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Immunohistochemical study of amoeboid microglial cells in fetal rat brain

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ABSTRACT

The present study examined the expression of different antigens in amoeboid microglial cells (AMC) in fetal rat brain extending from 12 to 20 d postconception (E12–E20) using a panel of monoclonal antibodies which recognised the major histocompatibility complex (MHC) class I (OX-18) and class II (OX-6) antigens, leucocyte common antigen (OX-1), CD4 receptor (OX-35), complement type 3 receptor (OX-42) or macrophage antigens of unknown function (ED1 and ED2). Of the above-mentioned antigens, ED1 and ED2-labelled AMC were observed in the neuroepithelia as early as embryonic day 12 (E12); other antigens were not detected at this stage. At E14, except for MHC class I antigen, all other antigens were expressed by AMC distributed predominantly in the developing white matter. At E16, AMC in the intermediate zone lateral to the striatum were endowed with all the above-mentioned antigens including MHC class I. At E18, the immunoreactivities of AMC stained with OX-6, OX-18, OX-35 and OX-42 antigens were noticeably reduced when compared with those cells at E16. At E20, amoeboid microglial cells exhibited full complement of antigen expression similar to those cells at E16; some of the labelled cells emitted a variable number of cytoplasmic processes. It is suggested that the successive and differential expression of various macrophage related antigens on AMC in fetal brain is related to the specific requirement of local environment in different stages of development.

Key words: Glia; antigen expression.

INTRODUCTION

The origin of ramified microglial cells or simply microglia has been a much debated issue since the pioneer study by del Rio-Hortega (1932) who stated that the cells are derived from invasion of pial elements. The more widely accepted view, however, is that they originate from circulating monocytes through the transitory stage as brain macrophages, namely, amoeboid microglial cells (AMC) (Ling & Wong, 1993). With advancing age, the monocyte-derived amoeboid microglial cells residing mainly in loosely organised subcortical white matter and circumventricular regions undergo morphological transformation to become ramified microglia which persist through adulthood. Immunohistochemical studies have shown the vigorous expression of major histocompatibility complex (MHC) class I antigen, leucocyte common antigen (LCA), complement receptor type 3 (CR3) and proteins of unknown function (ED1) in amoeboid microglial cells in postnatal rats (Ling et al. 1990, 1991). This has greatly amplified the view of the mononuclear phagocyte nature of the cell type. The expression of the above-mentioned macrophage related antigens, on the other hand, is at low levels or virtually undetectable in adult rats (Perry & Gordon, 1987; Flaris et al. 1993) when the cells progressively emitted their processes to become ramified microglia (Sminia et al. 1987; Ling et al. 1990, 1991; Milligan et al. 1991*a*). Interestingly, a similar down-regulation of membrane glycoprotein (lectin receptors) was also observed with the ramification of amoeboid microglia (Wu et al. 1992, 1994).

While much is known about the immunophenotypic features and development of amoeboid microglial cells in postnatal brain (Ling & Wong, 1993), very

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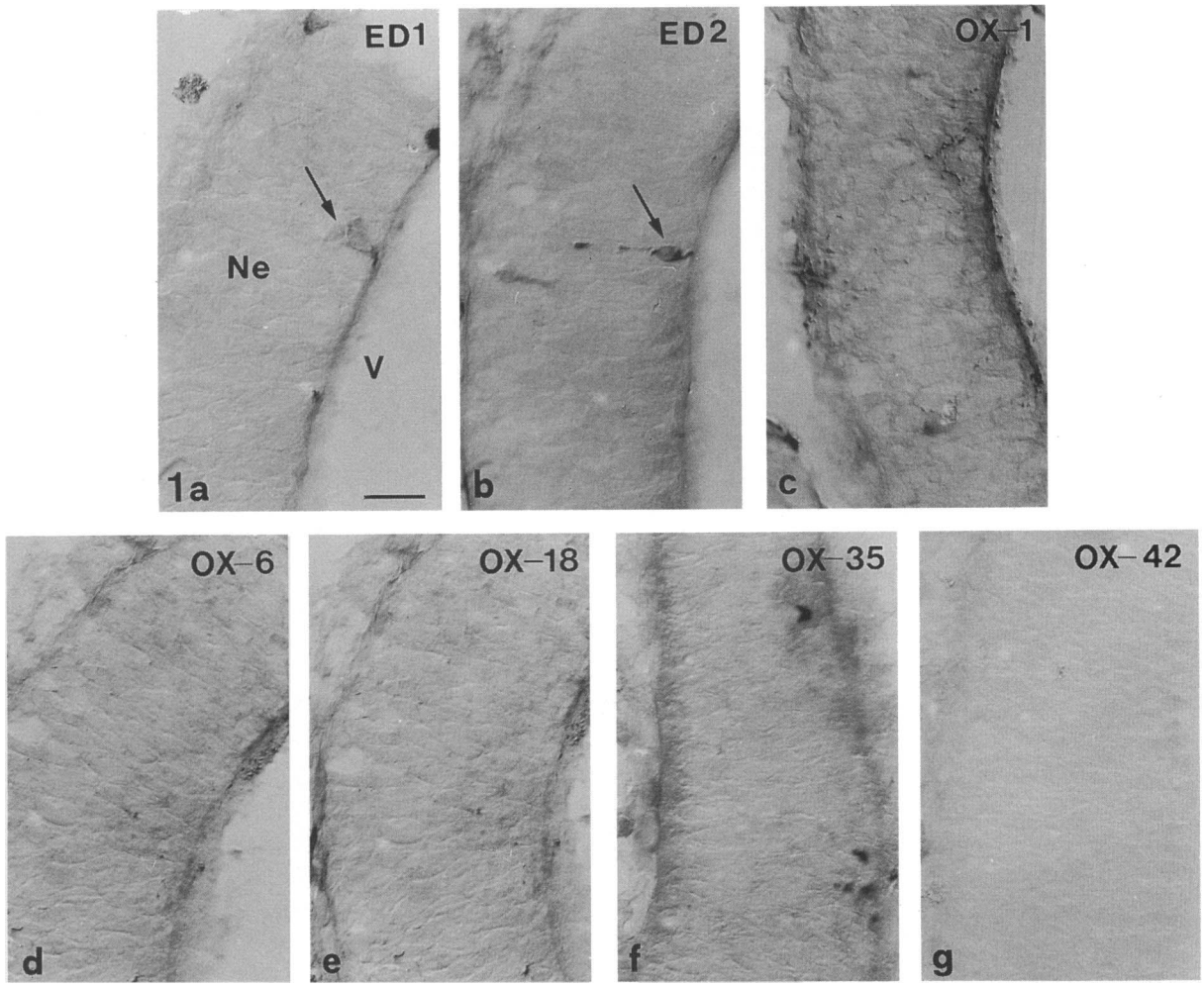

Fig. 1 *a–g*. Expression of macrophage antigens, marked by ED1 (*a*) or ED2 (*b*), in AMC at E12. The occasional immunolabelled cells occur at the junction of the telencephalon and diencephalon. Note that the ED1 or ED2 labelled cells (arrows) are adjacent to the ventricular surface (V). Labelled cells are absent when the adjacent sections are incubated in OX-1 (*c*), OX-6 (*d*), OX-18 (*e*), OX-35 (*f*) and OX-42 (*g*). Ne, neuroepithelium. Bar, 20 μm.

little is known about the same aspects of similar cells known to exist in fetal brain (Tseng et al. 1983; Ashwell, 1991; Boya, 1991). So far, most of the studies in fetal brain have been based on observations in circumscribed regions (Valentino & Jones, 1981) or confined to a limited period during development (Sminia et al. 1987). A systematic study of the expression of various macrophage related surface antigens on amoeboid microglial cells prenatally is therefore desirable. The immunophenotypic characterisation of the cell type in fetal stage is deemed to be crucial to a better understanding of their functional roles in early development which would help to unravel their mode of formation or ontogenesis.

MATERIALS AND METHODS

Animals

Albino Wistar rats were used in this study. Timed mating was carried out by placing 3 adult males with 1 female overnight. The day of insemination, as determined by the presence of vaginal plug in stool, was designated as embryonic day 0 (E0). Birth usually occurred on the 22nd embryonic day (E22). In addition, all rats were fed with standard laboratory diet.

Following deep anaesthesia of the mother rat achieved with an intraperitoneal injection of 7% chloral hydrate (0.4 ml/100 g body weight), fetuses ranging from E12 to E20 were quickly removed and immersed in fixative. The brain of some fetuses between E16 to E20 was removed after 1 h fixation and further immersed in toto in fresh fixative to

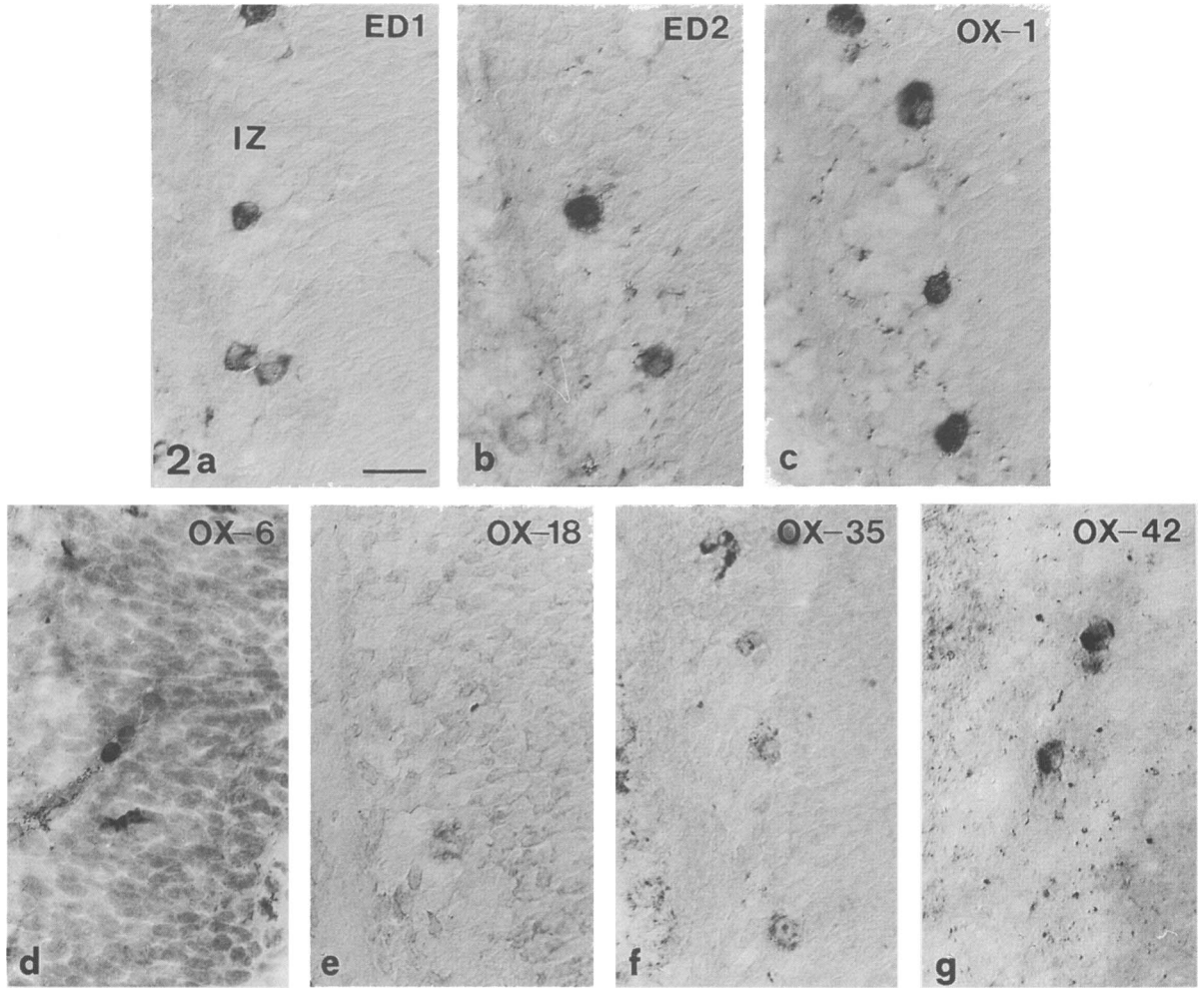

Fig. 2*a–g*. Expression of different antigens by AMC at E14. Except for MHC class I (OX-18, *e*), the cells express macrophage antigens detected with ED1 and ED2 (*a*, *b*), LCA (OX-1, *c*), MHC class II (OX-6, *d*), CD4 (OX-35, *f*) and CR3 (OX-42, *g*). The labelled cells are distributed predominantly in the intermediate zone (IZ). OX-6 labelled cells (*d*) are irregular in outline, while the other immunoreactive cells are round. Bar, 20 μm.

ensure better tissue preservation. The fixative was composed of a mixture of periodate-lysine-paraformaldehyde according to the method of McLean & Nakane (1974), with a concentration of 2% paraformaldehyde. After overnight fixation at 4 °C, the fetuses were kept overnight in 30% sucrose in 0.1 M phosphate buffer, pH 7.4, at 4 °C. The entire brain was then cut serially into 20–30 μm sections in the coronal plane with a cryostat and collected on gelatin-coated glass slides. These were stored at room temperature before immunohistochemical reaction.

Immunohistochemistry

The following mouse monoclonal antibodies (Sero Tec, USA) were used for immunohistochemical staining of amoeboid microglial cells: (1) ED1, directed against monocytes/macrophages, (2) ED2, directed against macrophages/perivascular cells, (3) MRC OX-1, directed against rat leucocyte common antigen (LCA), (4) MRC OX-6, directed against MHC class II antigens, (5) MRC OX-18, directed against MHC class I antigens, (6) MRC OX-35, recognising an epitope of rat CD4 antigen and shown to cross-react with microglial cells, and (7) MRC OX-42, recognising the rat complement type 3 receptor (CR3).

Before incubation with the respective monoclonal antibodies, the sections were pretreated with 1% H_2O_2 for 1 h to block any possible endogenous peroxidase and then with 2% normal horse serum for 1 h. The sections were then incubated in primary antibodies which were diluted 1:100, with phosphate-buffered saline (0.1 M, pH 7.4) containing 0.1% Triton X-100 for 18–20 h at room temperature. Subsequent antibody detection was carried out using the Vecta-

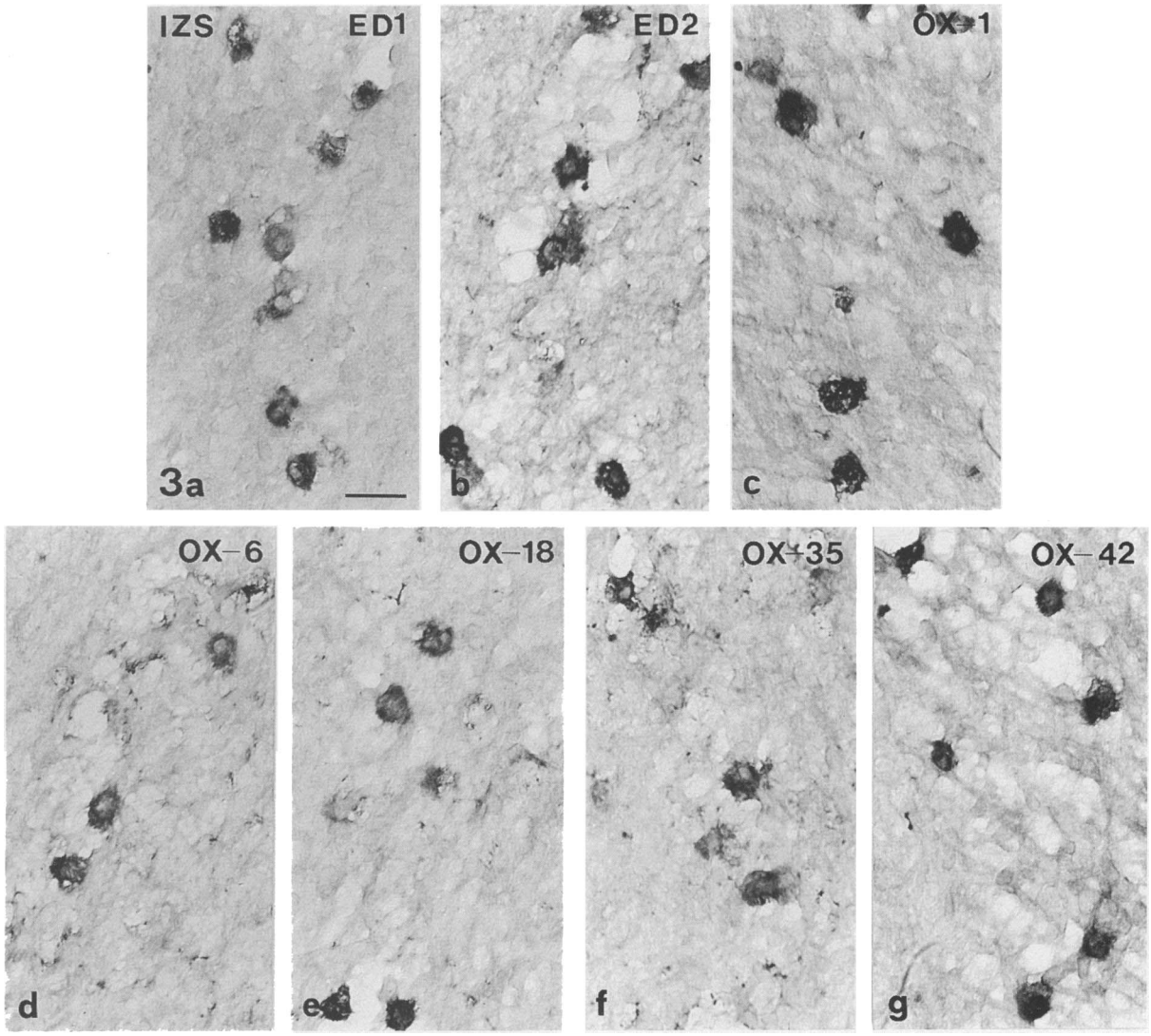

Fig. 3*a–g*. Expression of different antigens by AMC at E16. All the antigens examined are expressed on AMC in the intermediate zone lateral to the striatum (IZS). All labelled AMC appear round. Bar, 20 μm.

stain ABC kit (PK-4002, Vector Laboratories, Burlingane, CA) against mouse IgG with 3,3′-diaminobenzidine (DAB, Sigma-5367) as a peroxidase substrate, and intensified with nickel ammonium sulphate. Sections were finally counterstained with 0.05% thionin and examined under the light microscope.

RESULTS

The first appearance of cells of macrophagic nature was at E12, when sporadic ED1 or ED2 positive cells were observed at the junction of the telencephalon and diencephalon (Fig. 1*a*, *b*). These immunolabelled cells, tentatively referred to as amoeboid microglia, were round or displayed a variable number of pseudopodial processes. In some sections, the immunoreactive cells were located below the ventricular surface (Fig. 1*a*, *b*). Labelled cells , however, were not detected in the developing brain tissues using the antibodies OX-1, OX-6, OX-18, OX-35 and OX-42 (Fig. 1*c–g*).

At E14, many immunoreactive cells bearing the external morphology of AMC (hereafter referred to as immunolabelled AMC) were distributed predominantly in the developing white matter, e.g. the intermediate zone. They exhibited all the antigens tested except for MHC class I (OX-18) antigen (Fig. 2*a–g*). The immunolabelled cells were mostly round or oval (Fig. 2*a–c*, *f*, *g*), but the OX-6 labelled cells tended to be irregular in cell outline.

From E16 to E20, the immunolabelled AMC increased progressively in numbers, and the majority of them were localised in the developing white matter,

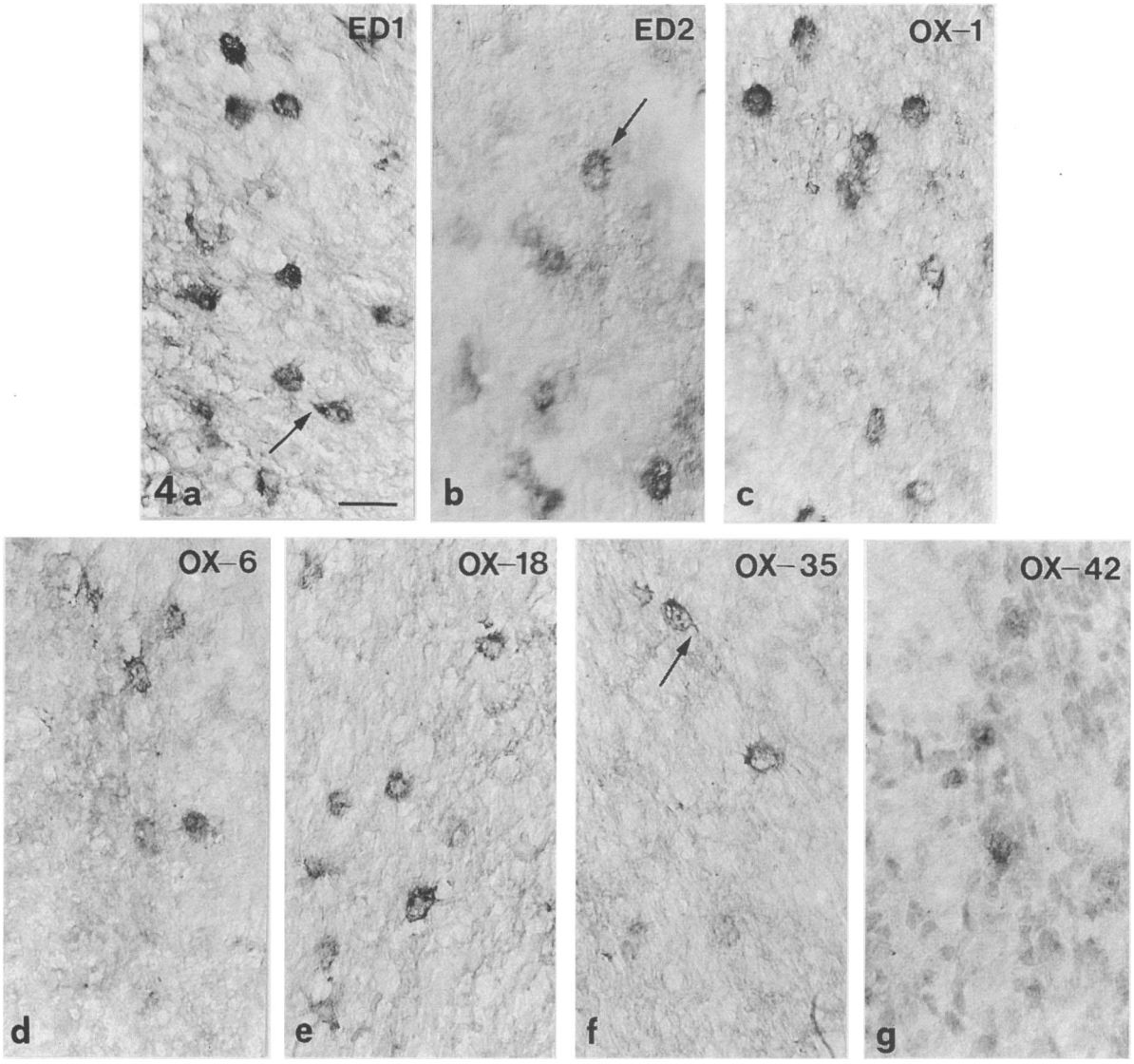

Fig. 4*a–g*. Expression of different antigens by AMC in the IZS at E18. The AMC retain the same antigens expression as those at E16. The staining intensities of labelled AMC bearing OX-6, OX-18, OX-35, and OX-42 antigens (*d*, *e*, *f*, *g*) are noticeably weaker than those of AMC at E16. The cell outlines of AMC (arrows) are irregular. Bar, 20 μm.

i.e. the intermediate zone lateral to the striatum (IZS). They were endowed with all the antigens tested (Figs 3–5). The immunoreactive AMC appeared round at E16 but with advancing age, the ED1, ED2, OX-6 and OX-18-labelled cells began to emit short and stout processes (Fig. 5*a*, *b*, *d*, *e*). At E18, the immunoreactivities of AMC with OX-6, OX-18, OX-35 and OX-42 were considerably reduced when compared with those at E16 (Figs 3, 4*d–g*). From E16 onwards, some cells in the meninges also displayed the immunoreactivities of all the antigens examined. At E20, the amoeboid microglial cells displayed the full complement of antigens detected with the above-mentioned antibodies. The Table summarises the immunophenotypic profiles of AMC in the course of development in fetal brain.

DISCUSSION

The present study has demonstrated that the first macrophage antigens expressed by AMC at E12 were ED1 and ED2 which are markers of monocytes/macrophages (Dijkstra et al. 1985). This strongly supports the monocytic origin of AMC. However, since the nervous tissue is avascular at the site of localisation of ED1 positive cells, it is suggested that they may be derived from invading monocytes from meninges into the avascular neuroepithelium as suggested by Sorokin et al. (1992). However, the possibility that ED1+ and ED2+ macrophages in the avascular neuroepithelium originate from so-called primitive/fetal macrophages as proposed by Takahashi & Naito (1993) should also be considered.

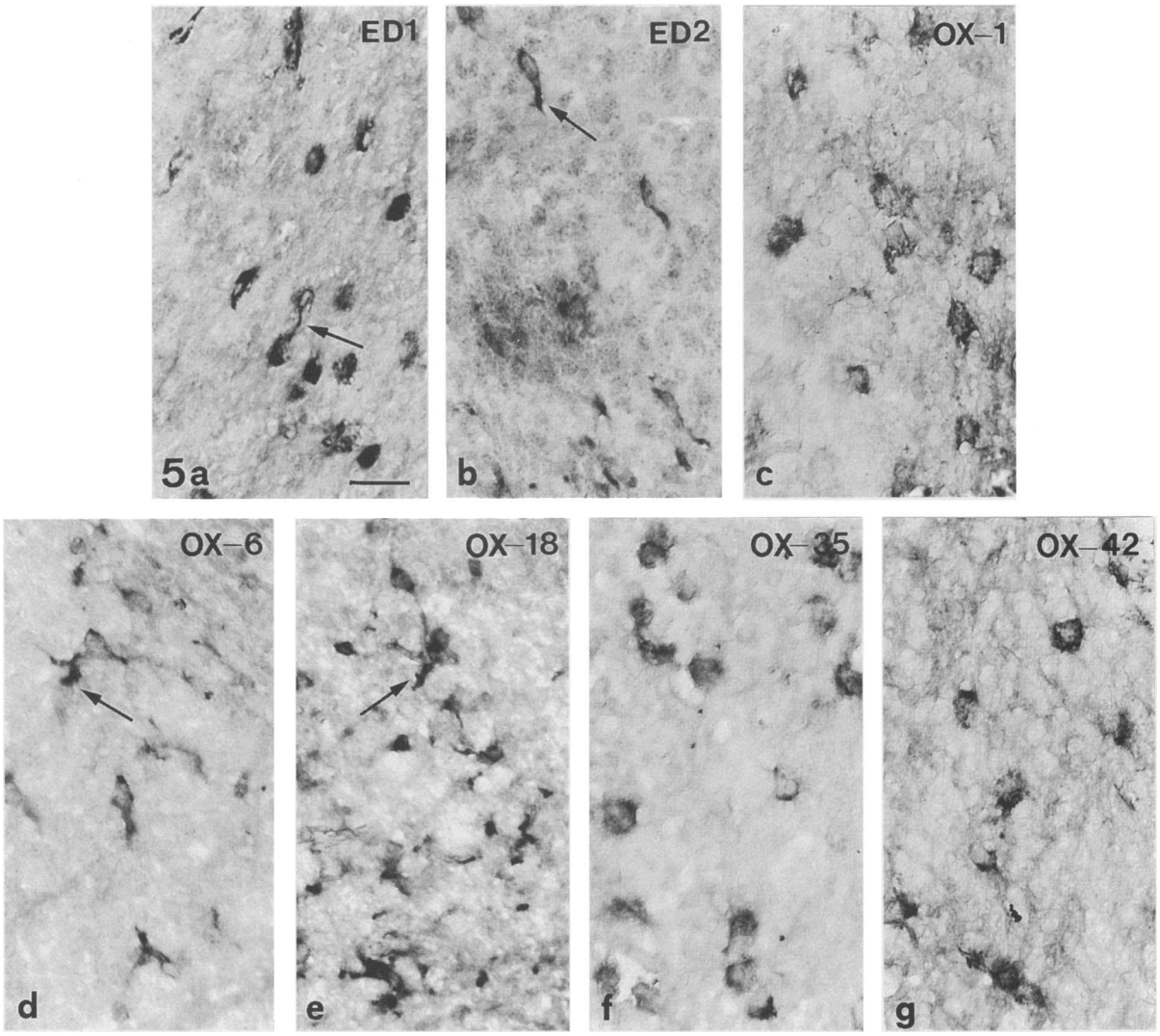

Fig. 5*a–g*. Expression of different antigens by AMC in the IZS at E20. During this stage, the AMC exhibit immunoreactivities comparable to those cells at E16. Note that the cells labelled by ED1, ED2, OX-6 or OX-18 immunomolecules (*a*, *b*, *d*, *e*, arrows) begin to project short and stout processes. Bar, 20 μm.

Table. *Immunophenotypic profiles of amoeboid microglial cells in fetal rat brain extending from E12 to E20*

Antibodies/age	E12	E14	E16	E18	E20
ED1	±	+	++	+++	+++
ED2	±	+	++	+	++
OX-1	−	+	++	++	+++
OX-6	−	+	+	+	++
OX-18	−	−	++	+	++
OX-35	−	+	++	+	+++
OX-42	−	+	++	+	++

±, sporadic cells; +, a few cells; ++, many cells; +++, large number of cells.

The latter authors described that primitive/fetal macrophages differentiated directly from their haematopoietic precursor cells without passing through the stages of promonocyte and monocyte in various tissues.

On infiltration into the developing nervous tissues, monocytes probably differentiate rapidly into typical macrophages, i.e. AMC, as reflected by the full expression of different antigens such as ED1, ED2, OX-1, OX-6, OX-35 and OX-42 at E14. A similar phenomenon has been documented by Kutteh et al. (1991), who demonstrated that human fetal macrophages not only possessed macrophage markers but also expressed mature macrophage functions in early gestation. The rapid expression of different antigens may have been induced by their ambient microenvironment as suggested by Oliver (1990), who demonstrated that human fetal macrophages in various tissues were extremely sensitive to environmental changes and responded rapidly by up-regulating their surface molecules. Thus the transient reduction in immunoreactivities of OX-6, OX-18, OX-35 and OX-42 antigens on AMC at E18 may have

resulted from the altered microenvironment with the establishment of nonfenestrated endothelium in the developing cerebrum (Yoshida et al. 1988).

The present study has shown the occurrence of ED1 or ED2 positive cells, albeit in small numbers, in the brain parenchyma as early as E12. Judging by their morphology, they can be identified with confidence as AMC. At E14, OX-6 labelled AMC made their first appearance in the neuroepithelia. In the same period, OX-1, OX-35 and OX-42 labelled cells were also detected in the intermediate zone of the fetal cortex. OX-18 labelled cells, however, were not detected until E16 in the IZS. This is contrary to the finding of Sminia et al. (1987) who reported the lack of MHC class II antigen of cells in prenatal rat brain. They reported that the first occurrence of ED1 or ED2 labelled macrophages was restricted to the meninges and choroid plexus at E16. Labelled cells were undetectable in the cerebral parenchyma until birth. The discrepancy may be due to different immunohistochemical procedures used.

With HRP-conjugated lectin (GSA-IB4) labelling method, AMC were first detected in the fetal rat forebrain as early as E11 (Ashwell, 1991). However, this study has shown that the first macrophage antigens (ED1, ED2) were not detected until E12, suggesting that the expression of lectin receptor precedes other surface antigens constitutively expressed by AMC. The reason for this is uncertain although it is speculated that lectin receptor is probably crucial to signal the early differentiation of the cell type. The functional significance of early expression of ED1 and ED2 on AMC remains speculative. It has been described that the amount of ED1 expression can be correlated with phagocytic activity (Milligan et al. 1991*b*; Bauer et al. 1994). Furthermore, the alteration of this antigen is indicative of cell activation (Graeber et al. 1990). The present results showed that early AMC, the majority being round cells and containing ED1 antigen, exhibited a stronger immunoreactivity when compared with those bearing short processes. It is thus possible that this may be related to their phagocytic activities in the course of cell differentiation.

A noteworthy feature in the present study was the occurrence of some AMC bearing MHC class II antigen (OX-6) which is hardly detectable in postnatal brain except when induced by lipopolysaccharide or interferon-gamma (Xu & Ling, 1994*a–c*). The functional significance of the prenatal expression of this antigen remains speculative. It has been demonstrated that MHC class II (Ia) antigen expression on microglial cells in vitro and in vivo may be induced by certain cytokines or factors (Wong et al. 1985; Steiniger & van der Meide, 1988; Frei & Fontana, 1989; Vass & Lassmann, 1990; Sethna & Lampson, 1991). Among these, interferon-gamma has been shown to be a potent inducer in Ia antigen expression on microglia. Against this view, however, is that so far interferon has not been detected in the fetus.

Microglial cells are known to express MHC class II antigen in inflammation (Perry & Gordon, 1988). The expression of Ia-antigen is indicative of cells involved in antigen presentation (Unanue, 1984). It is possible that microglia may serve as antigen-presenting cells in initiating the immune response in the CNS (Frei et al. 1987). Banati & Graeber (1994), however, found that microglia could sense even the slightest alteration in their microenvironment, such as changes in the extracellular concentration of ions, and responded rapidly by upregulating their surface molecules such as complement receptors and MHC molecules. They suggested that the expression of MHC antigen on microglia did not necessarily equal their antigen presentation capacity. Since the AMC in fetal rat brain constitutively expressed MHC class II antigen, it is not unreasonable to speculate that they are endowed with the capacity in antigen presenting function. On the other hand, it is also not impossible that the MHC antigen expression by these cells resulted from the alteration of the microenvironment, especially when the blood–brain barrier in prenatal brain is not fully developed, allowing the free access of serum-derived substances.

Finally, another interesting finding in this study was the demonstration of CD4 receptor on AMC, a feature hitherto undescribed in fetal brain. Unanue (1978) has categorised CD4 with LCA and MHC class II as antigens involved in antigen presentation. This being so, it is not surprising to note their coexistence in AMC.

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