Pergamon

Progress in Neurobiology Vol. 56, pp. 173 to 189, 1998
© 1998 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0301-0082/98/$19.00

PII: S0301-0082(98)00035-5

THE ORIGIN AND DIFFERENTIATION OF MICROGLIAL CELLS DURING DEVELOPMENT

MIGUEL A. CUADROS* and JULIO NAVASCUÉS

Departamento de Biología Celular, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain

(*Received* 30 *January* 1998)

Abstract—Some authors claim that microglia originate from the neuroepithelium, although most now believe that microglial cells are of mesodermal origin, and probably belong to the monocyte/macrophage cell line. These cells must enter the developing central nervous system (CNS) from the blood stream, the ventricular space or the meninges. Afterward microglial cells are distributed more or less homogeneously through the entire nervous parenchyma. Stereotyped patterns of migration have been recognized during development, in which long-distance tangential migration precedes radial migration of individual cells. Microglial cells moving through the nervous parenchyma are ameboid microglia, which apparently differentiate into ramified microglia after reaching their definitive location. This is supported by the presence of cells showing intermediate features between those of ameboid and ramified microglia. The factors that control the invasion of the nervous parenchyma, migration within the developing CNS and differentiation of microglial cells are not well known. These phenomena apparently depend on environmental factors such as soluble or cell-surface bound molecules and components of the extracellular matrix. Microglial cells within the developing CNS are involved in clearing cell debris and withdrawing misdirected or transitory axons, and presumably support cell survival and neurite growth. © 1998 Elsevier Science Ltd. All rights reserved

CONTENTS

1. Introduction 173
2. Origin of microglia 174
   2.1. Origin from monocytes/macrophages 174
   2.2. Origin from primitive hemopoietic cells 175
   2.3. Origin from neuroectodermal cells 176
3. Invasion of the developing CNS by microglial precursors 176
   3.1. Entry of microglial precursors from the blood stream 176
   3.2. Entry of microglial precursors from the ventricle 177
   3.3. Entry of microglial precursors from the meninges 178
   3.4. Factors guiding the entry into the developing CNS 178
4. Spreading of microglial precursors within the CNS: proliferation and migration 178
   4.1. Proliferation 178
   4.2. Migration 178
      4.2.1. Steps in the migration of microglial cells 179
      4.2.2. Factors that control migration of microglial cells 179
5. Differentiation of microglial cells 182
6. Role of microglial cells during development 183
7. Conclusions 185
   Acknowledgements 185
   References 185

ABBREVIATIONS

CNS	Central nervous system
CSF-1	Colony stimulating factor-1
G-CSF	Granulocyte-colony stimulating factor
ICAM-1	Intercellular adhesion molecule-1
IL-1	Interleukin-1
IL-6	Interleukin-6
LFA-1	Lymphocyte function-associated antigen-1
M-CSF	Macrophage-colony stimulating factor
MCP-1	Monocyte chemoattractant protein-1
NGF	Nerve growth factor
NT-3	Neurotrophin-3
PCNA	Proliferating cell nuclear antigen
TNF	Tumor necrosis factor

* Corresponding author. Tel.: +34.958.246332/ + 34.958.243232; Fax: 34 958 243258; e-mail: macuadro@goliat.ugr.es.

1. INTRODUCTION

Microglial cells were reported to exist within the central nervous system (CNS) nearly a century ago [see Barron (1995)]. Although del Rio-Hortega (1932) provided a complete framework for defining this cell type, many features of microglia are still controversial. An example is the two opposing hypotheses on the origin of microglial precursors that continue to be defended more than half a century after del Rio-Hortega's paper. One hypothesis states that microglial precursors are cells of neuroectodermal origin like neurons and the remaining glial cells; the other proposes that they proceed from mesodermal cells and therefore originate outside the developing nervous tissue. If the second theory is true, how and when microglia precursors enter the nervous parenchyma must be determined. Regardless of their origin, these cells must migrate through and differentiate in the entire CNS.

This review deals with the origin and development of microglia within the developing normal brain. It is convenient to distinguish between:

1. the production of microglia during development; and
2. the origin and differentiation of cells responsible for microglial turnover in adult life and microglial production under pathological conditions (cerebral ischemia, infection, mechanical or chemical injury,...), because different mechanism are probably involved in these situations.

This article refers only to the first topic; readers interested in the biology of the adult microglia and their function in the normal and pathological brain should consult recent reviews (Perry and Gordon, 1991; Thomas, 1992; Banati *et al*., 1993; Banati and Graeber, 1994; Barron, 1995; Gehrmann *et al*., 1995; Streit, 1995; Kreutzberg, 1996; Moore and Thanos, 1996).

2. ORIGIN OF MICROGLIA

As indicated already, two principal views about the origin of microglia currently exist:

1. microglial cells are of mesodermal origin; and
2. microglial cells originate from neuroepithelial cells.

View (1) is sustained by a large proportion of authors, who believe that microglia derive either from monocytes that leave the blood stream and colonize the nervous parenchyma, or from primitive (or stem) hemopoietic cells that differentiate as microglial cells within the CNS.

2.1. Origin from Monocytes/Macrophages

del Rio-Hortega (1932) postulated that "the microglia arises from polyblasts or embryonic cells of the meninges", stating that microglial cells were of mesenchymal, non-neuroectodermal origin. He remarked that microglial cells invading the brain from the meninges had features similar to these of blood leukocytes (del Rio-Hortega, 1932), and were therefore probably blood-borne cells. Microglial cells and cells of monocytic lineage share several features, such as the presence in monocytes and all or some types of microglia of particular enzymes, as nucleoside diphosphatase, non-specific esterase and acid phosphatase (Boya *et al*., 1979; Ling *et al*., 1982; Murabe and Sano, 1982a; Fujimoto *et al*., 1987, 1989; Castellano *et al*., 1991). Microglial and monocytic cells contain large amounts of vault particles (Chugani *et al*., 1991) and are labeled by several lectins (Streit and Kreutzberg, 1987; Hutchins *et al*., 1990; Acarin *et al*., 1994). Moreover, antibodies that recognize both microglia and monocytic cells have been developed in a number of species, as fish (Dowding *et al*., 1991), amphibians (Goodbrand and Gaze, 1991), birds (Jeurissen *et al*., 1988; Cuadros *et al*., 1992), rodents (Imamura *et al*., 1990; Gehrmann and Kreutzberg, 1991; Perry and Gordon, 1991; Flaris *et al*., 1993) and humans (Penfold *et al*., 1991; Paulus *et al*., 1992). These findings, together with the phagocytic properties of microglial cells, suggest that microglia are related to monocytic cells and belong to the mononuclear phagocytic system.

However, other studies appear to contradict the monocytic origin of microglia. First, ramified microglia do not contain some of the markers shared by ameboid microglia with cells of monocytic lineage (Oehmichen *et al*., 1979; Wood *et al*., 1979; Streit, 1995). Second, some markers that label macroglial cells have been reported also to label microglia (Dickson and Mattiace, 1989; Wolswijk, 1995). And third, microglial cells have features different from other cells of the mononuclear phagocytic system: they display particular morphological features *in vitro* (Giulian *et al*., 1995), proliferate spontaneously in culture (Giulian *et al*., 1995), and have a distinct ion channel pattern different from that of monocytes and macrophages in non-nervous tissues (Kettenmann *et al*., 1990; Brockhaus *et al*., 1993).

The *op*/*op* mutant mouse shows a systemic lack of colony stimulating factor-1 (CSF-1, also known as macrophage-colony stimulating factor, M-CSF) and macrophage populations are severely affected in different tissues (Wiktor-Jedrzejczak *et al*., 1992). However, the microglia population is apparently normal in the non-injured brain of mutant mice (Naito *et al*., 1991; Blevins and Fedoroff, 1995), suggesting, therefore, that microglia are different from macrophages in other tissues. Cultures of brain cortex cells from *op*/*op* mice demonstrated that microglial cells require the presence of CSF-1 for their development (Blevins and Fedoroff, 1995); the presence of normal numbers of microglia in the brain of *op*/*op* mice was explained proposing that the lack of CSF-1 was compensated *in vivo* by other factors produced locally in the CNS (Blevins and Fedoroff, 1995). Therefore, the results provided by the *in vivo* and *in vitro* studies are compatible both with the microglia belonging to the monocyte/macrophage cell line and also with them not being related to this cell line.

To elucidate whether microglial cells derive from monocytic cells, Ling *et al*. (Ling, 1979; Ling *et al*., 1980) injected either an ink solution or carbon-labeled monocytes into the blood stream of newborn rats. Because they found some ameboid and rami-

fied microglia which contained carbon particles, they concluded that microglia originated from monocytes which enter the nervous parenchyma from the blood stream (Ling, 1981).

The idea that microglial cells are of monocyte/macrophage lineage has received support from other kinds of studies. Cells with morphological features of microglia and with a pattern of membrane currents characteristic of microglia develop from monocytes or non-nervous tissue macrophages which are cultured on an astrocyte monolayer (Schmidtmayer *et al*., 1994; Sievers *et al*., 1994). At present, it is not known whether all cells of the macrophage/monocyte lineage can produce microglia cells, or whether this ability is limited to a special subset of such cells. Giulian *et al*. (1995) found that mononuclear phagocytes isolated from the brain of newborn rats gave rise to cells with particular morphological features which did not appear in cultured mononuclear phagocytes from non-nervous sources; these authors concluded that microglial precursors are a unique class of cell different from precursors of other types of mononuclear phagocytes. In this connection, it was found that a subpopulation of bone marrow-derived cells showed the same ion channel pattern as microglial cells, suggesting that the bone marrow contains precursors that are committed to produce microglia and that they are different from the precursors which produce macrophages for other body regions (Banati *et al*., 1991).

2.2. Origin from Primitive Hemopoietic Cells

However, macrophages/microglial cells appear within the CNS before it is vascularized (Ashwell, 1991; Sorokin *et al*., 1992; Cuadros *et al*., 1993; Wang *et al*., 1996) and before monocytes are produced in hemopoietic tissues (Sorokin *et al*., 1992; Hurley and Streit, 1996; Naito *et al*., 1996). Therefore, not all microglial cells can originate from circulating monocytes during development. Another possibility is that some or all microglial cells derive from undifferentiated hemopoietic cells which colonize the developing CNS independently of its vascularization (Hurley and Streit, 1996). In this regard Alliot *et al*. (1991) noted that hemopoietic cells which can differentiate into microglial cells are present in the bone marrow and in the nervous parenchyma of both the adult and developing CNS of mice. The presence of macrophages of hemopoietic origin within the early nervous parenchyma has been established using quail–chick embryo chimeras (Cuadros *et al*., 1993). Although it is possible that these embryonic macrophages give rise to the population of microglial cells in the adult, the connection between them and microglial cells has not been conclusively established. In fact, embryonic macrophages might also leave the CNS or degenerate after fulfilling their functions during development, and therefore they would not be microglial precursors.

The hypothesis that microglial cells are of hemopoietic origin has been tested in mammals using bone marrow chimeras (Matsumoto and Fujiwara, 1987; Hickey and Kimura, 1988; De Groot *et al*., 1992; Hickey *et al*., 1992; Unger *et al*., 1993; Krall *et al*., 1994). One of these studies (Matsumoto and Fujiwara, 1987) failed to observe microglial cells of bone marrow origin, and concluded that microglia were probably of neuroectodermal origin. Hickey and Kimura (1988) showed that the perivascular microglia are bone marrow-derived. In contrast, very few parenchymal microglial cells of donor origin were observed despite the fact that non-microglial cells derived from the graft were common within the nervous parenchyma of normal and pathological brains (Hickey and Kimura, 1988; Hickey *et al*., 1992; Lassmann *et al*., 1993). These authors suggested that parenchymal microglia were of neuroectodermal origin, or that they derived from bone marrow-derived precursors which enter the nervous system before or about the time of birth. In an attempt to explain the presence of microglial precursors in the embryonic or early postnatal brain, De Groot *et al*. (1992) transplanted bone marrow cells to irradiated adult and 1 day old mice. Only *ca* 10% of the resting microglia were of donor origin in both newborn and adult mice. Although the authors noted that microglial precursors might enter the CNS from fetal hemopoietic organs during embryonic stages, they sustained that the microglia comprise a heterogeneous population of mainly neuroectodermal origin. A study by Krall *et al*. (1994) showed that *ca* 20% of microglial cells (mainly perivascular cells) were replaced by cells of donor origin 6–8 months after bone marrow transplant in mice; these results were in accordance with those reported by Kennedy and Abkowitz (1997). Finally, a recent paper (Eglitis and Mezey, 1997) confirmed that transplant-derived cells colonize the CNS of bone marrow-chimeric mice; certain of the cells of donor origin were identified as microglia, but surprisingly, some astrocytes were also found to be of donor origin.

The differences in the results obtained from bone marrow chimeras may have resulted from differences in the species used in these studies, the age at grafting, and the strategies used to reveal donor-origin cells. In several of these studies antibodies against specific major histocompatibility class I antigens (MHC I) molecules were used to detect cells of donor origin (Matsumoto and Fujiwara, 1987; Hickey and Kimura, 1988; Hickey *et al*., 1992). Because MHC I are not constitutively expressed in normal rat brains (Flaris *et al*., 1993), it was necessary to induce upregulation of these molecules to disclose the presence of labeled cells. Differences in the induced expression of MHC I and the slow turnover of microglial cells in the adult brain (Lawson *et al*., 1992) may explain why Matsumoto and Ikuta (1985) found no labeled ramified microglia, whereas Hickey and co-workers (Hickey and Kimura, 1988; Hickey *et al*., 1992) did identify a few ramified microglia of donor origin.

The results obtained in bone marrow–chimera experiments do not rule out the possibility that microglia precursors originate in embryonic hemopoietic organs at early stages of development. In rodents, microglial precursors seem to enter the CNS from the last days of embryonic life until the third postnatal week, and most of the microglial precursors would presumably have left the bone marrow or other hemopoietic organs by the time De Groot *et*

al. (1992) irradiated newborn mice in order to construct chimeric mice. Thus the results from bone marrow–chimeric rodents do not have still provided conclusive results regarding the origin of microglia.

2.3. Origin from Neuroectodermal Cells

Several authors have sustained that at least some microglial cells are of neuroectodermal lineage. This view results from different sort of studies. Autoradiographic analyses of the genesis of microglia within the mouse hippocampus showed that microglial cells seemed to derive from glioblasts that also produce astrocytes; this conclusion was based on a presumed continuous morphological transition from proliferating glioblasts to resting microglia (Kitamura *et al.*, 1984). The finding of microglial cells within the matrix cell layer during development has been considered an indication of the neuroepithelial origin of microglia (Hutchins *et al.*, 1990). However, the microglial cells within the neuroepithelium may also be cells that are traversing the neuroepithelial layer after entering the nervous parenchyma from the ventricle (Cuadros *et al.*, 1994).

It was found that monoclonal antibodies against the protein lipocortin-1 label both a fraction of neuroepithelial cells and microglial cells in the developing rat brain, thus suggesting that microglial cells originate within the neuroepithelium (Fedoroff, 1995; McKanna and Fedoroff, 1996). In addition, some of the antibodies that recognize microglial cells also label a proportion of cells of neuroectodermal origin (Dickson and Mattiace, 1989; McKanna, 1993; Navascués *et al.*, 1994; Wolswijk, 1995). Although these observations appear to support the idea that microglial cells are of neuroectodermal lineage, it should be recalled that sharing some antigenic markers does not mean that two cell types also share the same origin. At the present more markers linking microglial cells to the macrophage/monocyte lineage are known than to other cell type.

Macrophage-like cells and/or microglia are produced in cultures of embryonic neuroepithelium (Hao *et al.*, 1991; Richardson *et al.*, 1993; Papavasiliou *et al.*, 1996). This finding suggests that microglial cells may derive from embryonic neuroepithelial cells. Moreover, macrophage/microglial cells were produced in mouse neuroepithelial cell cultures thought to be free of potential microglial precursors of mesenchymal origin after selective elimination of cells bearing the Mac-1 antigen, present in macrophages and microglial cells (Hao *et al.*, 1991). However, the macrophage-like cells produced in these cultures might derive from cells without Mac-1 immunoreactivity which had previously invaded the developing CNS. In this connection Alliot *et al.* (1991) inferred that microglial cells may derive from cells in the nervous parenchyma which have not yet acquired the Mac-1 epitope.

A recent article (Fedoroff *et al.*, 1997) has introduced new elements to the controversy about the origin of microglial cells, sustaining that astrocytes and microglial cells may derive from the same progenitor cell. This conclusion was supported by the finding of clones derived from single newborn mouse brain cells which contained both astrocytes (recognized by the labeling with anti-GFAP antibodies) and microglial cells (marked with Mac-1 antibody). These results may be explained in two ways:

1. that microglial cells (or a fraction of them) are of neuroepithelial origin, as astrocytes are thought to be;
2. that astrocytes, like microglial cells, can be derived from hematopoietic cells that have entered into the CNS.

This second possibility challenges the idea that astrocytes are of neuroectodermal lineage, but agrees with the finding by Eglitis and Mezey (1997) that astrocytes of donor origin appear in mice subjected to bone marrow grafting.

3. INVASION OF THE DEVELOPING CNS BY MICROGLIAL PRECURSORS

If microglial precursors originate outside the nervous parenchyma, they need to enter it at some point. This probably occurs for the most part before the nervous system is mature. In the quail many microglial precursors enter the nervous parenchyma during the last week of incubation (Cuadros *et al.*, 1994, 1997; Navascués *et al.*, 1995), and they appear for the first time within the rodent nervous system during the end of embryonic life and first days of postnatal life (Perry *et al.*, 1985; Milligan *et al.*, 1991a; Perry and Gordon, 1991). The entry of microglial precursors during embryonic or early postnatal life, together with proliferation of such cells within the CNS (Dalmau, 1997; Dalmau *et al.*, 1997a), should provide enough precursors to produce the mature microglial population, which shows slow turnover in the adult (Lawson *et al.*, 1992). In the adult, new microglial cells appear presumably through mechanisms different from those involved in the early migration of precursors, and will therefore not be discussed here.

Three routes of microglial precursor invasion have been proposed. Microglial precursors may enter the nervous parenchyma:

1. from the blood stream, by traversing the endothelial wall;
2. from the ventricles, by traversing the ventricular layer; or
3. from the meninges, by traversing the pial surface.

Each of these routes will be discussed. Invasion of the nervous parenchyma from the blood stream is apparently related with the hemopoietic (primitive or monocytic) origin of microglial precursors; however, the other routes of invasion do not preclude this origin, as hemopoietic cells may pass into the ventricle or migrate into the meninges after leaving the hemopoietic organs or blood stream, and then enter the developing nervous parenchyma.

3.1. Entry of Microglial Precursors from the Blood Stream

Migration from the blood stream is consistent with the blood-borne origin of microglial cells, and

has received experimental support from studies in which labeled microglial cells were observed within the nervous parenchyma after circulating blood cells were labeled (Ling, 1979; Ling *et al*., 1980). Cells entering via this route probably adhere to the vessel wall and subsequently gain access to the nervous parenchyma in a process similar to that described for the invasion of other tissues by cells of the macrophage/monocyte lineage (Cramer, 1992). The LFA-1/ICAM-1 (lymphocyte function-associated antigen-1/intercellular adhesion molecule-1) adhesion system, one of the systems involved in these processes, has been implicated in the entry of leukocytes into the lesioned brain parenchyma (Akiyama *et al*., 1994). This system may participate in the entry of microglial precursors into the developing rat brain from the blood stream: upregulation of ICAM-1 in the endothelial cells of the developing brain vessels coincides with the presence of numerous LFA-1-positive cells within the vessels and in the nervous parenchyma during the period when microglial precursors invade the brain (Dalmau *et al*., 1997b). Passage from the blood stream to the nervous parenchyma is easier in the developing brain than in the mature one, as the blood–brain barrier forms relatively late; therefore, large numbers of microglial precursors might use this route to invade the developing CNS. Blood vessels are distributed throughout the nervous parenchyma, allowing microglial precursors that migrate across the endothelial wall to reach their final location in the CNS without having to migrate over long distances.

However, microglial cells also appear in regions of the developing CNS devoid of vascularization (Hurley and Streit, 1996). In the mammalian retina microglial cells appear well before of vascularization (Ashwell *et al*., 1989; Schnitzer, 1989; Diaz-Araya *et al*., 1995), and they spread throughout the entire avascular retina of the quail (Navascués *et al*., 1994, 1995). Eventually, some microglial cells are seen adhering to or near blood vessels (Murabe and Sano, 1982b; Navascués *et al*., 1996; Dalmau *et al*., 1997a), but microglial cells are not distributed around blood vessels during development, as would be expected if they came from the blood stream. Therefore, although some microglial precursors appear to enter the nervous parenchyma by traversing the endothelial wall, they likely also use other routes.

3.2. Entry of Microglial Precursors from the Ventricle

The idea that microglial precursors may reach the nervous parenchyma from the ventricle is based on the presence of cells of the monocyte/macrophage lineage in the ventricular lumen (Jordan and Thomas, 1988). Experimental injections of microcarrier beads coated with crotoxin showed that ventricular macrophages adhered to the beads and acquired a ramified appearance similar to that of the ramified microglia in the nervous parenchyma (Kaur *et al*., 1990). In addition, microglial cells have seen apparently in the process of traversing the ventricular surface during brain development (Cuadros *et al*., 1994; Cavalcante *et al*., 1995; Navascués *et al*., 1996) [Fig. 1(A)]. The relative contribution of

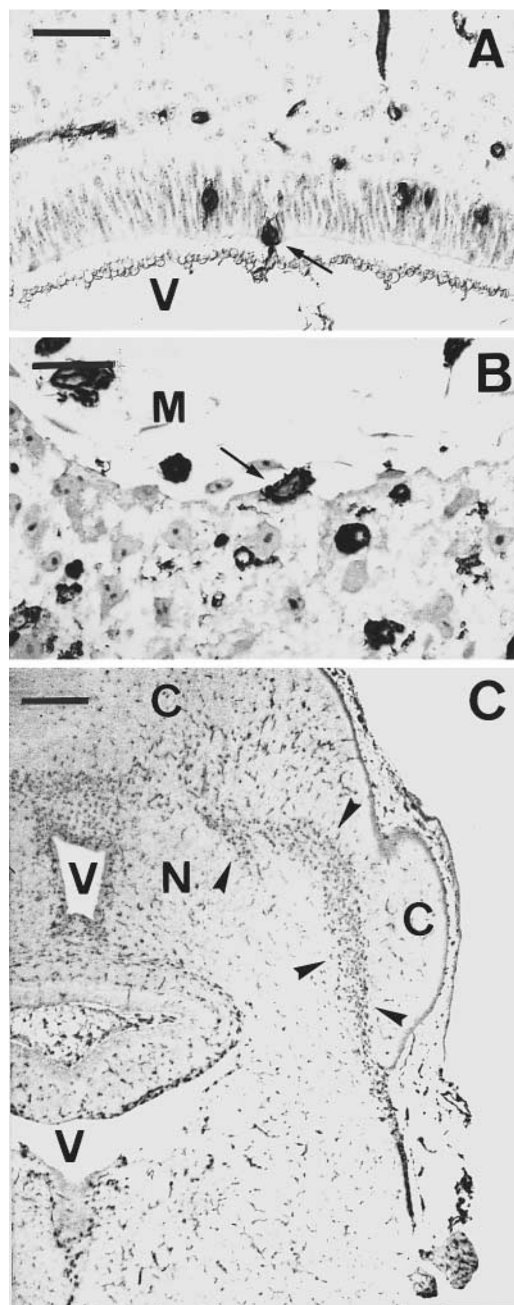

Fig. 1. (A) Microglial precursors labeled with the antibody QH1, located between the ventricle and nervous parenchyma in the optic tectum of a quail embryo after 11 days of incubation (E11). This image suggests that one microglial cell (arrow) is traversing the ventricular layer, presumably from the ventricular space (V) to the nervous parenchyma. Paraffin section. Scale bar: 50 μm. (B) Semithin section in the region of the basal cerebellar peduncles of an E11 quail embryo. QH1 immunocytochemistry. A QH1-positive cell (arrow) appears to be in the act of crossing the pial border. M, Meninges. Scale bar: 20 μm. (C) 'Stream' of QH1 labeled cells (arrowheads) within the cerebellar white matter in an E11 quail embryo. Note that the presumptive regions of the cerebellar nuclei (N) and cortical layers (C) are not invaded by the stream. V, Ventricle. Paraffin section. Scale bar: 300 μm.

precursors that use this route to the final number of microglial cells is not known, and presumably varies depending on the brain area considered.

3.3. Entry of Microglial Precursors from the Meninges

This route of entry of microglial precursors was first proposed by del Rio-Hortega (1921, 1932), who described the "sources of the microglia" as regions around the pial margin from which large numbers of microglial cells originated. This idea has been supported by later studies that showed microglial cells on the pial surface during the development (Boya *et al*., 1991; Cuadros *et al*., 1994, 1997; Navascués *et al*., 1996; Dalmau *et al*., 1997a), and by electron microscopic images that suggested that precursors of microglial cells traverse the pial border of the CNS (Boya *et al*., 1987). Large clusters of microglial precursors were found on both sides of the pial border at some points of the brain surface of the developing quail; some of the cells in these clusters were apparently in the act of traversing the pial surface [Fig. 1(B)]. Most microglial cells in the optic tectum (Cuadros *et al*., 1994) and cerebellum (Cuadros *et al*., 1997) seem to derive from precursors which have entered the nervous parenchyma by traversing the pial surface.

In addition to these points of 'massive' invasion through which large numbers of microglial cells apparently enter the nervous parenchyma, individual microglial precursors seem to traverse the pial surface at other points, such as the surface of the optic lobes (Cuadros *et al*., 1994) and the dorsal surface of the telencephalic vesicles (unpublished observations). This represents a 'diffuse' invasion through the pial surface. Diffuse invasion does not occur in the developing cerebellum (Boya *et al*., 1991; Cuadros *et al*., 1997), perhaps because the external granule layer, which is directly beneath the pial surface during embryonic life, prevents this invasion.

In all, there is evidence in support of each of the proposed routes of invasion of the developing CNS described above, and it seems that microglial precursors use all the three. It has been suggested that cells entering the nervous tissue via different pathways constitute different subpopulations of microglial cells (Provis *et al*., 1996).

3.4. Factors Guiding the Entry Into the Developing CNS

Because invasion of microglial precursors takes place in a consistent manner, there must be specific factors that guide the process. The number of available microglial precursors is probably highest during the invasion period, when the CNS is most easily invaded. Several authors have stressed that the appearance of large numbers of microglial precursors or brain macrophages within the developing brain coincides with cell death phenomena (Perry *et al*., 1985; Ashwell, 1991; Milligan *et al*., 1991a; Perry and Gordon, 1991). However, little is known about the relationship between cell death and microglial invasion. Both disease and injury of the CNS in rats and mice induces the expression of genes that code for a monocyte/macrophage chemoattractant factor, monocyte chemoattractant protein-1 (MCP-1) (Glabinski *et al*., 1996; Ivacko *et al*., 1997); this or similar chemoattractants are likely involved in the recruitment of mononuclear phagocytes from the blood stream, and perhaps also from the surrounding nervous parenchyma (see Section 4.2.2). However, it is not known whether these factors released during the process of cell degeneration come into play during development.

4. SPREADING OF MICROGLIAL PRECURSORS WITHIN THE CNS: PROLIFERATION AND MIGRATION

Microglial cells appear in all regions of the mature CNS, although their density varies between areas of the nervous parenchyma (Lawson *et al*., 1990). Two mechanisms may contribute to the spreading of microglial cells throughout the nervous parenchyma: proliferation and migration.

4.1. Proliferation

Microglia show proliferative activity in the normal adult brain (Lawson *et al*., 1992) which greatly increases when microglia activate in injured brain (Streit *et al*., 1988; Reid *et al*., 1993; Amat *et al*., 1996). Brain macrophages and immature microglia in the developing CNS also have mitotic activity, as revealed by the uptake of labeled nucleotides or by the presence of proliferating cell nuclear antigen (PCNA) (Mander and Morris, 1996; Cossmann *et al*., 1997; Dalmau, 1997; Marín-Teva *et al*., 1998). The relative contribution of proliferation to the increase in number of microglial cells during development has not been established.

The proliferation of microglial cells within the developing CNS depend on external factors. *In vitro* studies have characterized several mitogens for brain macrophages/microglia (Suzumura *et al*., 1990; Elkabes *et al*., 1996). As some of these molecules are present in the developing CNS (Giulian *et al*., 1991), they would be involved in the control of the proliferation of microglial cells during development.

4.2. Migration

Evidently, the distance that the microglial cells have to cover through the developing nervous parenchyma depends on their initial and final location points. Microglial cells that enter the CNS by traversing the endothelial wall may leave the blood stream in regions near their final location, and therefore need not migrate through long distances in the nervous parenchyma. In contrast, microglial precursors that enter through the ventricular or meningeal surfaces must cover long distances to colonize areas far from their entry point. Several studies have shown that microglial cells move through the nervous tissue: microglial cell migration has been demonstrated in the frog CNS after the injection of cobalt (Lázár and Pál, 1996), after resection of the facial nucleus of the rat (Angelov *et al*., 1995), after

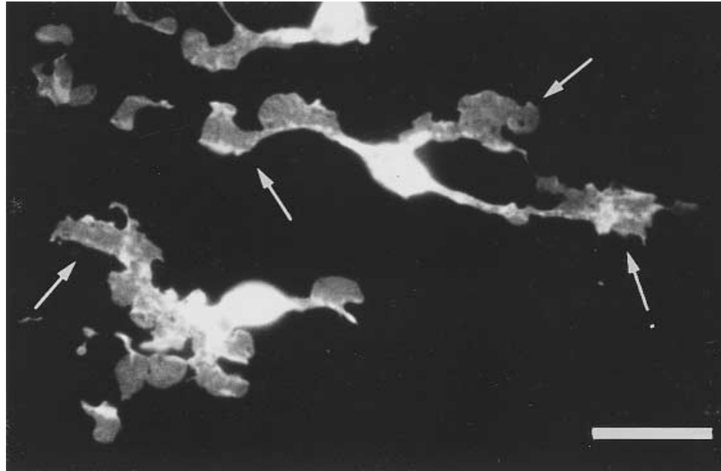

Fig. 2. Migrating ameboid microglial cells in the nerve fiber layer of an E10 quail embryo. The general orientation of the cells is from the retinal center towards the periphery, although the length and shape of cell processes vary. Note the presence of numerous lamellipodia (arrows). QH1 labeling with a fluorescein-conjugated secondary antibody on a preparation of the vitreal part of the retina as described in Marín-Teva *et al.* (1998). Scale bar: 17 μm.

focal lesion in the rabbit retina (Humphrey and Moore, 1996), and in the retina of rats with inherited photoreceptor degeneration (Thanos, 1992; Thanos and Richter, 1993; Roque *et al.*, 1996).

The mode of microglial cell migration in the developing CNS has been investigated in embryonic quail retinas (Marín-Teva *et al.*, 1998). This study reveals that microglial cells move on Müller cells endfeet like fibroblasts migrating on a two-dimensional substratum (Fig. 2). Little is known, however, about the movement of microglial cells through regions of the CNS with a more complex organization.

4.2.1. *Steps in the Migration of Microglial Cells*

Studies in the developing quail have documented long-distance migration of microglial precursors in several CNS regions such as the retina (Navascués *et al.*, 1995), optic tectum (Cuadros *et al.*, 1994) and cerebellum (Cuadros *et al.*, 1997) (Fig. 3). Migration of the microglial precursors in these regions occurs in two different steps. During the first step, called ‘tangential migration’, many microgial precursors move together from their entry point within the nervous parenchyma. In this step the cells move on an oriented substrate: microglial precursors apparently migrate parallel to the axon fascicles of the nerve fiber layer in the retina (Navascués *et al.*, 1995) [Fig. 3(A)], of the stratum album centrale in the tectum (Cuadros *et al.*, 1994) [Fig. 3(B)] and of the cerebellar white matter (Cuadros *et al.*, 1997) [Fig. 1(C), Fig. 3(C and D)]. These oriented substrates may act as ‘highways’ which lead the microglial precursors deep within the nervous parenchyma, far from their entry points.

In the second step, ‘radial migration’, individual microglial cells change direction and move towards the pial (scleral in the retina) surface. As a consequence, microglial precursors reach all regions of the nervous parenchyma. Radial migration is not as easily observed as tangential migration, because each cell moves independently. In addition, it is more difficult to determine whether microglial cells move along preexisting substrates during radial migration, although preliminary observations in the developing retina suggest that the precursors move along the radial processes of Müller cells (Navascués *et al.*, 1996). Thus, microglial precursors leave the ‘highways’ and enter ‘secondary roads’ to reach their final destination within the nervous parenchyma (Fig. 4).

The sequence of events described above is apparent in regions of the developing quail brain with a laminar organization. At present we do not know whether it also occurs during the development of microglia in all regions of the developing CNS, and whether similar steps occur in other species, although similar events may explain observations in the developing brain of mammals (Murabe and Sano, 1982b; Perry *et al.*, 1985; Lent *et al.*, 1985; Milligan *et al.*, 1991a; Caggiano and Brunjes, 1993; Cavalcante *et al.*, 1995).

4.2.2. *Factors that Control Migration of Microglial Cells*

The fact that microglial cells migrate in a stereotypical manner suggests that migration is controlled by specific factors, although the nature of such factors is not known. Factors released during cell death may influence microglial migration, as several authors have reported that microglial precursors are attracted towards regions of the nervous parenchyma where intense cell death occurs (Hume *et al.*, 1983; Ashwell, 1991; Perry and Gordon, 1991; Pearson *et al.*, 1993), and accumulations of macrophages/microglial cells appear in regions of the developing CNS where intense cell death occurs (Ashwell, 1990, 1991; Milligan *et al.*, 1991a; Johnson and Berman, 1996) or where transitory axonal projections are being eliminated (Innocenti *et al.*, 1983). In addition, microglial cells increase in number and/or move in response to cell death pro-

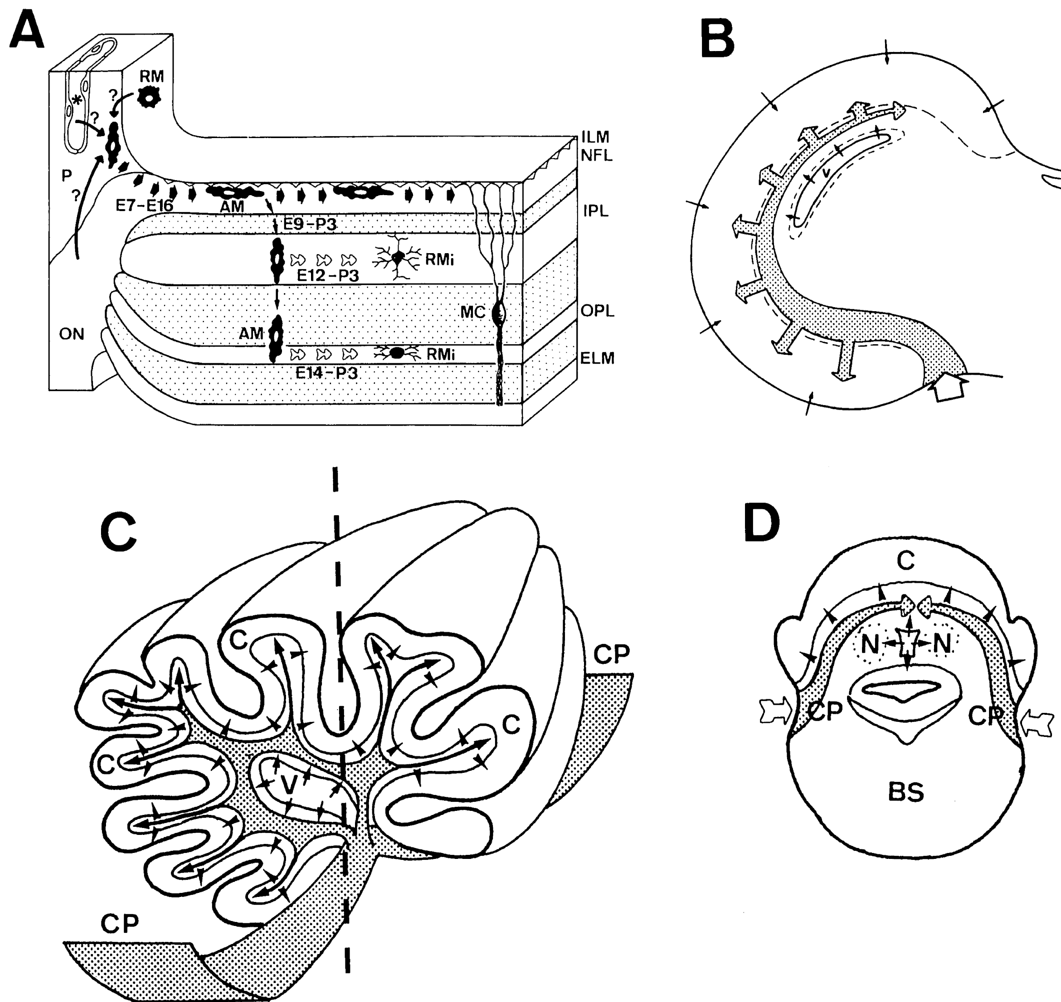

Fig. 3. Schematic representation of the routes of migration of microglial precursors during development of (A) the retina, (B) the optic tectum and (C and D) the cerebellum in the quail. (A) Microglial precursors seem to enter the retina from the pecten (P). Curved arrows with question marks indicate that these precursors may reach the pecten by different routes: they can migrate from the optic nerve (ON), from the blood vessels of the pecten (*), or from the vitreal space, where they appear as round macrophages (RM). In any case ameboid macrophages (AM) enter the retina between the embryonic day 7 (E7) and E16, and migrate tangentially (thick solid arrows) through the nerve fiber layer (NFL) until they reach the peripheral regions of the retina. Beginning at E9 and until posthatching day 3 (P3), individual cells change the direction of their movement and migrate radially to colonize the inner (IPL) and outer (OPL) plexiform layers. Morphological differentiation of microglial cells (indicated by thick open arrows) begins at E12 in the IPL, and at E14 in the OPL, and is complete in both layers at P3, when only ramified microglia (RMi) are seen in these layers. ILM, internal limiting membrane; ELM, external limiting membrane; MC, Müller cell. (B) Most microglial precursors apparently enter the tectum from the pial surface at the caudal ventromedial tectum (thick open arrow) and invade the entire stratum album centrale (dotted region), where they migrate tangentially. From there they migrate radially toward more external regions (dotted arrows). In addition, single microglial cells may enter the tectum by traversing the ventricular surface and by crossing the pial surface at other points (thin arrows). (C) Three-dimensional drawing of an E11–12 cerebellum sectioned along a parasagittal plane. (D) Transverse section at the level marked by the vertical dotted line in (C). Large number of microglial precursors enter the cerebellum from the meninges at the base of the cerebellar peduncles (CP) at the points marked by arrows in (D). These cells migrate tangentially through the white matter; the area of this migration corresponds to the stippled region in (C) and (D). Finally, single microglial cells migrate radially (arrowheads in C and D), colonizing the cortical layers (C) where they differentiate. In addition, other microglial precursors may enter by traversing the ventricular layer (small arrows in C and D) or the endothelial wall (not shown). BS, Brainstem; N, cerebellar nuclei; V, ventricle. (A) Taken from Cuadros *et al.* (1994); (B) from Navascués *et al.* (1995); (C and D) from Cuadros *et al.* (1997).

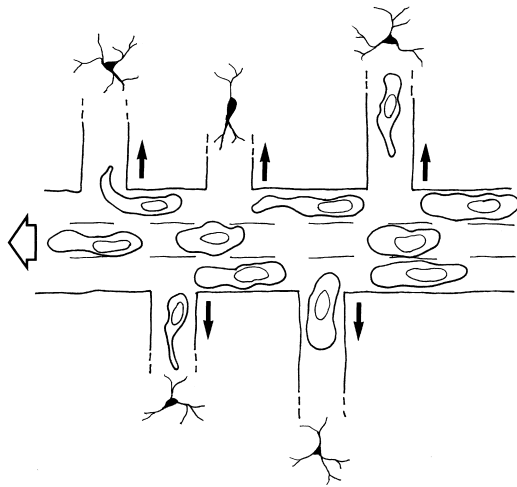

Fig. 4. Schematic drawing illustrating the two steps of migration of microglial precursors described in the text. Tangential migration occurs along the 'highway,' where all cells move in the same direction (indicated by the thick open arrow). At some points individual cells leave the highway and move along 'secondary roads' (whose direction is indicated by arrows) to reach specific points within the nervous parenchyma. Finally, microglial cells differentiate in these specific points.

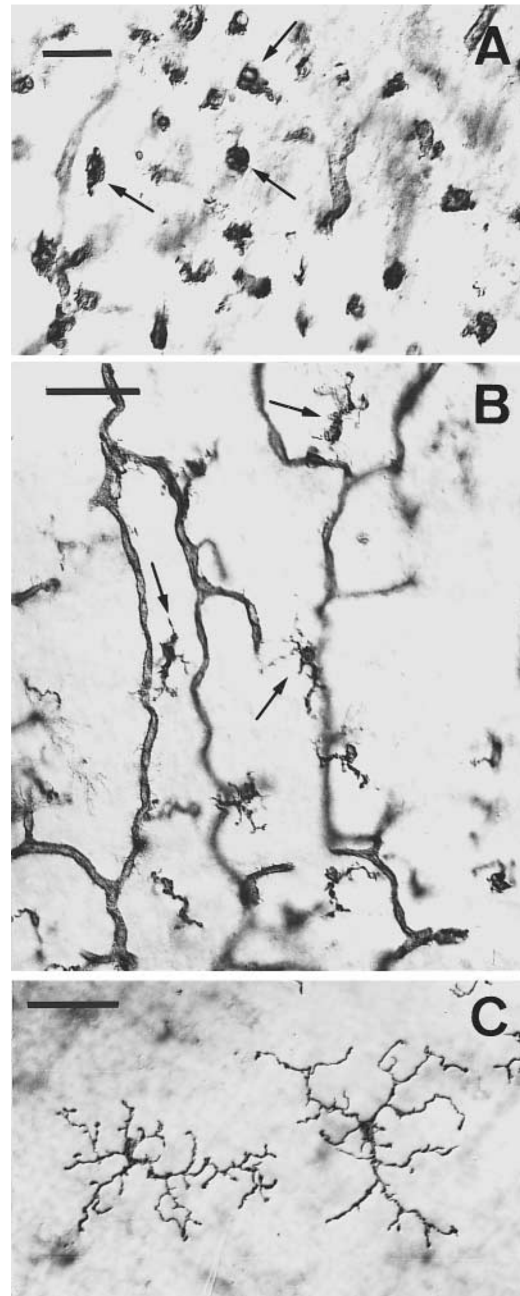

Fig. 5. Morphological features of microglial cells during development of the quail CNS as seen with QH1 immunocytochemistry. (A) Ameboid microglia (arrows) in the optic tectum of an E12 embryo. Scale bar: 30 μm. (B) Poorly ramified cells (some labeled by arrows) located in the optic tectum of an E12 embryo. Scale bar: 50 μm. (C) Mature microglia in the outer nerve layer of the adult retina. In this layer nearly all microglial processes are within the same plane, making it possible to observe the pattern of ramification. Scale bar: 50 μm. (A and B) Vibratome sections; (C), retinal wholemount.

cesses (Thanos, 1992; Thanos and Richter, 1993; Ferrer *et al.*, 1995; Nitatori *et al.*, 1995; Finsen *et al.*, 1996; Lázár and Pál, 1996; Roque *et al.*, 1996). Microglial cells would therefore behave like embryonic neurons whose migration in the the adult brain is guided by cell death phenomena (Sheen and Macklis, 1995).

Several factors have been isolated in the injured brain that may mediate the concentration of microglia in the affected area. Increase of chemoattractant chemokines (such as MCP-1) occurs associated with degenerative processes (Hulkower *et al.*, 1993; Glabinski *et al.*, 1996; Mallat *et al.*, 1996). These factors are produced by brain macrophages/microglial cells, which therefore contribute to the recruitment of cells of the same type, and also by non-microglial cells (Ransohoff *et al.*, 1993; Mallat *et al.*, 1996). It has been demonstrated that some of these factors are also released in the immature brain after injury (Ivacko *et al.*, 1997), but at the moment we do not know if they influence the migration of microglial cells in the developing normal brain.

However, the distribution of microglial cells during development frequently does not coincide with intense cell death (Milligan *et al.*, 1991a; Cuadros *et al.*, 1994, 1997; Parnaik *et al.*, 1996), and no relationship was found between the density of microglial cells and the intensity of cell death in particular regions during development (Lawson *et al.*, 1990). Therefore, other factors in addition to degeneration phenomena must also participate in controlling migration.

Microglial cells may recognize contact-guidance factors bound to the substrate on which they move. In this respect tangential migration of interneuron precursors in the mouse olfactory bulb requires NCAM-polysialic acid, whereas radial migration of these cells does not depend on the presence of this molecule (Hu *et al.*, 1996). We currently know of no molecules with a similar effect on the migration of microglial precursors, although microglial cells express receptors for molecules of the extracellular

matrix and the surface of other cells (Akiyama and McGeer, 1990; Sébire *et al*., 1993), and produce thrombospondin, which increases adhesion and migration, during development (Chamak *et al*., 1995). These facts have been related mainly to phagocytosis and the promotion of neurite growth from differentiating neurons, but they may also be involved in the migration of microglial precursors within the developing CNS. In addition, microglia in culture produce and activate enzymes which degrade components of the extracellular matrix (Nakajima *et al*., 1992; Gottschall *et al*., 1995; Washington *et al*., 1996). These enzymes enhance neurite outgrowth (Nakajima *et al*., 1993; Seeds *et al*., 1996), are involved in the spread and migration of glial precursors (Amberger *et al*., 1997), and probably also facilitate the migration of microglial cells through the nervous parenchyma, although their production by migrating ameboid cells during development has not yet been established.

5. DIFFERENTIATION OF MICROGLIAL CELLS

Microglial cells apparently pass through different stages of development (Fig. 5). In the first stage, ameboid microglia, have morphological, histochemical and immunological features similar to those of macrophages outside the CNS, and are therefore also known as brain macrophages. Ameboid microglia are round or have short, broad processes; presumably, cells of dendritic or elongated morphology also belong to this type of microglia, as seen in the developing retina, tectum and cerebellum of the quail (Cuadros *et al*., 1994, 1997; Navascués *et al*., 1995; Marín-Teva *et al*., 1998). Part of the ameboid cells present in the embryonic brain may degenerate (Dalmau, 1997), as occurs with activated microglia in the adult brain (Reid *et al*., 1993; Gehrmann and Banati, 1995; Jones *et al*., 1997), but many of them would develop thin processes and become ramified. These phenomena may lead to the disappearance of

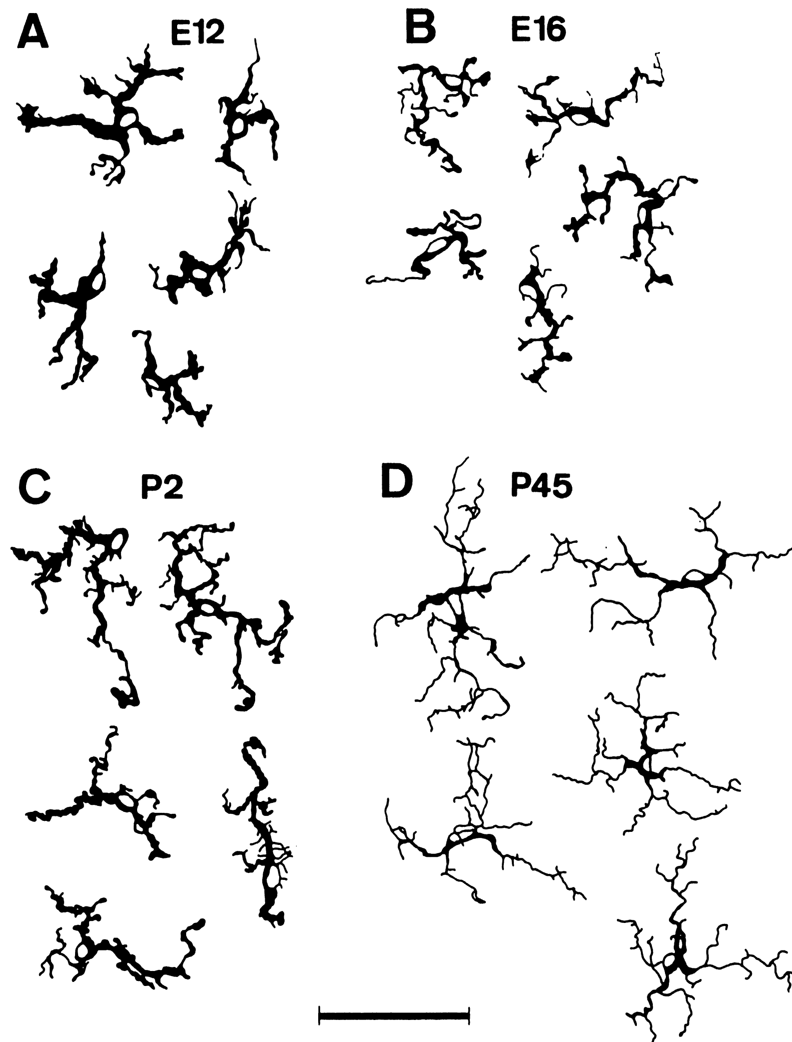

Fig. 6. Camera lucida drawings of differentiating ramified microglial cells in the developing optic tectum of the quail. Cells from (A) E12, (B) E16, (C) P2 and (D) adult quails are illustrated. Note the progressive increase in branching pattern complexity and the tapering of cell processes during development. Scale bar: 50 μm. Taken from Cuadros *et al*. (1994).

all ameboid microglia, which are no longer detectable in mature brains. The complexity of ramification of the microglial processes increases during development, making it possible to distinguish poorly ramified from fully ramified, or mature, microglia (Fig. 6).

These events were described by del Rio-Hortega (1932), and received later support from morphological observations of intermediate forms between ameboid and ramified microglial cells (Ling, 1981; Perry *et al*., 1985; Leong and Ling, 1992; Wu *et al*., 1992, 1993; Cuadros *et al*., 1994). The transformation of ameboid microglia into ramified microglia is now largely accepted (Perry and Gordon, 1988; Ling and Wong, 1993; Davis *et al*., 1994; Barron, 1995). *In vitro* studies have shown that cultured microglial cells become ramified under certain conditions, such as culture on astrocyte monolayers (Sievers *et al*., 1994, 1996; Tanaka and Maeda, 1996), when certain factors are added to the culture medium (Suzumura *et al*., 1991; Giulian *et al*., 1995; Fujita *et al*., 1996; Eder *et al*., 1997), and in the presence of certain extracellular matrix molecules (Chamak and Mallat, 1991; Giulian *et al*., 1995). The addition of serum to the culture medium inhibits the appearance of the ramified phenotype (Chamak and Mallat, 1991; Giulian *et al*., 1995; Fujita *et al*., 1996; Ilschner and Brandt, 1996), although one recent study reported that microglial cells do not ramify when serum is absent (Wilms *et al*., 1997). The distribution and properties of microtubules change during the transformation of microglial cells in culture: ramified microglia show more stable microtubules, with a higher rate of acetylation and detyrosination than in ameboid microglia, and microtubules in ramified cells are apparently not anchored to microtubule organizing centers (Ilschner and Brandt, 1996). The transformations observed *in vitro* probably reflect changes that occur during CNS development.

It has been suggested that the variability in microglial cells in different regions of the brain depends on environmental factors (Lawson *et al*., 1990; Sievers *et al*., 1996; Vela *et al*., 1995). Microglial cells in regions exposed to plasma proteins are less ramified than microglia in regions of the brain parenchyma with a complete blood–brain barrier which excludes plasma proteins (Perry and Gordon, 1991). Because the CNS is a changing environment during development, its properties at each developmental age and in each area might influence microglial differentiation.

Several authors have concluded that ameboid cells or brain macrophages are not the origin of ramified microglia. In support of this view is that ameboid microglia do not show the same histochemical and immunocytochemical characteristics as ramified microglia. For example, Matsumoto and Ikuta (1985) claimed that brain macrophages (ameboid microglia) appearing during brain development are not related to microglial cells in the adult brain because they do not share certain antigens with ramified microglia. Therefore, these authors concluded that ameboid microglia do not produce ramified microglia.

An immunocytochemical study by Wu *et al*. (1994) described gradual modifications (downregulation) in the membrane glycoprotein(s) recognized by the isolectin B_4 of *Bandeiraea simplicifolia* during transformation of ameboid microglia into ramified microglia. The labeling of ramified microglia with this lectin was weaker than that of brain macrophages. Similar phenomena must occur with other molecules that label both ameboid and ramified microglia, as labeling intensity normally decreases as ramification becomes more complex. The decrease in labeling intensity may lead to a lack of staining in ramified microglia, as occurs with the antibody ED-1 in the rat (Flaris *et al*., 1993), although traces of ED-1 labeling may be recognized in some ramified microglia (Milligan *et al*., 1991a). Many of the histochemical and immunohistochemical features present in ameboid microglia but lost in ramified microglia reappear in the latter cells after activation (Streit *et al*., 1988; Davis *et al*., 1994). Microglial cells become labeled after they phagocytose axons which have been previously marked. A few days after phagocytosis the only labeled cells are ameboid microglial cells, whereas labeled ramified microglia appear in later stages (Bechmann and Nitsch, 1997). These observations suggest that ameboid microglial cells became ramified resting cells. Similar conclusions have been drawn from experiments in which prelabeled ameboid-like microglial cells were placed in organotypic hippocampal cultures; after 9 days *in vitro*, they gradually transformed into highly ramified cells (Hailer *et al*., 1997). Therefore, ameboid and ramified microglia are apparently two different stages of the same cell type.

6. ROLE OF MICROGLIAL CELLS DURING DEVELOPMENT

Microglial cells participate in many of the complex morphogenetic and histogenetic processes which take place during the development of CNS in order to establish the complex network of connections present in the adult. They remove dead cell fragments (Ferrer *et al*., 1990; Ashwell, 1990, 1991) and eliminate transitory or aberrant axons (Innocenti *et al*., 1983; Ashwell, 1990). In addition to acting as scavengers of cell and axon debris, microglial cells may also play more active roles during cell degeneration, for example by inducing the death of some cells (Mallat and Chamak, 1994; Ashwell and Bobryshev, 1996). In this connection it has been reported that macrophages elicit cell death of endothelial cells during regression of transient blood vessels in the developing rodent eye (Lang *et al*., 1994), and ameboid microglia favor the death of some kinds of developing neurons *in vitro* (Théry *et al*., 1991).

Microglial cells are also involved in phenomena which increase the complexity of the CNS. They produce trophic factors that support the development and normal function of neurons and glia (Nagata *et al*., 1993; Elkabes *et al*., 1996; Jonakait *et al*., 1996; Mazzoni and Kenigsberg, 1997). Much evidence suggests that microglial cells participate in the growth and guidance of neurites within the developing CNS. Macrophages direct the growth of neurites from motoneurons (Stolz *et al*., 1991) and

transform CNS regions which did not previously allow axonal growth into permissive substrates (David *et al.*, 1990). Moreover, factors released by microglial cells during development or injury may help promote axon growth (Nakajima *et al.*, 1993; Chamak *et al.*, 1994, 1995). Recent findings have demonstrated that microglial cells enhance axon growth *in vivo* (Rabchevsky and Streit, 1997). Microglial cells also promote the proliferation of astrocytes, increase myelinogenesis and stimulate the vascularization of the CNS (Giulian *et al.*, 1988; Hamilton and Rome, 1994).

Many of the effects of microglial cells mentioned here are apparently mediated by active substances. Microglial cells in culture produce a number of such substances as nerve growth factor (NGF), neurotrophin-3 (NT-3), interleukins 1 and 6 (IL-1 and IL-6), macrophage and granulocyte colony-stimulating factors (M-CSF and G-CSF) and tumor necrosis factor α (TNF-α) (Mallat *et al.*, 1989, 1996; Lee *et al.*, 1993; Benveniste, 1995; Elkabes *et al.*, 1996). In addition to acting on other populations of the nervous system, products released by microglial cells also regulate their own proliferation and function (Elkabes *et al.*, 1996). Brain macrophages express the gene that codes for M-CSF during development (Théry *et al.*, 1990), but it has not yet been established what factors are in fact produced by macrophages/microglia during development *in vivo*, and what their role is in the building of the CNS.

The distribution of microglial cells during development probably depends on the functions that they fulfil, but at least in part, this distribution may simply be a consequence of their migration to their final locations in the adult CNS. This is similar to the situation in neuroblasts, which play no specific role before their reach their final location. Therefore the presence of microglial cells in a particular region of the developing CNS may not be related with phenomena other than their own development.

It is ameboid microglia which play active roles during development. When they differentiate into ramified microglia, they become less active and progressively acquire the behavior of adult microglia. This transformation is reflected by the fact that microglial cells in the adult and developing brain respond differently to similar stimuli (Milligan *et al.*, 1991b; Lawson and Perry, 1995).

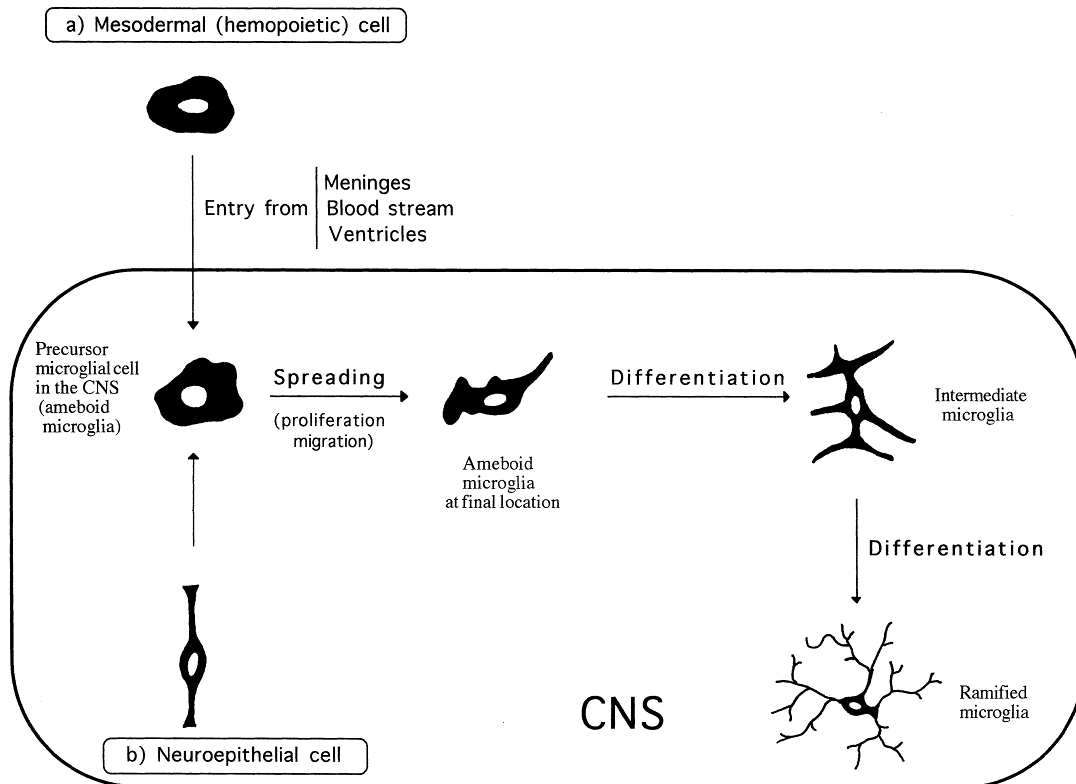

Fig. 7. Schema showing the proposed differentiation process of microglial cells during development. Most authors believe that microglial precursors within the CNS originate from mesodermal cells (hypothesis a) which enter the developing CNS through different routes. Some authors, however, support that these microglial precursors are of neuroepithelial origin (hypothesis b). The term 'ameboid microglia' designates cells of microglial lineage that show morphological features different from these of mature microglia and, therefore, should include the first microglial precursors in the nervous parenchyma and the early stages of microglial differentiation. Ameboid microglia spread through the CNS ('spreading') to reach their final location, where they differentiate ('differentiation') until they become fully ramified microglia.

7. CONCLUSIONS

Figure 7 provides a summary of the proposed process of microglial cell appearance during development. Although controversy still exists, some events are likely to occur:

1. Microglial cells derive from blood cells, or more likely, from cells of the blood cell lineage.
2. During development microglial precursors enter the CNS via different routes: from the meninges, from the ventricular lumen or from the blood stream.
3. Precursors migrate within the nervous parenchyma to their final location.
4. Microglial cells show features of ameboid microglia while they move through the nervous parenchyma, but differentiate and become mature ramified microglia once they reach their final location.

Particular aspects of this sequence of events may be restricted to some species and/or regions of the developing brain, and other aspects remain to be fully documented. Many of these processes are believed to occur in other systems, as they would explain many observations regarding microglial cells during development. More work remains to be done to precisely determine the early origin of microglial cells, the mechanisms used by microglial precursors to enter the nervous parenchyma and migrate through it, and the role of microglial cells within the developing CNS. Because of increasing interest in the microglia, many of these questions about the origin and differentiation of microglia may well be answered in the coming years.

Acknowledgements—Thanks are due to A. Almendros, R. Calvente, J. L. Marín-Teva, A. Moujahid, A. Quesada and J. Rodríguez-Ruiz, who participated in original work at our laboratory that is reported here. The authors are grateful to Dr B. Castellano for his critical reading of the manuscript. They would also like to thank Karen Shasok for improving the English style of the manuscript. Work at our laboratory was supported by Grant No. PB94-0789 from the Dirección General de Investigación Científica y Técnica (Spain).

REFERENCES

Acarin, L., Vela, J. M., González, B. and Castellano, B. (1994) Demonstration of poly-*N*-acetyl lactosamine residues in ameboid and ramified microglial cells in the rat brain by tomato lectin binding. *J. Histochem. Cytochem.* **42,** 1033–1041.

Akiyama, H. and McGeer, P. L. (1990) Brain microglia constitutively express β-2 integrins. *J. Neuroimmunol.* **30,** 81–93.

Akiyama, H., Tooyama, I., Kondo, H., Ikeda, K., Kimura, H., McGeer, E. G. and McGeer, P. L. (1994) Early response of brain resident microglia to kainic acid-induced hippocampal lesions. *Brain Res.* **635,** 257–268.

Alliot, F., Lecain, E., Grima, B. and Pessac, B. (1991) Microglial progenitors with a high proliferative potential in the embryonic and adult mouse brain. *Proc. Natl Acad. Sci. U.S.A.* **88,** 1541–1545.

Amat, J. A., Ishiguro, H., Nakamura, K. and Norton, W. T. (1996) Phenotypic diversity and kinetics of proliferating microglia and astrocytes following cortical stab wounds. *Glia* **16,** 368–382.

Amberger, V. R., Avellana-Adalid, V., Hensel, T., Baron-Van Evercooren, A. and Schwab, M. E. (1997) Oligodendrocyte-type 2 astrocyte progenitors use a metalloendoprotease to spread and migrate on CNS myelin. *Eur. J. Neurosci.* **9,** 151–162.

Angelov, D. N., Gunkel, A., Stennert, E. and Neiss, W. F. (1995) Phagocytic microglia during delayed neuronal loss in the facial nucleus of the rat: Time course of the neuronofugal migration of brain macrophages. *Glia* **13,** 113–129.

Ashwell, K. (1990) Microglia and cell death in the developing mouse cerebellum. *Dev. Brain Res.* **55,** 219–230.

Ashwell, K. (1991) The distribution of microglia and cell death in the fetal rat forebrain. *Dev. Brain Res.* **58,** 1–12.

Ashwell, K. W. S. and Bobryshev, Y. V. (1996) The developmental role of microglia. In: *Topical Issues in Microglia Research*, pp. 65–82. Eds. E. A. Ling, C. K. Tan and C. B. C. Tan. Singapore Neuroscience Association, Singapore.

Ashwell, K. W. S., Holländer, H., Streit, W. and Stone, J. (1989) The appearance and distribution of microglia in the developing retina of the rat. *Vis. Neurosci.* **2,** 437–448.

Banati, R. B. and Graeber, M. B. (1994) Surveillance, intervention and cytotoxicity: Is there a protective role of microglia? *Dev. Neurosci.* **16,** 114–127.

Banati, R. B., Hoppe, D., Gottman, K., Kreutzberg, G. W. and Kettenmann, H. (1991) A subpopulation of bone marrow-derived macrophage-like cells share a unique ion channel pattern with microglia. *J. Neurosci. Res.* **30,** 593–600.

Banati, R. B., Gehrmann, J., Schubert, P. and Kreutzberg, G. W. (1993) Cytotoxicity of microglia. *Glia* **7,** 111–118.

Barron, K. D. (1995) The microglial cell. A historical review. *J. Neurol. Sci.* **134**(Suppl.), 57–68.

Bechmann, I. and Nitsch, R. (1997) Astrocytes and microglial cells incorporate degenerating fibers following entorhinal lesion: a light, confocal and electron microscopical study using a phagocytosis-dependent labeling technique. *Glia* **20,** 145–154.

Benveniste, E. N. (1995) Cytokine production. In: *Neuroglia*, pp. 700–713. Eds. H. Kettenmann and B. R. Ransom. Oxford University Press, New York.

Blevins, G. and Fedoroff, S. (1995) Microglia in colony-stimulating factor-1 deficient *op/op* mice. *J. Neurosci. Res.* **40,** 535–544.

Boya, J., Calvo, J. and Prado, A. (1979) The origin of microglial cells. *J. Anat.* **129,** 177–186.

Boya, J., Carbonell, A. L., Calvo, J. and Borregón, A. (1987) Ultrastructural study on the origin of rat microglial cells. *Acta Anat.* **130,** 329–335.

Boya, J., Calvo, J. L., Carbonell, A. L. and Borregón, A. (1991) A lectin histochemistry study on the development of rat microglial cells. *J. Anat.* **175,** 229–236.

Brockhaus, J., Ilschner, S., Banati, R. B. and Kettenmann, H. (1993) Membrane properties of ameboid microglial cells in the corpus callosum slice from early postnatal mice. *J. Neurosci.* **13,** 4412–4421.

Caggiano, A. O. and Brunjes, P. C. (1993) Microglia and the developing olfactory bulb. *Neuroscience* **52,** 717–724.

Castellano, B., González, B., Jensen, M. B., Pedersen, E. B., Finsen, B. R. and Zimmer, J. (1991) A double staining technique for simultaneous staining of astrocytes and microglial cells in vibratome brain sections and astroglial cell cultures. *J. Histochem. Cytochem.* **39,** 561–568.

Cavalcante, L. A., Santoro, G. F., Barradas, P. C., Santos-Silva, A. and Carvalho, S. L. (1995) Lectin histochemistry of microglia in superior colliculus of the developing opossum. *Cienc. Cult.* **47,** 240–245.

Chamak, B. and Mallat, M. (1991) Fibronectin and laminin regulate the *in vitro* differentiation of microglial cells. *Neuroscience* **45,** 513–527.

Chamak, B., Morandi, V. and Mallat, M. (1994) Brain macrophages stimulate neurite growth and regeneration by secreting thrombospondin. *J. Neurosci. Res.* **38,** 221–233.

Chamak, B., Dobbertin, A. and Mallat, M. (1995) Immunohistochemical detection of thrombospondin in microglia in the developing rat brain. *Neuroscience* **69,** 177–187.

Chugani, D. C., Kedersha, N. L. and Rome, L. H. (1991) Vault immunofluorescence in the brain: New insights regarding the origin of microglia. *J. Neurosci.* **11,** 256–287.

Cramer, E. B.(1992) Cell biology of phagocyte migration from the bone marrow, out of the bloodstream, and across organ epithelia. In: *Inflammation: Basic Principles and Clinical Correlates* (2nd ed.), pp. 341–351. Eds. J. I. Gallin, I. M. Goldstein and R. Snyderman. Raven Press, New York.

Cossmann, P. H., Eggli, P. S., Christ, B. and Kurz, H. (1997) Mesoderm-derived cells proliferate in the embryonic central nervous system: confocal microscopy and three-dimensional visualization. *Histochem. Cell Biol.* **107,** 205–213.

Cuadros, M. A., Moujahid, A., Martín-Partido, G. and Navascués, J. (1992) Microglia in the mature and developing

quail brain as revealed by a monoclonal antibody recognizing hemopoietic cells. *Neurosci. Lett.* **148,** 11–14.

Cuadros, M. A., Martin, C., Coltey, P., Almendros, A. and Navascués, J. (1993) First appearance, distribution, and origin of macrophages in the early development of the avian central nervous system. *J. Comp. Neurol.* **330,** 113–129.

Cuadros, M. A., Moujahid, A., Quesada, A. and Navascués, J. (1994) Development of microglia in the quail optic tectum. *J. Comp. Neurol.* **348,** 207–224.

Cuadros, M. A., Rodríguez-Ruiz, J., Calvente, R., Almendros, A., Marín-Teva, J. L. and Navascués, J. (1997) Microglia development in the quail cerebellum. *J. Comp. Neurol.* **389,** 390–401.

Dalmau, I. (1997) Las celulas de microglia en el cerebro de la rata en desarrollo. Doctoral Thesis. Universidad Autonoma de Barcelona, Barcelona, Spain.

Dalmau, I., Finsen, B., Tønder, N., Zimmer, J., Gonzalez, B. and Castellano, B. (1997a) Development of microglia in the prenatal rat hippocampus. *J. Comp. Neurol.* **377,** 70–84.

Dalmau, I., Vela, J. M., Gonzalez, B. and Castellano, B. (1997b) Expression of LFA-1a and ICAM-1 in the developing rat brain: a potential mechanism for the recruitment of microglial cell precursors. *Dev. Brain Res.* **103,** 163–170.

David, S., Bouchard, C., Tsatas, O. and Giftochristos, N. (1990) Macrophages can modify the nonpermissive nature of the adult mammalian central nervous system. *Neuron* **5,** 463–469.

Davis, E. J., Foster, T. D. and Thomas, W. E. (1994) Cellular forms and functions of brain microglia. *Brain Res. Bull.* **34,** 73–78.

De Groot, C. J. A., Huppes, W., Sminia, T., Kraal, G. and Dijkstra, C. D. (1992) Determination of the origin and nature of brain macrophages and microglial cells in mouse central nervous system, using non-radioactive *in situ* hybridization and immunoperoxidase techniques. *Glia* **6,** 301–309.

Diaz-Araya, C. M., Provis, J. M., Penfold, P. L. and Billson, F. A. (1995) Development of microglial topography in human retina. *J. Comp. Neurol.* **363,** 53–68.

Dickson, D. W. and Mattiace, L. A. (1989) Astrocytes and microglia in human brain share an epitope recognized by a B-lymphocyte-specific monoclonal antibody (LN-1). *Am. Pathol.* **135,** 135–147.

Dowding, A. J., Maggs, A. and Scholes, J. (1991) Diversity amongst the microglia in growing and regenerating fish CNS: immunohistochemical characterization using FL.1, an anti-macrophage monoclonal antibody. *Glia* **4,** 345–364.

Eder, C., Klee, R. and Heinemann, U. (1997) Distinct soluble astrocytic factors induce expression of outward K+ currents and ramification of brain macrophages. *Neurosci. Lett.* **226,** 147–150.

Eglitis, M. A. and Mezey, E. (1997) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc. Natl Acad. Sci. U.S.A.* **94,** 4080–4085.

Elkabes, S., DiCicco-Bloom, E. M. and Black, I. B. (1996) Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J. Neurosci.* **16,** 2508–2521.

Fedoroff, S. (1995) Development of microglia. In: *Neuroglia*, pp. 162–181. Eds. H. Kettenmann and B. R. Ransom. Oxford University Press, New York.

Fedoroff, S., Zhai, R. and Novak, J. P. (1997) Microglia and astroglia have a common progenitor cell. *J. Neurosci. Res.* **50,** 477–486.

Ferrer, I., Bernet, E., Soriano, E., Del Rio, T. and Fonseca, M. (1990) Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. *Neuroscience* **39,** 451–458.

Ferrer, I., Olivé, M., Blanco, R., Ballabriga, J., Cinós, C. and Planas, A. M. (1995) Ameboid microglial response following X-ray-induced apoptosis in the neonatal rat brain. *Neurosci. Lett.* **193,** 109–112.

Finsen, B., Lehrmann, E., Castellano, B., Kiefer, R. and Zimmer, J. (1996) The role of microglial cells and brain macrophages in transient global cerebral ischemia. In: *Topical Issues in Microglia Research*, pp. 297–318. Eds. E. A. Ling, C. K. Tan and C. B. C. Tan. Singapore Neuroscience Association, Singapore.

Flaris, N. A., Densmore, T. L., Molleston, M. C. and Hickey, W. F. (1993) Characterization of microglia and macrophages in the central nervous system of rats: definition of the differential expression of molecules using standard and novel monoclonal antibodies in normal CNS and in four models of parenchymal reaction. *Glia* **7,** 34–40.

Fujimoto, E., Miki, A. and Mizoguti, H. (1987) Histochemical studies of the differentiation of microglial cells in the cerebral hemispheres of chick embryos and chicks. *Histochemistry* **87,** 209–216.

Fujimoto, E., Miki, A. and Mizoguti, H. (1989) Histochemical study of the differentiation of microglial cells in the developing human cerebral hemispheres. *J. Anat.* **166,** 253–264.

Fujita, H., Tanaka, J., Toku, K., Tateishi, N., Suzuki, Y., Matsuda, S., Sakanaka, M. and Maeda, N. (1996) Effects of GM-CSF and ordinary supplements on the ramification of microglia in culture: a morphometrical study. *Glia* **18,** 269–281.

Gehrmann, J. and Banati, R. B. (1995) Microglial turnover in the injured CNS: activated microglia undergo delayed DNA fragmentation following peripheral nerve injury. *J. Neuropathol. Exp. Morphol.* **54,** 680–688.

Gehrmann, J. and Kreutzberg, G. W. (1991) Characterisation of two new monoclonal antibodies directed against rat microglia. *J. Comp. Neurol.* **313,** 409–430.

Gehrmann, J., Matsumoto, Y. and Kreutzberg, G. W. (1995) Microglia: intrinsic immunoeffector cell of the brain. *Brain Res. Rev.* **20,** 269–287.

Giulian, D., Woodward, J., Young, D., Krebs, J. F. and Lachman, L. B. (1988) Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. *J. Neurosci.* **8,** 2485–2490.

Giulian, D., Johnson, B., Krebs, J. F., George, J. K. and Tapscott, M. (1991) Microglial mitogens are produced in the developing and injuried mammalian brain. *J. Cell Biol.* **112,** 323–333.

Giulian, D., Bartel, S., Broker, J., Li, X. and Kirkpatrick, J. B. (1995) Cell surface morphology identifies microglia as a distinct class of mononuclear phagocyte. *J. Neurosci.* **15,** 7712–7726.

Glabinski, A. R., Balasingam, V., Tani, M., Kunkel, S. L., Strieter, R. M., Yong, V. W. and Ransohoff, R. M. (1996) Chemokine monocyte chemoattractant protein-1 is expressed by astrocytes after mechanical injury to the brain. *J. Immunol.* **156,** 4363–4368.

Goodbrand, I. A. and Gaze, R. M. (1991) Microglia in tadpoles of *Xenopus laevis*: normal distribution and the response to optic nerve injury. *Anat. Embryol.* **184,** 71–82.

Gottschall, P. E., Yu, X. and Bing, B. (1995) Increased production of gelatinase B (matrix metalloproteinase-9) and interleukin-6 by activated rat microglia in culture. *J. Neurosci. Res.* **42,** 335–342.

Hailer, N. P., Heppner, F. L., Haas, D. and Nitsch, R. (1997) Fluorescent dye prelabelled microglial cells migrate into organotypic hippocampal slice cultures and ramify. *Eur. J. Neurosci.* **9,** 863–866.

Hamilton, S. P. and Rome, L. H. (1994) Stimulation of *in vitro* myelin synthesis by microglia. *Glia* **11,** 326–335.

Hao, C., Richardson, A. and Fedoroff, S. (1991) Macrophage-like cells originate from neuroepithelium in culture: characterization and properties of the macrophage-like cells. *Int. J. Dev. Neurosci.* **9,** 1–14.

Hickey, W. F. and Kimura, H. (1988) Perivascular microglial cells of the CNS are bone marrow derived and present antigen *in vivo*. *Science* **239,** 290–292.

Hickey, W. F., Vass, K. and Lassmann, H. (1992) Bone marrow-derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. *J. Neuropathol. Exp. Neurol.* **51,** 246–256.

Hu, H., Tomasiewicz, H., Magnuson, T. and Rutishauser, U. (1996) The role of polysialic acid in migration of olfactory bulb interneuron precursors in the subventricular zone. *Neuron* **16,** 735–743.

Hulkower, K., Brosnan, C. F., Aquino, D. A., Cammer, W., Kulshrestha, S., Guida, M. P., Rapoport, D. A. and Berman, J. W. (1993) Expression of CSF-1, c-fms, and MCP-1 in the central nervous system of rats with experimental allergic encephalomyelitis. *J. Immunol.* **150,** 2525–2533.

Hume, D. A., Perry, V. H. and Gordon, S. (1983) Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. *J. Cell Biol.* **97,** 253–257.

Humphrey, M. F. and Moore, S. R. (1996) Microglial responses to focal lesions of the rabbit retina: correlation with neural and macroglial reactions. *Glia* **16,** 325–341.

Hurley, S. D. and Streit, W. J.(1996) Microglia and the mononuclear phagocytic system. In: *Topical Issues in Microglia Research*, pp. 1–19. Eds. E. A. Ling, C. K. Tan and C. B. C. Tan. Singapore Neuroscience Association, Singapore.

Hutchins, K. D., Dickson, D. W., Rashbaum, W. K. and Lyman, W. D. (1990) Localization of morphologically distinct microglial populations in the developing human fetal brain: implications for ontogeny. *Dev. Brain Res.* **55,** 95–102.

Ilschner, S. and Brandt, R. (1996) The transition of microglia to a ramified phenotype is associated with the formation of stable acetylated and detyrosinated microtubules. *Glia* **18,** 129–140.

Imamura, K., Ito, M., Suzumura, A., Asai, J. and Takahashi, A. (1990) Generation and characterization of monoclonal antibodies against rat microglia and ontogenic distribution of positive cells. *Lab. Invest.* **63,** 853–861.

Innocenti, G. M., Clarke, S. and Koppel, H. (1983) Transitory macrophages in the white matter of the developing visual cortex. II. Development and relations with axonal pathways. *Dev. Brain Res.* **11,** 55–66.

Ivacko, J., Szaflarski, J., Malinak, C., Flory, C., Warren, J. S. and Silverstein, F. S. (1997) Hypoxic-ischemic injury induces monocyte chemoattractant protein-1 expression in neonatal rat brain. *J. Cereb. Blood Flow Metab.* **17,** 759–770.

Jeurissen, S. H. M., Jase, E. M., Koch, G. and De Boer, G. F. (1988) The monoclonal antibody CVI-CHNL-68.1 recognizes cells of the monocyte–macrophage lineage in chickens. *Dev. Comp. Immunol.* **12,** 855–864.

Johnson, J. K. and Berman, N. E. J. (1996) A transient phase of cell death in the developing medial forebrain of the perinatal ferret. *Dev. Brain Res.* **94,** 159–165.

Jonakait, G. M., Luskin, M. B., Wei, R., Tian, X. F. and Ni, L. (1996) Conditioned medium from activated microglia promotes cholinergic differentiation in the basal forebrain *in vitro*. *Dev. Biol.* **177,** 85–95.

Jones, L. L., Banati, R. B., Graeber, M. B., Bonfanti, L., Raivich, G. and Kreutzberg, G. W. (1997) Population control of microglia: does apoptosis play a role? *J. Neurocytol.* **26,** 755–770.

Jordan, F. L. and Thomas, W. E. (1988) Brain macrophages: questions of origin and interrelationship. *Brain Res. Rev.* **13,** 165–178.

Kaur, C., Ling, E. A., Gopalakrishnakone, P. and Wong, W. C. (1990) Response of intraventricular macrophages to crotoxin-coated microcarrier beads injected into the lateral ventricle of postnatal rats. *J. Anat.* **168,** 63–72.

Kennedy, D. W. and Abkowitz, J. L. (1997) Kinetics of central nervous system microglia and macrophage engrafment: analysis using a transgenic bone marrow transplantation model. *Blood* **90,** 986–993.

Kettenmann, H., Hoppe, D., Gottmann, K., Banati, R. and Kreutzberg, G. W. (1990) Cultured microglial cells have a distinct pattern of membrane channels different from peritoneal macrophages. *J. Neurosci. Res.* **26,** 278–287.

Kitamura, T., Miyake, T. and Fujita, S. (1984) Genesis of resting microglia in the gray matter of mouse hippocampus. *J. Comp. Neurol.* **226,** 421–433.

Krall, W. J., Challita, P. M., Perlmutter, L. S., Skelton, D. C. and Kohn, D. B. (1994) Cell expressing human galactocerebrosidase from a retroviral vector repopulate macrophages and central nervous system microglia after murine bone marrow transplantation. *Blood* **83,** 2737–2748.

Kreutzberg, G. W. (1996) Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* **19,** 312–318.

Lang, R., Lustig, M., Francois, F., Sellinger, M. and Plesken, H. (1994) Apoptosis during macrophage-dependent ocular tissue remodeling. *Development* **120,** 3395–3403.

Lassmann, H., Schmied, M., Vass, K. and Hickey, W. F. (1993) Bone marrow derived elements and resident microglia in brain inflammation. *Glia* **7,** 19–24.

Lawson, L. J. and Perry, V. H. (1995) The unique characteristics of inflammatory responses in mouse brain are acquired during postnatal development. *Eur. J. Neurosci.* **7,** 1584–1595.

Lawson, L. J., Perry, V. H., Dri, P. and Gordon, S. (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39,** 51–170.

Lawson, L. J., Perry, V. H. and Gordon, S. (1992) Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* **48,** 405–415.

Lázár, G. and Pál, E. (1996) Removal of cobalt-labeled neurons and nerve fibers by microglia from the frog's brain and spinal cord. *Glia* **16,** 101–107.

Lee, S. C., Liu, W., Roth, P., Dickson, D. W., Berman, J. W. and Brosnan, C. F. (1993) Macrophage colony-stimulating factor in human fetal astrocytes and microglia: differential regulation by cytokines and lipopolysaccharide, and modulation of class II MHC on microglia. *J. Immunol.* **150,** 594–604.

Lent, R., Linden, R. and Cavalcante, L. A. (1985) Transient populations of presumptive macrophages in the brain of the developing hamster, as indicated by endocytoxis of blood-borne horseradish peroxidase. *Neuroscience* **15,** 1203–1215.

Leong, S. K. and Ling, E. A. (1992) Amoeboid and ramified microglia: their interrelationship and response to brain injury. *Glia* **6,** 39–47.

Ling, E. A. (1979) Transformation of monocytes into ameboid microglia and into microglia in the corpus callosum of postnatal rats as shown by labelling monocytes by carbon particles. *J. Anat.* **128,** 847–858.

Ling, E. A. (1981) The origin and nature of microglia. In: *Advances in Cell Neurobiology*, Vol. 2, pp. 33–82. Eds. S. Fedoroff and L. Hertz. Academic Press, London.

Ling, E. A. and Wong, W. C. (1993) The origin and nature of ramified and ameboid microglia: a historical review and current concepts. *Glia* **7,** 9–18.

Ling, E. A., Penney, D. and Leblond, C. P. (1980) Use of carbon labelling to demonstrate the role of blood monocytes as precursors of the "amoeboid cells" present in the corpus callosum of postnatal rats. *J. Comp. Neurol.* **193,** 631–657.

Ling, E. A., Kaur, C. and Wong, W. C. (1982) Light and electron microscopic demonstration of non-specific esterase in ameboid microglial cells in the corpus callosum in postnatal rats: a cytochemical link to monocytes. *J. Anat.* **135,** 385–394.

Mallat, M. and Chamak, B. (1994) Brain macrophages: neurotoxic or neurotrophic effector cells? *J. Leukoc. Biol.* **56,** 416–422.

Mallat, M., Houlgatte, R., Brachet, P. and Prochiantz, A. (1989) Lipopolysaccharide-stimulated rat brain macrophages release NGF *in vitro*. *Dev. Biol.* **133,** 309–311.

Mallat, M., Calvo, C. F. and Dobbertin, A. (1996) Recruitment of brain macrophages: roles of cytokines and extracellular matrix proteins produced by glial or neuronal cells. *Braz. J. Med. Biol. Res.* **29,** 1173–1177.

Mander, T. H. and Morris, J. F. (1996) Development of microglia and macrophages in the postnatal rat pituitary. *Cell Tissue Res.* **286,** 347–355.

Marín-Teva, J. L., Almendros, A., Calvente, R., Cuadros, M. A. and Navascués, J. (1998) Tangential migration of ameboid microglia in the developing quail retina: mechanism of migration and migratory behavior. *Glia* **22,** 31–52.

Matsumoto, Y. and Ikuta, F. (1985) Appearance and distribution of fetal brain macrophages in mice. *Cell Tissue Res.* **239,** 271–278.

Matsumoto, Y. and Fujiwara, M. (1987) Absence of donor-type major histocompatibility complex class I antigen-bearing microglia in the rat central nervous system of radiation bone marrow chimeras. *J. Neuroimmunol.* **17,** 71–82.

Mazzoni, I. E. and Kenigsberg, R. L. (1997) Microglia from the developing rat medial septal area can affect cholinergic and GABAergic neuronal differentiation *in vitro*. *Neuroscience* **76,** 147–157.

McKanna, J. A. (1993) Primitive glial compartments in the floor plate of mammalian embryos: distinct progenitors of adult astrocytes and microglia support the notoplate hypothesis. *Persp. Dev. Neurobiol.* **1,** 245–255.

McKanna, J. A. and Fedoroff, S. (1996) Development of microglia *in situ* and in cultures. In: *Topical Issues in Microglia Research*, pp. 21–42. Eds. E. A. Ling, C. K. Tan and C. B. C. Tan. Singapore Neuroscience Association, Singapore.

Milligan, C. E., Cunningham, T. J. and Levitt, P. (1991a) Differential immunochemical markers reveal the normal distribution of brain macrophages and microglia in the developing rat brain. *J. Comp. Neurol.* **314,** 125–135.

Milligan, C. E., Levitt, P. and Cunningham, T. J. (1991b) Brain macrophages and microglia respond differently to lesions of the developing and adult visual system. *J. Comp. Neurol.* **314,** 136–146.

Moore, S. and Thanos, S. (1996) The concept of microglia in relation to central nervous system disease and regeneration. *Prog. Neurobiol.* **48,** 441–460.

Murabe, Y. and Sano, Y. (1982a) Morphological studies on microglia. V. Microglial cells in the cerebral cortex of the rat, with special reference to their possible involvement in synaptic function. *Cell Tissue Res.* **223,** 493–506.

Murabe, Y. and Sano, Y. (1982b) Morphological studies on neuroglia. VI. Postnatal development of microglial cells. *Cell Tissue Res.* **225,** 469–485.

Nagata, A., Takei, N., Nakajima, K., Saito, H. and Kohsaka, S. (1993) Microglial conditioned medium promotes survival and development of cultured mesencephalic neurons from embryonic rat brain. *J. Neurosci. Res.* **34,** 357–363.

Naito, M., Hayashi, S. I., Yoshida, H., Nishikawa, S. I., Schults, L. D. and Takahashi, K. (1991) Abnormal differentiation of tissue macrophage populations in "osteopetrosis" (*op*) mice defective in the production of macrophage colony-stimulating factor. *Am. J. Pathol.* **139,** 657–667.

Naito, M., Umeda, S., Yamamoto, T., Moriyama, H., Umezu, H., Hasegawa, G., Usuda, H., Shultz, L. D. and Takahashi, K. (1996) Development, differentiation, and phenotypic heterogeneity of murine tissue macrophages. *J. Leukoc. Biol.* **59,** 133–138.

Nakajima, K., Tsuzaki, N., Shimojo, M., Hamanoue, M. and Kohsaka, S. (1992) Microglia isolated from rat brain secrete a urokinase-type plasminogen activator. *Brain Res.* **577,** 285–292.

Nakajima, K., Nagata, K., Hamanoue, M., Takemoto, N. and Kohsaka, S. (1993) Microglia-derived elastase produces a low-molecular-weight plasminogen that enhances neurite outgrowth in rat neocortical explant cultures. *J. Neurochem.* **61,** 2155–2163.

Navascués, J., Moujahid, A., Quesada, A. and Cuadros, M. A. (1994) Microglia in the avian retina: immunocytochemical demonstration in the adult quail. *J. Comp. Neurol.* **350,** 171–186.

Navascués, J., Moujahid, A., Almendros, A., Marín-Teva, J. L. and Cuadros, M. A. (1995) Origin of microglia in the quail retina: central-to-peripheral and vitreal-to-scleral migration of microglial precursors during development. *J. Comp. Neurol.* **354,** 209–228.

Navascués, J., Cuadros, M. A. and Almendros, A. (1996) Development of microglia: Evidence from studies in the avian central nervous system. In: *Topical Issues in Microglia Research*, pp. 43–64. Eds. E. A. Ling, C. K. Tan and C. B. C. Tan. Singapore Neuroscience Association, Singapore.

Nitatori, T., Sato, N., Waguri, S., Karasawa, Y., Araki, H., Shibanai, K., Kominami, E. and Uchiyama, Y. (1995) Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. *J. Neurosci.* **15,** 1001–1011.

Oehmichen, M., Wiethölter, H. and Greaves, M. F. (1979) Immunological analysis of human microglia: lack of monocytic and lymphoid membrane differentiation antigens. *J. Neuropathol. Exp. Neurol.* **38,** 99–103.

Papavasiliou, A. K., Mehler, M. F., Dobrenis, K., Marmur, R., Mabie, P. C. and Kessler, J. A. (1996) Microglial lineage species are expressed in mammalian epidermal growth factor-generated embryonic neurospheres. *J. Neurosci. Res.* **46,** 49–57.

Parnaik, R., Neophytou, C., Scholes, J. and Raff, M. C. (1996) Clearance of apoptotic cells in the CNS. *Soc. Neurosci. Abst.* **22,** 1226.

Paulus, W., Roggendorf, W. and Kirchner, T. (1992) Ki-M1P as a marker for microglia and brain macrophages in routinely processed human tissues. *Acta Neuropathol.* **84,** 538–544.

Pearson, H. E., Payne, B. R. and Cunningham, T. J. (1993) Microglial invasion and activation in response to naturally occurring neuronal degeneration in the ganglion cell layer of the postnatal cat retina. *Dev. Brain Res.* **76,** 249–255.

Penfold, P. L., Madigan, M. C. and Provis, J. M. (1991) Antibodies to human leucocyte antigens indicate subpopulations of microglia in human retina. *Vis. Neurosci.* **7,** 383–388.

Perry, V. H. and Gordon, S. (1988) Macrophages and microglia in the nervous system. *Trends Neurosci.* **11,** 273–277.

Perry, V. H. and Gordon, S. (1991) Macrophages and the nervous system. *Int. Rev. Cytol.* **125,** 203–244.

Perry, V. H., Hume, D. A. and Gordon, S. (1985) Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience* **15,** 313–326.

Provis, J. M., Diaz, C. M. and Penfold, P. L. (1996) Microglia in human retina: a heterogeneous population with distinct ontogenies. *Persp. Dev. Neurobiol.* **3,** 213–222.

Rabchevsky, A. G. and Streit, W. J. (1997) Grafting of cultured microglial cells into the lesioned spinal cord of adults rats enhances neurite outgrowth. *J. Neurosci. Res.* **47,** 34–48.

Ransohoff, R. M., Hamilton, T. A., Tani, M., Stoler, M. H., Shick, H. E., Major, J. A., Estes, M. L., Thomas, D. M. and Tuohy, V. K. (1993) Astrocyte expression of mRNA encoding cytokines IP-10 and JE/MCP-1 in experimental autoimmune encephalomyelitis. *FASEB J.* **7,** 592–600.

Reid, D. M., Perry, H. H., Andersson, P. B. and Gordon, S. (1993) Mitosis and apoptosis of microglia *in vivo* induced by an anti-CR3 antibody which crosses the blood–brain barrier. *Neuroscience* **56,** 529–533.

Richardson, A., Hao, C. and Fedoroff, S. (1993) Microglia progenitor cells: a subpopulation in cultures of mouse neopallial astroglia. *Glia* **7,** 25–33.

del Rio-Hortega, P. (1921) El tercer elemento de los centros nerviosos. Histogénesis y evolución normal; éxodo y distribución regional de la microglía. *Mem. Real Soc. Esp. Hist. Nat.* **11,** 213–268.

del Rio-Hortega, P. (1932) Microglia. In: *Cytology and Cellular Pathology of the Nervous System*, Vol. 2, pp. 483-534. Ed. W Penfield. Paul B. Hoeber, New York.

Roque, R. S., Imperial, C. J. and Caldwell, R. B. (1996) Microglial cells invade the outer retina as photoreceptors degenerate in Royal College of Surgeons rats. *Invest. Ophthalmol. Vis. Sci.* **37,** 196–201.

Schmidtmayer, J., Jacobsen, C., Miksch, G. and Sievers, J. (1994) Blood monocytes and spleen macrophages differentiate into microglia-like cells on monolayers of astrocytes: membrane currents. *Glia* **12,** 259–267.

Schnitzer, J. (1989) Enzyme-histochemical demonstration of microglial cells in the adult and postnatal rabbit retina. *J. Comp. Neurol.* **282,** 249–263.

Sébire, G., Héry, C., Peudenier, S. and Tardieu, M. (1993) Adhesion proteins on human microglial cells and modulation of their expression by IL1α and TNFα. *Res. Virol.* **144,** 47–52.

Seeds, N. W., Friedman, G., Hayden, S., Thewke, D., Haffke, S., McGuire, P. and Kystosek, A. (1996) Plasminogen activators and their interaction with the extracellular matrix in neural development, plasticity and regeneration. *Semin. Neurosci.* **8,** 405–412.

Sheen, V. L. and Macklis, J. D. (1995) Targeted neocortical cell death in adult mice guides migration and differentiation of transplanted embryonic neurons. *J. Neurosci.* **15,** 8378–8392.

Sievers, J., Parwaresch, R. and Wottge, H. U. (1994) Blood monocytes and spleen macrophages differentiate into microglia-like cells on monolayers of astrocytes: morphology. *Glia* **12,** 245–258.

Sievers, J., Hartmann, D., Wilms, H., Wollmer, A. and Schmidtmayer, J. (1996) Is the ramification of microglia an special case of macrophage morphology in epithelia? In: *Topical Issues in Microglia Research*, pp. 105–125. Eds. E. A. Ling, C. K. Tan and C. B. C. Tan. Singapore Neuroscience Association, Singapore.

Sorokin, S. P., Hoyt, R. F., Jr., Blunt, D. G. and McNelly, N. A. (1992) Macrophage development: II. Early ontogeny of macrophage populations in brain, liver, and lungs of rat embryos as revealed by a lectin marker. *Anat. Rec.* **232,** 527–550.

Stolz, B., Erulkar, S. and Kuffler, D. P. (1991) Macrophages direct process elongation from adult frog motoneurons in culture. *Proc. R. Soc. Lond. B* **244,** 227–231.

Streit, W. J. (1995) Microglial cells. In: *Neuroglia*, pp. 85–96. Eds. H. Kettenmann and B. R. Ransom. Oxford University Press, New York.

Streit, W. J. and Kreutzberg, G. W. (1987) Lectin binding by resting and reactive microglia. *J. Neurocytol.* **16,** 249–260.

Streit, W. J., Graeber, M. B. and Kreutzberg, G. W. (1988) Functional plasticity of microglia: a review. *Glia* **1,** 301–307.

Suzumura, A., Sawada, M., Yamamoto, H. and Marunouchi, T. (1990) Effects of colony stimulating factors on isolated microglia *in vitro*. *J. Neuroimmunol.* **30,** 237–244.

Suzumura, A., Marunouchi, T. and Yamamoto, H. (1991) Morphological transformation of microglia *in vitro*. *Brain Res.* **545,** 301–306.

Tanaka, J. and Maeda, N. (1996) Microglial ramification requires nondiffusible factors derived from astrocytes. *Exp. Neurol.* **137,** 367–375.

Thanos, S. (1992) Sick photoreceptors attract activated microglia from the ganglion cell layer: a model to study the inflammatory cascades in rat with inherited retinal dystrophy. *Brain Res.* **588,** 21–28.

Thanos, S. and Richter, W. (1993) The migratory potential of vitally labelled microglial cells within the retina of rats with hereditary photoreceptor dystrophy. *Int. J. Dev. Neurosci.* **11,** 671–680.

Théry, C., Hétier, E., Evrard, C. and Mallat, M. (1990) Expression of macrophage colony-stimulating factor gene in the mouse brain during development. *J. Neurosci. Res.* **26,** 129–133.

Théry, C., Chamak, B. and Mallat, M. (1991) Cytotoxic effect of brain macrophages on developing neurons. *Eur. J. Neurosci.* **3,** 1155–1164.

Thomas, W. E. (1992) Brain macrophages: evaluation of microglia and their functions. *Brain Res. Rev.* **17,** 61–74.

Unger, E. R., Sung, J. H., Manivel, J. C., Chenggis, M. L., Blazar, B. R. and Krivit, W. (1993) Male donor-derived cells in the brains of female sex-mismatched bone marrow transplant

recipients: a Y-chromosome specific *in situ* hybridization study. *J. Neuropathol. Exp. Neurol.* **52,** 460–470.

Vela, J. M., Dalmau, I., González, B. and Castellano, B. (1995) Morphology and distribution of microglial cells in the young and adult mouse cerebellum. *J. Comp. Neurol.* **361,** 602–616.

Wang, C. C., Wu, C. H., Shieh, J. Y., Wen, C. Y. and Ling, E. A. (1996) Immunohistochemical study of ameboid microglial cells in fetal rat brain. *J. Anat.* **189,** 567–574.

Washington, R. A., Becher, B., Balabanov, R., Antel, J. and Dore-Duffy, P. (1996) Expression of the activation marker urokinase plasminogen-activator receptor in cultured human central nervous system microglia. *J. Neurosci. Res.* **45,** 392–399.

Wiktor-Jedrzejczak, W., Ratajczak, M. Z., Ptasznik, A., Sell, K. W., Ahmed-Ansari, A. and Ostertag, W. (1992) CSF-1 deficiency in the *op/op* mouse has differential effects on macrophage populations and differentiation stages. *Exp. Hematol.* **20,** 1004–1010.

Wilms, H., Hartmann, D. and Sievers, J. (1997) Ramification of microglia, monocytes and macrophages *in vitro*: influences of various epithelia and mesenchymal cells and their conditioned media. *Cell Tissue Res.* **287,** 447–458.

Wolswijk, G. (1995) Strongly G_{D3} + cells in the developing and adult rat cerebellum belong to the microglial lineage rather than to the oligodendrocyte lineage. *Glia* **13,** 13–26.

Wood, G. W., Gollahon, K. A., Tilzer, S. A., Vats, T. and Morantz, R. A. (1979) The failure of microglia in normal brain to exhibit mononuclear phagocyte markers. *J. Neuropathol. Exp. Neurol.* **38,** 369–376.

Wu, C. H., Wen, C. Y., Shieh, J. Y. and Ling, E. A. (1992) A quantitative and morphometric study of the transformation of amoeboid microglia into ramified microglia in the developing corpus callosum in rats. *J. Anat.* **181,** 423–430.

Wu, C. H., Wen, C. Y., Shieh, J. Y. and Ling, E. A. (1993) A quantitative study of the differentiation of microglial cells in the developing cerebral cortex in rats. *J. Anat.* **182,** 403–413.

Wu, C. H., Wen, C. Y., Shieh, J. Y. and Ling, E. A. (1994) Down-regulation of membrane glycoprotein in ameboid microglia transformation into ramified microglia in postnatal rat brain. *J. Neurocytol.* **23,** 258–269.