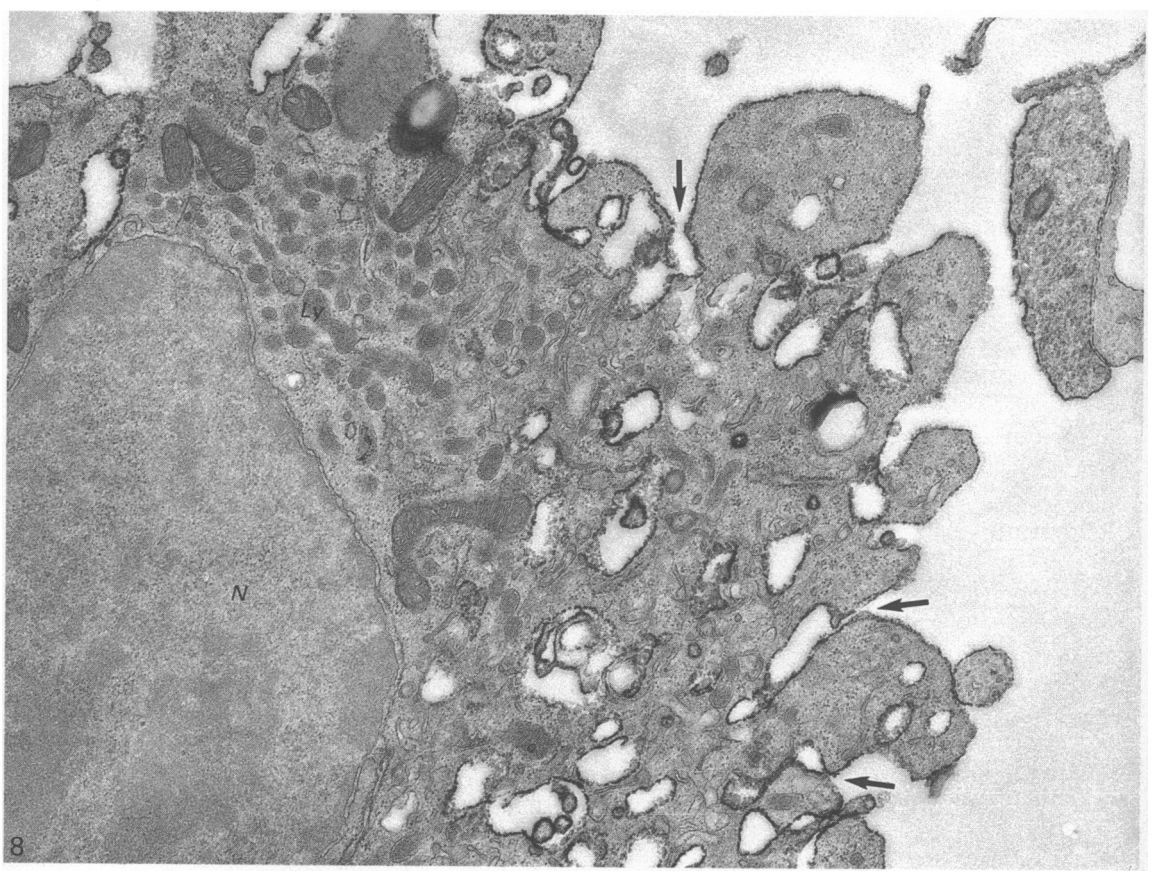
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with OX-1. The labelled cells in the cavum septum pellucidum usually formed cell clusters (Fig. 12).

Apart from a few weakly stained cells that were observed in the corpus callosum (Fig. 13) and cavum septum pellucidum (Fig. 14) in 2 and 5-day-old rats, the postnatal brain appeared to be negative to OX-6 and OX-3 monoclonal antibodies. In animals that were older than 5 days, so far we have not observed any positive cells on incubation with these monoclonal antibodies.

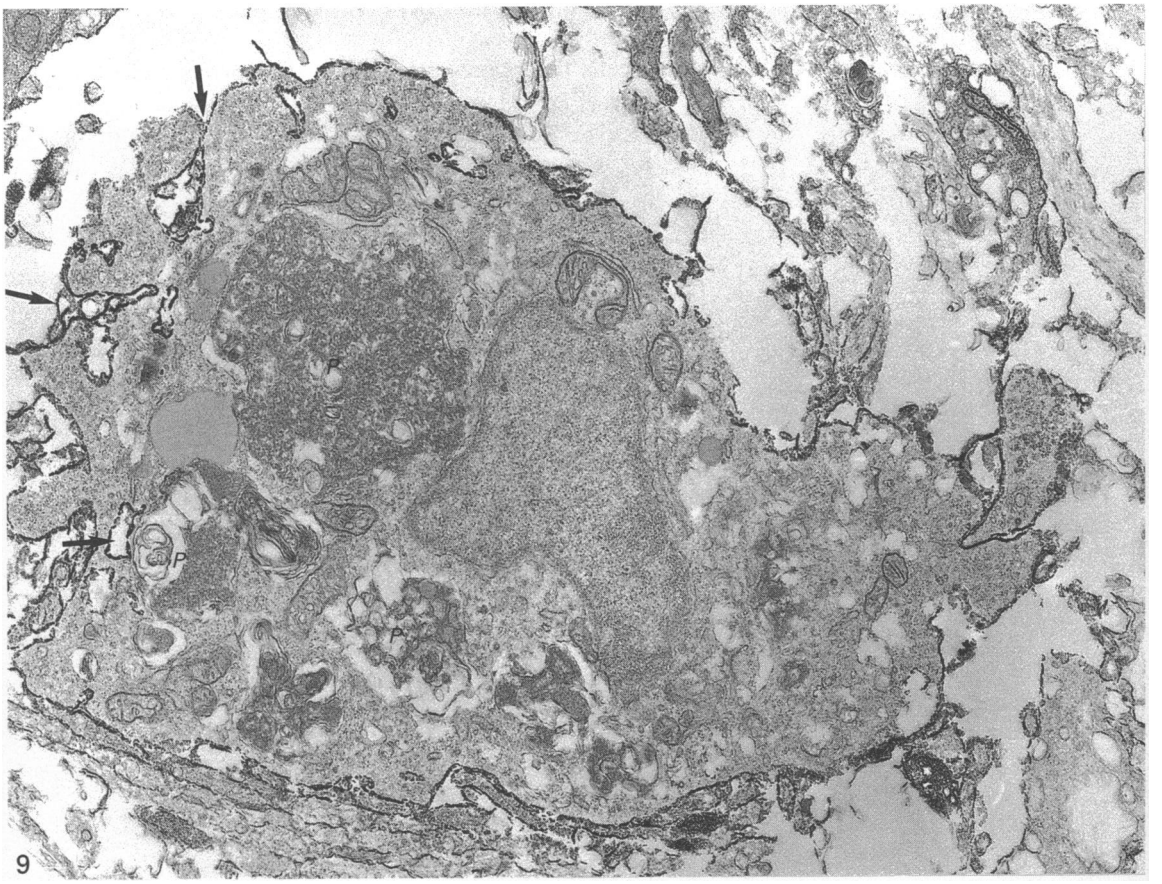
DISCUSSION

It is generally believed that the CNS in normal animals is an immunologically privileged site and that the molecules of the major histocompatibility complex (MHC) necessary for restricted antigen recognition are almost undetectable (Lampson & Fisher, 1984; Lampson & Hickey, 1986; Hickey & Kimura, 1988). Such a concept may require reappraisal in view of the recent findings by Hayes *et al.* (1987) that the microglial cells in the normal human white matter expressed MHC class II antigens. Akiyama & McGeer (1989), however, showed that apart from the vascular endothelial cells, very few microglia could be detected expressing MHC antigens in the normal rat brain. A definite feature of microglial cells was their enhanced expression of MHC antigens in neurodegenerative disorders (for references, see Introduction). In other words, vigorous expression of MHC antigens was only observed in reactive or activated microglia induced by neurotoxic agents (Akiyama *et al.* 1988; Akiyama & McGeer, 1989; Streit *et al.* 1989) or intracerebral transplantation (Poltorak & Freed, 1989).

The results of the present study demonstrated the intense immunoreactivity of amoeboid microglial cells in the postnatal rat brain with OX-18, a monoclonal antibody that detects the MHC class I antigens. It is known that MHC class I antigens serve as the restriction elements of cytotoxic/suppressor lymphocytes (Sell, 1987; Stites, Stobo & Wells, 1987; Akiyama *et al.* 1988; Weinstein *et al.* 1990). The expression of MHC class I antigens on amoeboid microglial cells shows the putative role of these cells to interact with T-cytotoxic/suppressor cells. Studies of the postnatal corpus callosum (Ling, 1976, 1981), however, did not identify any lymphocytes amongst the amoeboid microglia. It is therefore possible that the presence of MHC encoded class I antigens on the latter may be related directly to their primary phagocytic function rather than an immunological role in the postnatal rat brain. The present electron microscopic immunocytochemical study showed that some of the OX-18 positive amoeboid microglial cells contained phagosomes most probably derived from degenerating cellular elements and nerve fibres. Our earlier study (Ling, Kaur, Yick & Wong, 1990) demonstrated the presence of complement type 3 (CR3) membrane receptors on amoeboid microglia. It was suggested that these may be involved also in the endocytotic activity of the cells. In the present study, all the amoeboid microglial cells in the postnatal corpus callosum as well as in the cavum septum pellucidum were stained with the monoclonal antibody OX-18. A similar

Fig. 7. A round vacuolated amoeboid microglial cell showing immunoreactivity at its plasma membrane with OX-18 (arrowheads). *N*, nucleus. 5-day-old rat. × 17500.

Fig. 8. Several pseudopodial processes of different sizes project from the surface of an amoeboid microglial cell. Note the intense immunoreactive reaction with OX-18 at the plasma membrane and its tubular invaginations (arrows). The latter are deeply extended into the cytoplasm near the nucleus (*N*). *Ly*, lysosomes. 5-day-old rat. × 21000.



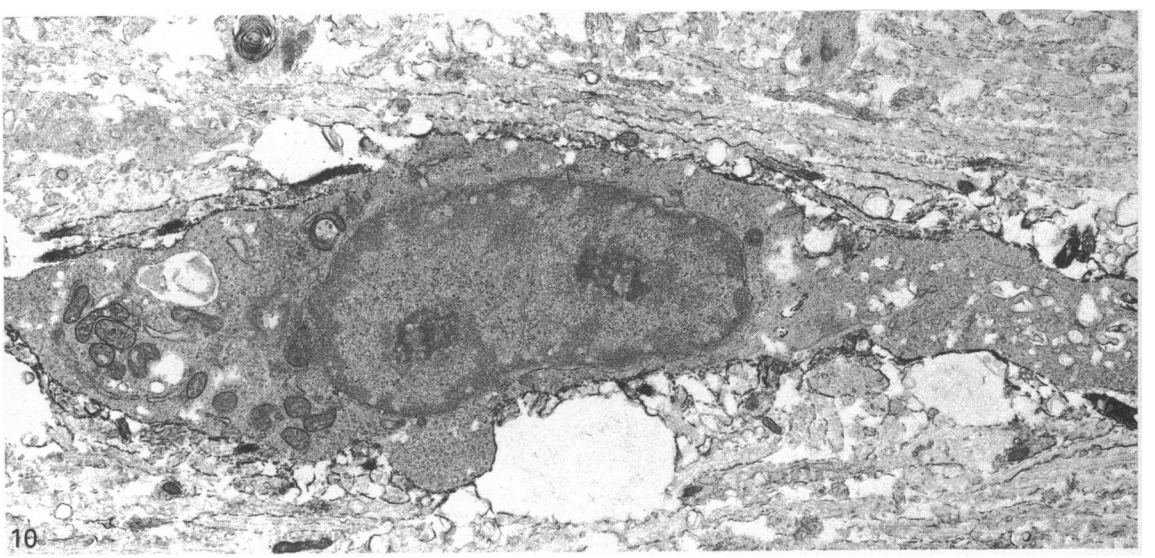
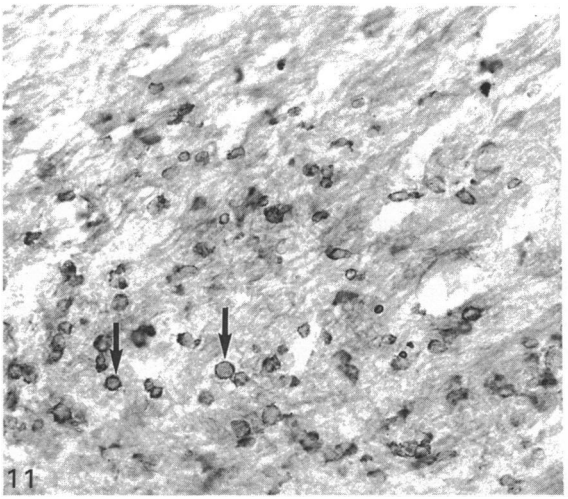
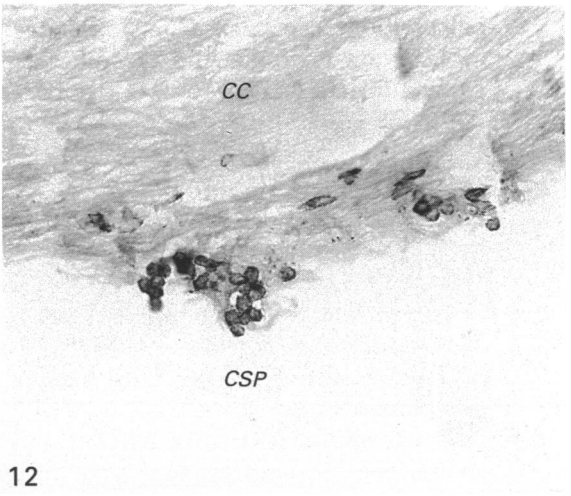
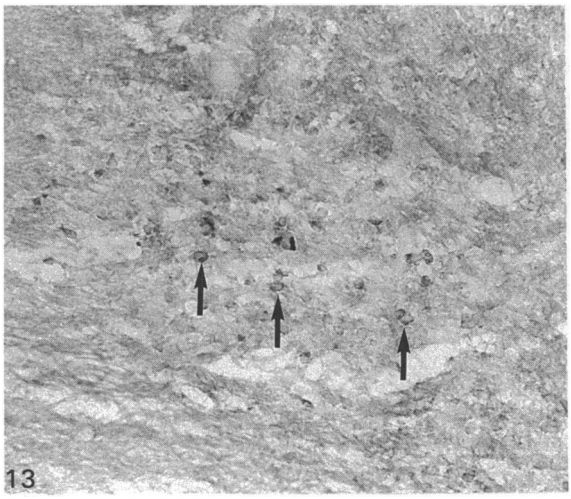


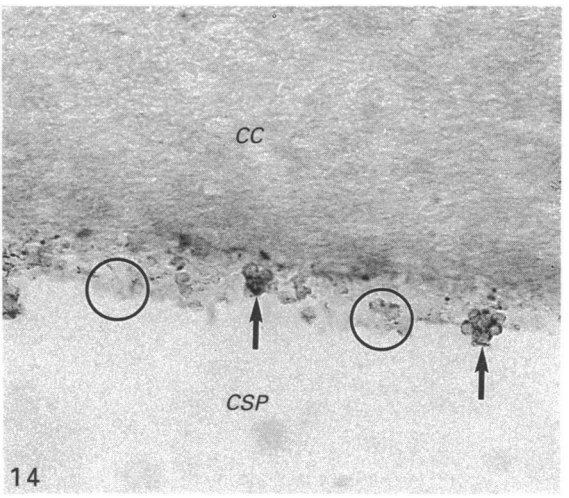
Fig. 9. An immunoreactive amoeboid microglial cell stained with OX-18. The immunoreactivity is localized at the plasma membrane and its invaginations (arrows). The abundant cytoplasm contains masses of phagosomes (*P*). 9-day-old. ×12750.

Fig. 10. An elongated amoeboid microglial cell similar to those shown in Figure 4. The cytoplasm accumulates at both poles. Immunoreactivity with OX-18 is localized at the plasma membrane which does not show the extensive tubular invagination as observed in Figures 8 and 9. 15-day-old rat. ×8750.









Figs 11, 12. Staining of amoeboid microglial cells with OX-1 in the corpus callosum (Fig. 11) and the cavum septum pellucidum (Fig. 12) in a 2-day-old rat. Arrows indicate the localization of the immunoreactivity at the plasma membrane of the cells. The OX-1 positive cells in the cavum are often in cell clusters (Fig. 12). *CC*, corpus callosum; *CSP*, cavum septum pellucidum. × 194.

Fig. 13. MHC Ia antigen staining in amoeboid microglial cells in the corpus callosum with OX-6 in a 2-day-old rat. Arrows indicate a few weakly stained cells among the callosal fibres. The immunoreactivity is barely visible. × 110.

Fig. 14. MHC Ia antigen staining in amoeboid microglial cells in the cavum septum pellucidum (*CSP*) with OX-3 in a 2-day-old rat. Arrows indicate two clusters of cells that are weakly stained, others (circle) are unstained. *CC*, corpus callosum. × 194.

staining pattern was observed with OX-42 in our earlier study (Ling *et al.* 1990) suggesting that both types of receptors are colocalized in the plasma membrane of amoeboid microglia.

In the present study, MHC class Ia antigens expression was negligible, if any, on amoeboid microglia as shown by the extremely faint reaction when the monoclonal antibody OX-3 or OX-6 was applied. These receptors are required for the presentation of foreign antigens to T-helper/inducer lymphocytes (Weinstein *et al.* 1990). It is therefore rather unlikely that in normal developing brain the amoeboid microglial cells

would have the ability to carry out antigen presentation and to react with T-helper/inducer to generate an immune response. The expression of immune associated antigens on microglia, on the other hand, may be induced by the stimulation of gamma interferon (Suzumura, Mezitis, Gonatas & Silberberg, 1987; Steiniger & Van der Meide, 1988).

Another feature of the amoeboid microglial cells in the brain was their intense immunoreactivity when stained with the monoclonal antibody OX-1, a marker for leukocyte common antigen (LCA). LCA is known to appear on polymorphonuclear leukocytes, lymphocytes, monocytes and macrophages (Sunderland, McMaster & Williams, 1979; Akiyama *et al.* 1988). This finding supports the proposed monocytic origin of amoeboid microglia (Ling, 1981). As glioblasts, astrocytes and oligodendrocytes did not express MHC I and LCA membrane antigens, it can be confidently deduced that they have a gene expression distinct from amoeboid microglia.

The temporal pattern of MHC I and LCA antigens on amoeboid microglia was comparable. In both instances, the antigen expression was considerable in the early postnatal rats when the majority of the cells were found with abundant cytoplasm. With the progressive change of their morphology from the round to oval and ramified form which is considered to be a resting form of microglia (see review, Ling, 1981), there is a gradual reduction of the immunoreactivity with the growth of the brain. The last trace of reaction of amoeboid microglia with both the immunological markers, OX-1 and OX-18, was at the age of weaning. By this age, very low expression of MHC I and LCA antigens could be detected in the corpus callosum or in the cavum septum pellucidum which is known to be almost obliterated at the age of 15th postnatal day (Tseng, Ling & Wong, 1983). The diminution of the immunoreactivity of amoeboid microglia with age is probably a down regulation of the surface antigens, a similar phenomenon that was observed for the complement type 3 receptors in the cells (Ling *et al.* 1990).

The significance of the vigorous expression of leukocyte antigens in amoeboid microglia in the early postnatal rat is only speculative. Their phenotypic expression supports their monocytic origin as described earlier (Ling, Penney & Leblond, 1980; Ling, 1981). It is possible that the cells retain in them the surface antigens soon after their entry into the CNS in the perinatal period. The neuroectodermal glioblasts, oligodendrocytes and astrocytes are unreactive with the monoclonal antibody OX-1. This indicates the absence of LCA in these cells.

Finally, the considerable expression of MHC encoded antigens, particularly class I antigens, on amoeboid microglia in postnatal rat brain leads us to propose that the postnatal rat brain is not immunologically privileged. It may, on the other hand, acquire such an immunological privilege by the age of weaning when it does not show any significant and detectable MHC encoded antigens except in neurodegenerative disorders.

SUMMARY

The expression of major histocompatibility complex (MHC) antigen and leukocyte common antigen (LCA) was observed in the amoeboid microglial cells in postnatal rat brain. Considerable MHC class I surface antigen was detected at the plasma membrane and its tubular invaginations in the amoeboid microglia in the corpus callosum using the monoclonal antibody OX-18. In early postnatal (2 and 5 day) rats, the OX-18 positive cells were mostly round but a few possessed stout processes. With

increasing age (9 and 15 days) the OX-18 positive amoeboid microglia assumed an oval or elongated form. By the time of weaning (21 days) and in older animals, the immunoreactivity was extremely weak and was detectable only on some branched microglia bearing fine processes. The presence of MHC class Ia antigens with OX-3 and OX-6 was hardly detectable except for a few weakly stained cells in the corpus callosum and cavum septum pellucidum in early postnatal rats. The expression of LCA was observed in amoeboid microglia using the monoclonal antibody OX-1 and this followed a similar temporal pattern to that with OX-18. The additional phenotypic features of amoeboid microglial cells in the present study support their monocytic origin. These cells are endowed with MHC class I antigens which may serve as the restriction elements for T lymphocytes, at least in the early postnatal brain.

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