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BRAIN RESEARCH REVIEWS

Review

The origin and cell lineage of microglia—New concepts

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ARTICLE INFO

Article history:
Accepted 14 November 2006
Available online 26 December 2006

Keywords:
Microglia
Ontogeny
Embryonic mouse
Myeloid lineage
Transcription factors

ABSTRACT

Despite intense study, the precise origin and cell lineage of microglia, the resident mononuclear phagocytes of the nervous system, are still a matter for debate. Unlike macroglia (astrocytes and oligodendrocytes) and neurons, which are derived from neuroectoderm, microglial progenitors arise from peripheral mesodermal (myeloid) tissue. The view still commonly held is that tissue-resident mononuclear phagocytes (including microglia) are derived from circulating blood monocytes and these take up residence late in gestation and postnatally. However, microglial progenitors colonise the nervous system primarily during embryonic and fetal periods of development. Recent evidence indicates differences between the lineage of mononuclear phagocytes during the embryonic and fetal period from that in the neonate and adult—mononuclear phagocytes that take up residence within tissues are derived from a lineage of myeloid cells that is independent of the monocyte lineage. Our own findings on the development and differentiation of microglial progenitors, taken together with findings by other investigators, and in the context of the heterogeneity between myeloid differentiation in the fetus and in the adult, support the view that microglia are derived prenatally from mesodermal progenitors that are distinct from monocytes. Furthermore, microglial progenitors colonise the nervous system via extravascular routes initially. These findings challenge the concept that resident microglia in the nervous system are derived from circulating blood monocytes. Work is still underway to establish the tissue of origin and lineage of microglial progenitors in vivo. This information is critical not only from a developmental perspective, but significantly from a therapeutic viewpoint, as (i) the unique property of microglial progenitors to colonise the nervous system from the periphery allows these cells to be exploited as a biological and non-invasive means for cell therapy by delivering genes to the nervous system (microglial engraftment), and (ii) there are indications that microglial progenitors are specifically able to home to the nervous system. Use of microglial progenitors for therapeutic purposes becomes feasible only if the origin and cell lineage of these microglial progenitors are known and these cells can be isolated and manipulated in vitro (i.e., to express specific trophic factors) prior to

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doi:10.1016/j.brainresrev.2006.11.002

therapeutic transfer (e.g., intravenously) in vivo. In this paper, we shall briefly consider the existing concepts on the origin and lineage of microglial progenitors and discuss new hypotheses in the light of emerging data that suggest clear differences between fetal and adult ontogeny of myeloid cells.

Contents

1. Introduction: existing concepts on the origin of microglia (the monocytic theory)

Microglia are widely regarded as the resident mononuclear phagocytes (also referred to as 'tissue macrophages') of the nervous system. Under normal conditions in the adult, microglia possess highly ramified morphology and are distributed ubiquitously throughout the nervous system. In pathological conditions, microglia become progressively activated (they retract cellular processes, upregulate or express *de novo* distinct profiles of cell surface 'phenotypic' markers) and motile and adopt full immune effector functions. Microglial activation is graded and these cells transform into phagocytic cells capable of presenting antigen (in association with MHC class II molecules) to circulating T cells, and of production of a plethora of inflammatory cytokines, reactive oxygen intermediates and nitric oxide. Activated microglia express cell surface markers that are also found on other mononuclear phagocytes (specifically macrophages). In their fully activated states, they may contribute towards and exacerbate damage to neurons and other non-neuronal cells. Nevertheless, under certain conditions, microglia may also exert neuroprotective and neurotrophic functions (Rezaie and Male, 2002a; Rezaie, 2003; Streit, 2002), that are distinct from their potential to cause harm within the nervous system.

The origin of microglia has been at the centre of debate for several decades (Cuadros and Navascues, 1998; Kaur et al., 2001; Rezaie, 2003; Rezaie and Male, 2002b; Streit, 2001). This topic has been extensively reviewed elsewhere (see Rezaie and Male, 2002b; Rezaie, 2003). Briefly, proposals for the origin of microglia have considered these cells to be derived from (i) neuroectoderm (similar to oligodendrocytes and astrocytes), (ii) vascular adventitia, (iiii) an intrinsic population of hematopoietic stem cells resident within the nervous system, (iv) peripheral mesodermal/mesenchymal tissues or (v) from circulating blood monocytes. The latter proposal – that microglia, like other tissue-resident mononuclear phagocytes, are derived from circulating blood monocytes that originate from the bone marrow and are seeded within the nervous system around the time of birth and postnatally – gained acceptance for many years. Consensus opinion currently holds that microglia residing in the central nervous system are derived from progenitors that arise in the periphery (from mesodermal/mesenchymal sources). *In the neonate and adult*, microglia are considered to derive from circulating blood progenitors (monocytes) originating primarily within the bone marrow, that traverse the wall of blood vessels and populate the nervous system (see Perry et al., 1985; Rezaie and Male, 2002b). However, resident microglial cells in the adult have a slow turnover at rest and are capable of proliferation and self-renewal. Thus, while there is a varying degree of recruitment of bone marrow progenitors to the adult nervous system under pathological conditions (Beck et al., 2003; Flügel et al., 2001; Lassmann and Hickey, 1993; Priller et al., 2001), the population of microglia at rest in the adult (i.e., under normal conditions) can be replenished *intrinsically* and does not require significant turnover from circulating blood progenitors (Kennedy and Abkowitz, 1997; Lassmann and Hickey, 1993).

The lack of phenotypic distinction (in terms of expression of cell surface markers) between mononuclear phagocytes that infiltrate the adult nervous system under pathological conditions and activated resident microglial cells has further confounded attempts to discriminate between resident and infiltrating cells and their corresponding tissue source and cell lineage (Rezaie and Male, 2002a,b). However, postnatal and adult microglia differ from other populations of mononuclear phagocytes (notably circulating and tissue-resident macrophages) in their electrophysiological characteristics—they possess inward-rectifying potassium channels, a property that is also present in a subset of bone marrow progenitors (Banati et al., 1991). This finding has lent support to the notion that microglia in the postnatal and adult nervous system are derived from a discrete population of progenitors that reside within the bone marrow. Nevertheless, even though sophisticated chimera and transgenic experiments have shown that progenitors from the bone marrow can be traced to cells located *within* the nervous system in postnatal and adult CNS, these cells are not only few in number but the majority are located in or around the blood vessels of the brain and spinal cord (e.g., they give

rise to perivascular macrophages and a small number of perivascular parenchymal microglia) and do not truly represent the endogenous population of ramified microglia found widely dispersed in the CNS (Albini et al., 2005; Kennedy and Abkowitz, 1997; Lassmann and Hickey, 1993) (although this view has been challenged recently (Simard and Rivest, 2004)). In contrast, other populations of mononuclear phagocytes associated with the CNS (i.e., perivascular macrophages, meningeal macrophages and choroid plexus macrophages) are replaced relatively rapidly (within a few months) from circulating blood progenitors (Kennedy and Abkowitz, 1997; Lassmann and Hickey, 1993). Studies in humans have found similar paucity and distribution of bone-marrow-derived microglia within the CNS of patients who have undergone bone marrow transplantation (Krivit et al., 1995; Unger et al., 1993), reviewed in Rezaie and Male (2002a).

2. Microglia are likely to be of mesodermal (myeloid) origin

These aforementioned findings relate to microglial turnover in the *postnatal and adult* nervous system. Such findings suggest that microglia under normal conditions are very slowly repopulated by cells derived from bone-marrow progenitors following birth and in the adult. The progenitors are commonly thought to be monocytes in keeping with the mononuclear phagocyte hypothesis for tissue macrophages. However, it has been established that microglial progenitors actually populate the nervous system during *embryonic and fetal* development, well before birth (Morris et al., 1991; Rezaie, 2003; Takahashi, 2001). Research in our laboratory and by independent investigators, into the development of microglia

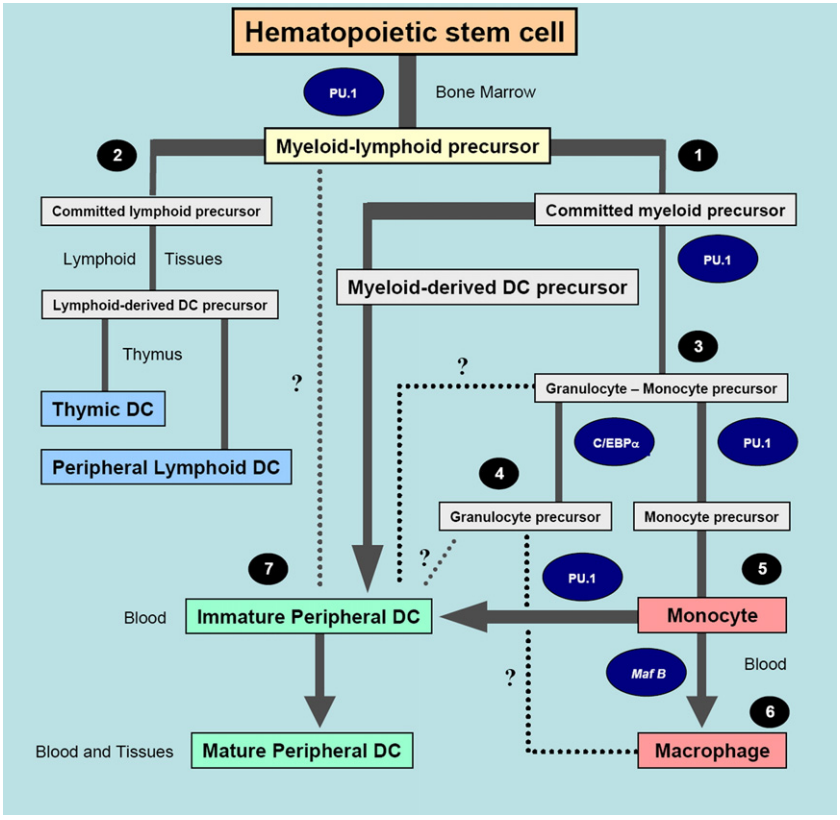

Fig. 1 – Origin of dendritic cells (DC) and macrophages in the neonate and adult. (1) These cells are committed to the myeloid lineage; (2) thymic dendritic cells and a subset of peripheral lymphoid DCs originate from a precursor with restricted lymphoid/NK/DC potential; (3) these are the most mature common progenitors of mononuclear phagocytes; (4) there are some indications that cells beyond this stage (neutrophil development) may still mature into macrophages or DCs; (5) current view holds that monocytes develop into tissue macrophages, seeded during ontogeny and maintained by local proliferation of mature macrophages and/or precursors; (6) the heterogeneous morphology, functional properties and cell surface phenotype of these cells reflects tissue localization and specialized roles; and (7) the development of myeloid-derived dendritic cells appears to be complex and may involve multiple pathways, which may overlap with macrophage development, and contribute to the heterogeneity of these two cell types. Recent findings have shown that the expression of specific transcription factors is important for lineage commitment in myeloid cells. For example, the transcription factor PU.1 is required for the development of myeloid-derived dendritic cells and macrophages, but not cells of lymphoid origin. The transcription factor C/EBP-alpha may on the other hand, inhibit the function of PU.1 and direct hematopoietic cells (CD34+ progenitors) to the granulocyte lineage. Another transcription factor—MafB, is now thought to act as an inducer of monocytic differentiation. It is possible that a balance of these transcription factors is necessary in order to direct the lineage of myeloid cells towards dendritic cells, macrophages or granulocytes—high levels of PU.1 determines a dendritic cell fate, high expression and activity of MafB determines a macrophage cell fate.

indicates that there is a developmental window (during the latter half of the first trimester and throughout the early part of second trimester in man, and between embryonic days 10–19 (E10–E19) in rodents) during which microglial progenitors populate the nervous system (Corbisiero et al., 2003; Jeetle et al., 2002; Rezaie, 2003; Rezaie et al., 2005b). Furthermore, there is evidence to support the existence of at least two separate 'populations' of microglia during embryonic and fetal development—one population derived from progenitors that are of myeloid/mesenchymal origin (not necessarily derived from blood-borne progenitors or monocytes) and a second population that represent a developmental and transitory form of fetal macrophage (derived from blood-borne precursors, possibly monocytes), which may be related to the ameboid microglial population seen in the postnatal period in rodents (Rezaie et al., 2005b).

Although microglia-like cells can be derived *in vitro* from embryonic 'stem cells' (Tsuchiya et al., 2005) and microglia are most probably of hematopoietic origin (Asheuer et al., 2004; Hess et al., 2004; Vallieres and Sawchenko, 2003; Vitry et al., 2003), the precise tissue of origin and cell lineage of microglial progenitors during the prenatal period, remain uncertain. Some have suggested the yolk sac as the tissue of origin (Alliot et al., 1999; Kaur et al., 2001), or otherwise that microglia are derived from an intrinsic pool of hematopoietic progenitors seeded within the CNS itself (see Rezaie and Male, 2002a). Microglia are likely to be of myeloid cell lineage on account that they express the myeloid-specific transcription factor PU.1 (Walton et al., 2000) and share certain phenotypic cell surface characteristics with mononuclear phagocytes, namely macrophages and myeloid-derived dendritic cells (DCs) (Rezaie and Male, 2002a). In particular, the observation that microglia share certain characteristics with 'immature' dendritic cells (DCs), coupled with the findings that some populations of dendritic cells are derived from myeloid (mesenchymal) progenitors within hematopoietic tissues, may indicate a common progenitor for microglia and myeloid DCs (Servet-Delprat et al., 2002, see also Rezaie et al., 2005a).

3. Differences between mononuclear phagocyte development in the fetus and adult

Over the past few years, the concept has emerged (and it is now recognised) that there are differences in characteristics

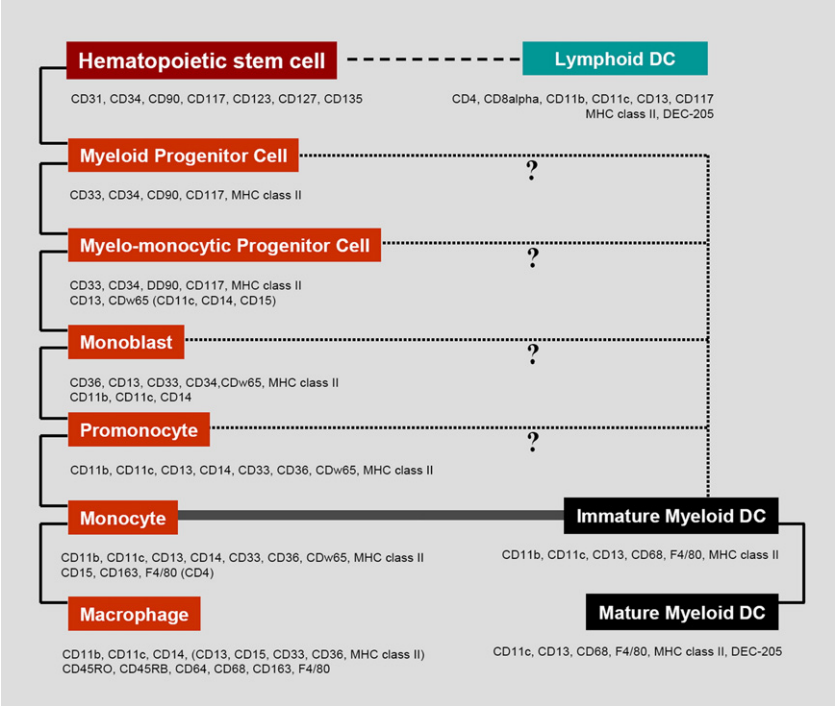

Fig. 2 – Phenotypic differentiation markers of myeloid-derived DCs, macrophages and their progenitors in the adult and neonate. Monocytes are typically identified by their expression of CD14, dendritic cells through expression of CD11c and CD205. *General markers of the monocyte/macrophage lineage in rodents include the following*: CD1d, CD2, CD4, CD11a, CD11b, CD13, CD15s, CD16, CD18, CD24, CD25, CD31, CD32, CD38, CD43, CD44, CD45, CD45R/B220, CD45RB, CD48, CD49d, CD49f, CD51, CD54, CD62L, CD64, CD71, CD80, CD86, CD90, CD107a, CD107b, CD119, CD121b, CD122, CD126, CD127, CD132, CD163, Crry/p65, Forssman Ag, Ly-6C, Ly-6G, MHC class I, MHC class II, NRK-P1, F4/80 antigen. *Maturation and subset discriminatory markers of the monocyte/macrophage lineage in rodents include*: CD2, CD4, CD11a, CD11c, CD13, CD14, CD25, CD31, CD32, CD38, CD44, CD45RB/B220, CD49f, CD51, CD64, CD68, CD71, CD80, CD86, CD90, CD107a, CD107b, CD127, CD163, Crry/p65, Forsmann Ag, Ly-6C, Ly-6G, HIS 36, MHC class II, NRK-P1, F4/80 antigen, MOMA-1. *General markers of the dendritic cell lineage in the rodent include*: CD1d, CD4, CD8a, CD11a, CD11c, CD13, CD15s, CD18, CD24, CD25, CD35, CD40, CD44, CD45, CD45RB, CD48, CD54, CD80, CD83, CD86, CD119, CD121a, CD132, Crry/p65, MHC class I, MHC class II, MY S35/TSA-1, OX-62 (CD85), CD205 (DEC-205), CD206, CD207, CD208, CD209.

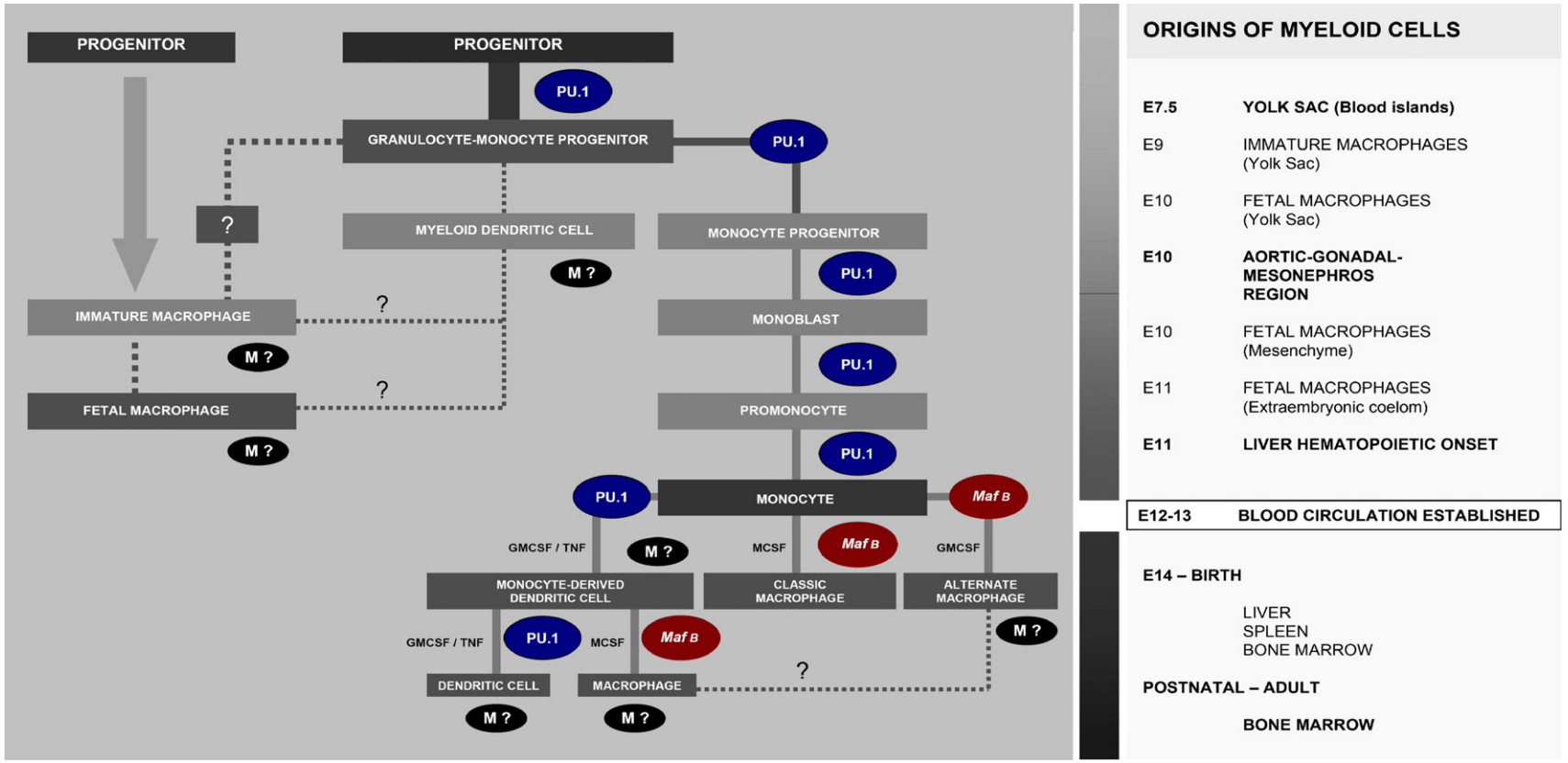

Fig. 3 – Proposed origins of myeloid-derived mononuclear phagocytes in the mouse. Microglia (M?) could be derivatives of immature/fetal macrophages derived from mesenchymal progenitors, myeloid dendritic cells derived from granulocyte–monocyte progenitors, or monocyte-derived dendritic cells, or a form of 'alternate' macrophage.

between mononuclear phagocytes, namely tissue-resident macrophages derived during development (termed 'fetal macrophages') from those in the adult, and indications that these cells may derive from separate progenitors/cell lineages (Faust et al., 1997; Naito et al., 1996; Takahashi et al., 1996; Takahashi, 2001; Shepard and Zon, 2000). Indeed, it has been proposed that fetal macrophages arise from progenitors that appear prior to vascularization of organs and independent of a monocyte-derived lineage (Faust et al., 1997; Naito et al., 1996; Takahashi et al., 1996; Takahashi, 2001; Shepard and Zon, 2000). This discrepancy between fetal and adult myeloid cell differentiation suggests that myelopoiesis is more complex than previously considered. The cell lineage and phenotypic differentiation of myeloid-derived macrophages and dendritic cells are still being unravelled in the *neonate and adult*, where the bone marrow is the principal source of hematopoiesis (Fig. 1). The differentiation of myeloid cells can be followed by their expression of general as well as maturation and subset discriminatory cell surface markers (Fig. 2). By comparison, little is known of the lineage specification and differentiation of these cells during embryonic and fetal development, where hematopoiesis is initiated within the yolk sac and sequentially adopted by other tissues (including liver and spleen) and only commences within the bone marrow late in organogenesis around the time of birth and postnatally (Takahashi, 2001) (Fig. 3).

4. Microglia in prenatal development: clues as to their origin and cell lineage

Microglial progenitors seed the nervous system primarily during the embryonic and fetal periods of development. While there is consensus that microglia are derived from mesodermal (myeloid) progenitors, such progenitors have yet to be unequivocally identified in the prenatal period. The lack of a precise lineage-related marker for microglial progenitors is a key issue underlying debate as to the lineage of these

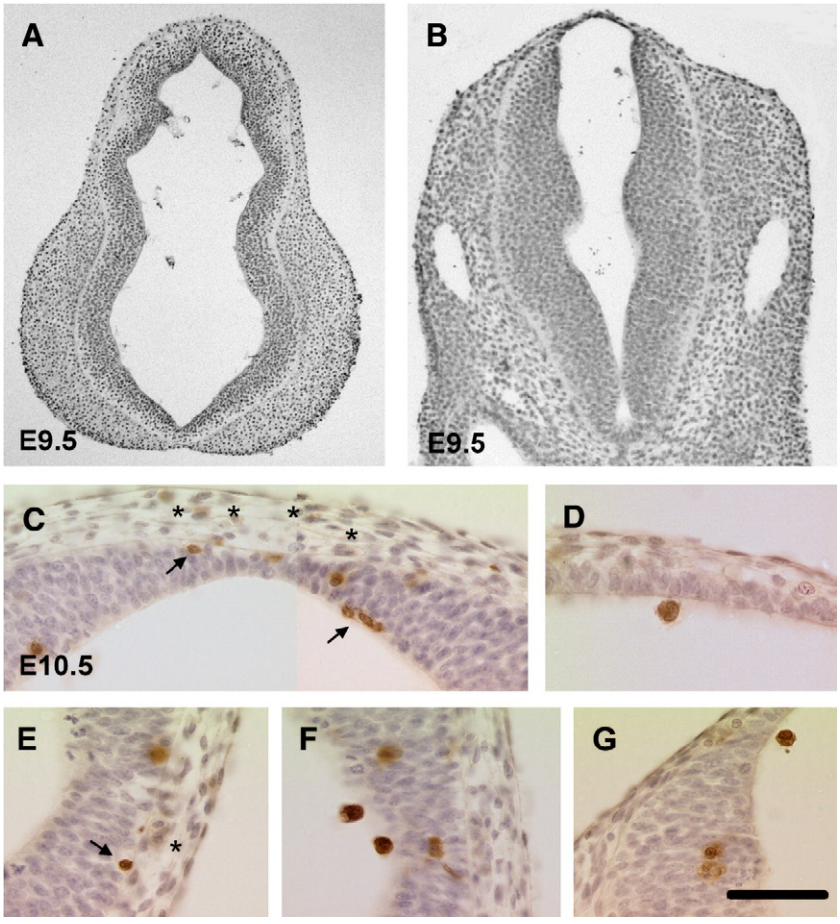

Fig. 4 – Microglial progenitors in the embryonic mouse (E9.5–E10.5). (A, B) Frontal sections showing different levels of the cranial neural tube at E9.5 (no Iba1-positive cells are present at this age). Iba1-positive cells are apparent at E10.5. Regions of the neural tube where such cells can be found are shown in panels C–G. In particular, Iba1-positive cells accumulate within the mesenchyme surrounding the neural tube (asterisks) and microglial progenitors can be detected within the developing nervous tissues proper (arrows), particularly in and around the roof of the hindbrain (arrows in C) and occasionally, also adjacent to or within the ventricular linings at E10.5 (D, F, G). Immunoreactivity with Iba1 antibody, nuclei counterstained with hematoxylin. Scale bar in panel G represents approximately 215 μm in panel A, 125 μm in panel B, 50 μm in panel C, 40 μm in panel D and 45 μm in panels E–G.

cells. Thus, it is uncertain (i) whether microglia belong to the myeloid dendritic cell lineage or to the monocyte-macrophage lineage of cells, (ii) whether microglia represent immature bipotential progenitors with the capacity to differentiate to myeloid dendritic cells or macrophages or indeed (iii) whether there are subpopulations of these cells residing within the central nervous system, that are heterogeneous as to lineage as well as function. Likewise, the precise tissue of origin for microglial progenitors is uncertain: they could derive from progenitors located in the yolk sac, the mesenchyme surrounding the nervous system or from progenitors that originate within the liver or spleen. Indeed it is also unclear whether microglia may derive from more than one type of progenitor cells during embryonic and fetal development. Certainly, the timing of the start of colonisation of the nervous system by microglia (~E10 to E12 in rodents; see Fig. 3, and beginning of the second trimester in man) also precludes the bone marrow as the primary source of these progenitors (Morris et al., 1991; Rezaie et al., 2005b; Takahashi, 2001). It is possible therefore that microglia may alternatively represent a population(s) of 'immature' or 'fetal macrophage' derived from a myeloid progenitor that is distinct from the monocyte-macrophage bone-marrow lineage of the adult (Fig. 3). Work in our laboratories is currently underway to determine the origin and source of microglial progenitors and whether there may indeed be more than one population of such progenitor cells.

As mentioned, most of the current understanding of the development of microglia stems from the perinatal and postnatal periods of development. We have been focusing our investigations on prenatal development. In our previous studies on human fetal microglia, we have shown that lectin histochemistry identifies a subpopulation of parenchymal microglia derived from extravascular progenitors. Immunohistochemistry to detect mononuclear phagocyte markers including CD11b, CD45, CD64, CD68, MHC class II antigen has identified another subset of microglia (commonly termed 'ameboid' microglia, thought to be derived from monocytes/macrophage) (Rezaie et al., 2005b). We have also shown that CD11b-deficiency does not significantly impair colonization of the developing mouse brain between E15 and birth (Jeetle et al., 2002). Indeed in both normal developing and CD11b-deficient mice, differentiating microglia can be detected within the CNS as early as E15 (Corbisiero et al., 2003; Jeetle et al., 2002). Our subsequent investigations between E9.5 and E14.5 have revealed that mononuclear phagocytes seed the developing murine nervous system as early as E10.5 (Fig. 4). Significant populations of Iba1 (Figs. 5 and 6) or tomato lectin (not shown)-positive microglial progenitors can be detected within the developing nervous system between E11 and E14. There appears to be discrepancy between the expression profiles and morphologies of progenitors that colonise the nervous system during this initial period. This will require further investigation, and corresponding data will be pre-

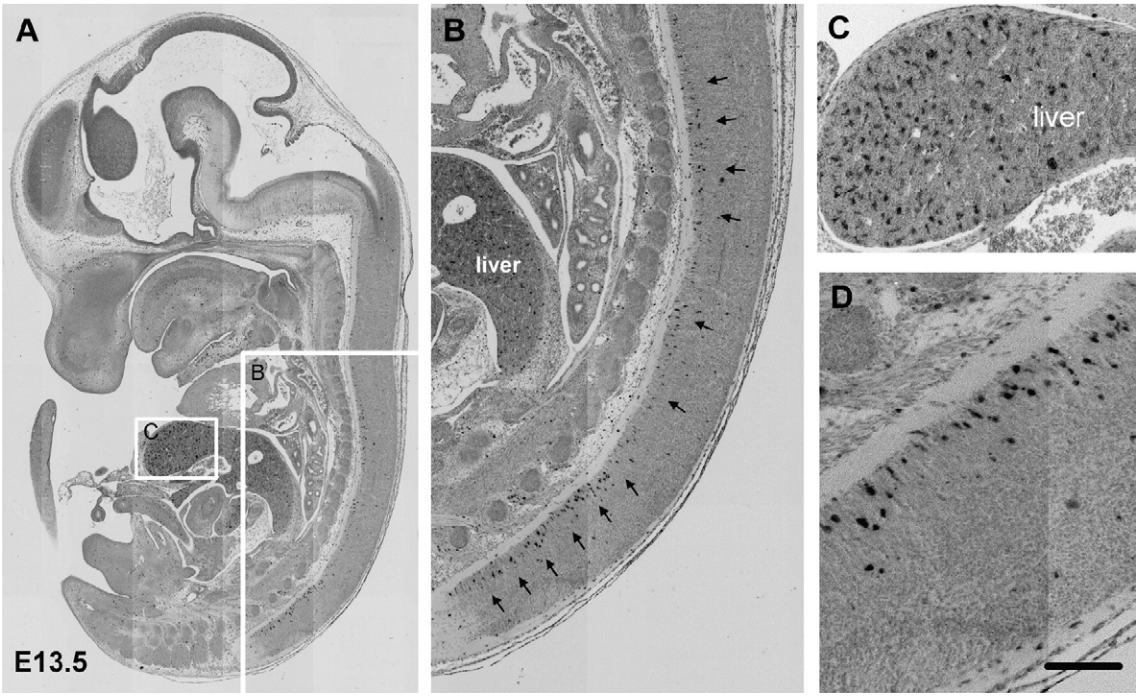

Fig. 5 – Microglial progenitors in the spinal cord of the embryonic mouse (E13.5). Composite images to show the distribution of Iba1-positive cells in sagittal sections of the embryonic mouse at E13.5. (A) Iba1-positive microglial progenitors can be detected at this stage in significant numbers within the superficial mantle layer, subjacent to the marginal layer of the spinal cord (see inset B on panel A, and panels B and D), from the sacral region through to the lumbar and thoracic segments (arrows in panel B). Mononuclear phagocytes can also be readily detected within the liver (see inset C in panel A, and panel C). Immunoreactivity with Iba1 antibody, nuclei counterstained with hematoxylin. Scale bar in panel D represents approximately 860 μm in panel A, 400 μm in panel B, 210 μm in panel C and 130 μm in panel D.

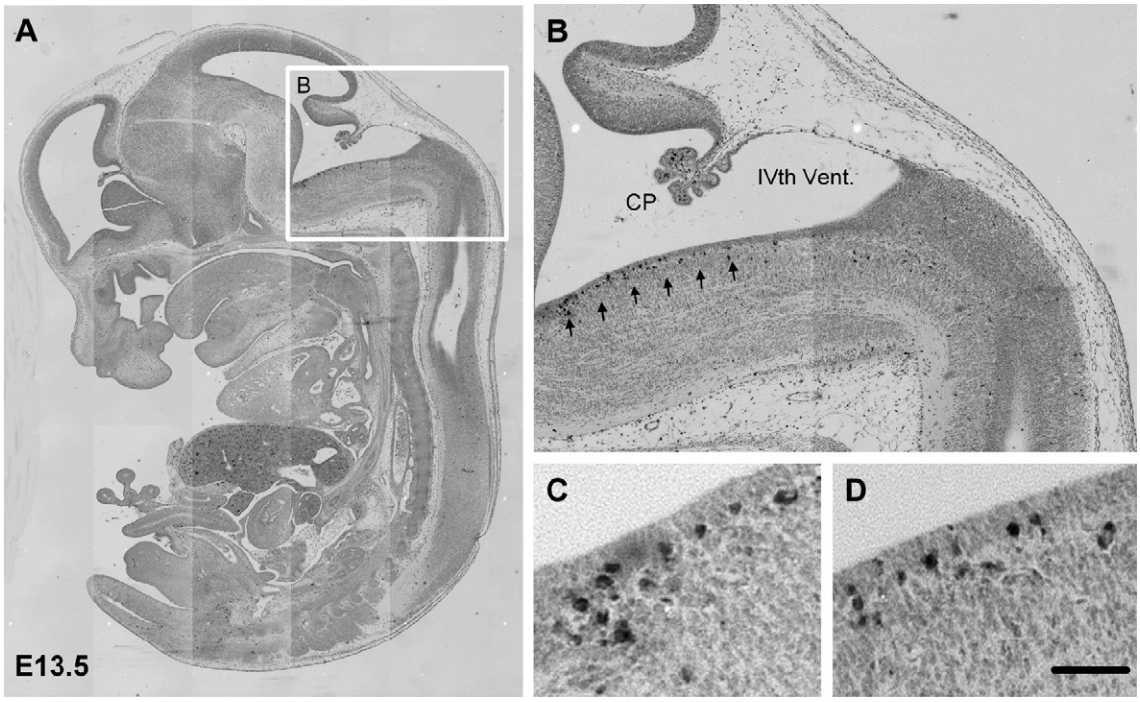

Fig. 6 – Microglial progenitors in the embryonic mouse brain (E13.5). Composite images to show the distribution of Iba1-positive cells in sagittal sections of the embryonic mouse at E13.5. (A–D) Iba1-positive microglial progenitors can be detected at this stage within the ventricular lining of the fourth ventricle (medulla oblongata)—see inset B in panel A, panel B (arrows). Higher power photos of Iba1-positive cells in this region are shown in panels C and D. CP: choroid plexus; IVth Vent: fourth ventricle. Immunoreactivity with Iba1 antibody, nuclei counterstained with hematoxylin. Scale bar in panel D represents approximately 830 μm in panel A, 290 μm in panel B and 48 μm in panels C and D.

sented elsewhere. Taken together, our findings in the human and rodent CNS suggest that there may exist separate progenitor populations in embryos for microglia. The source, phenotype and lineage of these different populations are not known.

Recent findings have shown that the expression of specific transcription factors is important for lineage commitment in myeloid cells. For example, the transcription factor PU.1 is required for the development of myeloid-derived dendritic cells and macrophages (Anderson et al., 2000; Fisher and Scott, 1998; Guerriero et al., 2000; Reddy et al., 2002) (including microglia; Walton et al., 2000) (Figs. 1 and 3). The transcription factor C/EBP-alpha may on the other hand, inhibit the function of PU.1 and direct hematopoietic cells (CD34+ progenitors) to the granulocyte cell lineage (Reddy et al., 2002) (Fig. 1). Another transcription factor—MafB, which was functionally cloned and characterised in 2000 (Huang et al., 2000) is now thought to act as an inducer of monocytic differentiation (Hamada et al., 2003; Kelly et al., 2000). It is possible that a balance of these transcription factors is necessary in order to direct the lineage of myeloid cells towards dendritic cells, macrophages or granulocytes. For example, Bakri et al. (2005) have shown that a balance of MafB and PU.1 determines cell fate of myeloid progenitors to develop into macrophages or dendritic cells—high levels of PU.1 determines a dendritic cell fate, high expression and activity of MafB determines a macrophage cell fate. Therefore, it is likely that the lineage commitment of microglial progenitors is determined by a predominance of one or more of these identified transcription factors. Consequently, the conditional temporal knockdown of these transcription factors *in vivo* together with subsequent screening of hematopoietic tissues (e.g., yolk sac, liver and spleen) and the nervous system for microglial progenitors during the known developmental 'window' (E10–E19) will not only lead us to identify the microglial progenitor ('stem cell'), but will also inform us of its lineage. Work in our laboratories is currently underway to determine the origin and source of microglial progenitors and whether there may indeed be more than one population of such progenitor cells.

5. Concluding remarks: potential therapeutic roles for microglial progenitors

To summarize, there is strong evidence to support that microglial progenitors seed the nervous system primarily during prenatal development. At least two subpopulations of microglia can be identified during prenatal and early postnatal development. These subpopulations may be discriminated based on heterogeneity in (i) phenotype, (ii) morphological characteristics and (iii) with respect to their locations (distribution patterns) within the developing nervous system. One population is derived mainly from extravascular progenitors (of myeloid/mesenchymal but not monocytic origin) during fetal development, progressively differentiates and

persists into adulthood as parenchymal microglia. A second, transitory population (commonly termed 'ameboid' microglia) is derived from circulating progenitors (probably monocytes and/or fetal macrophages) and can be found sporadically during the fetal period, but predominantly occurs during perinatal and postnatal development. Evidence for the differentiation of these ameboid cells to parenchymal microglia remains inconclusive. The tissue(s) of origin and cell lineage of progenitors that give rise to these populations of microglia during development have yet to be established conclusively. In the adult, under normal conditions, a very small proportion of microglia (predominantly perivascular microglia) may also be repopulated by circulating progenitors that are derived from the bone marrow. Therefore, the routes by which the nervous system is colonised by microglial progenitors, as well as the source and lineage of these progenitor cells appear to differ in prenatal development from that observed in the adult.

If the tissue of origin and lineage of the resident parenchymal microglia can be correctly identified, this finding will also have significant bearing for potential therapeutic use (Dobrenis, 1998). Microglial progenitors can be isolated, manipulated *in vitro* and employed for corrective cell and gene therapy (Cucchiarini et al., 2003; Watanabe et al., 2002). Unlike neuroectoderm-derived glial cells and neurons, microglial progenitors colonise the nervous system from the periphery. As such, they may be targeted as a biological tool for non-invasive delivery of genes to the nervous system (for example, in lysosomal and peroxisomal storage disorders (Krivit et al., 1995), metachromatic leukodystrophy (Biffi et al., 2004), certain brain tumours (Fathallah-Shakh et al., 2000), in repairing damage following stroke injury (Beck et al., 2003) or in neurodegenerative conditions) in order to arrest progression of disease and aid in recovery.

It has been proposed that microglial progenitors are able to specifically home to the central nervous system (see Rezaie and Male, 2002a). There is also preliminary evidence which indicates that isolated adult microglia may also home to the nervous system when introduced peripherally (intravenously) (Imai et al., 1997, 1999; Sawada et al., 1998), although the factors that enable these cells to be recruited to the nervous system remain unknown and both of these hypotheses need to be explored further. Even though mature microglial cells may possess a higher affinity for the brain than macrophages in experimental models when introduced exogenously into the blood circulation (Imai et al., 1997, 1999; Sawada et al., 1998), it would be impractical to isolate primary microglia from patients for re-introductory transplantation. Established differentiated human microglial cell lines could, in theory, represent an alternative source of cells that can be manipulated *in vitro* for microglial engraftment *in vivo*. However, the cross-transfer of such transformed cells could cause unwanted immune rejection, and considering the higher proliferative status of such cells, may possibly give rise to 'microgliomas' (a form of microglial tumour), if not appropriately regulated. As discussed above, although remnant microglial progenitors may also exist in the adult bone marrow, the extremely low turnover of parenchymal microglia from these cells indicates that they are too few in number to be able to be isolated and characterized readily. This is not the case in the well-defined period of development proposed here. The evidence from initial microglial cell transplants in the adult (Imai et al., 1997, 1999; Sawada et al., 1998) does argue for the hypothesis that microglial cells are a specialized branch of the myeloid lineage whose progenitors are destined for the CNS during the fetal period, and this progenitor, once correctly identified, would represent the most feasible and viable biological tool for specific gene delivery to the CNS.

Acknowledgments

We would like to thank Prof. David Male (Department Biological Sciences, Open University) for providing useful comments on the manuscript, Dr. Jon Golding (Department Biological Sciences, Open University) for helpful discussions and Ms. Jacki Brown for excellent technical assistance. We also thank Dr. Yoshinori Imai (currently at School of Medicine, Ehime University, Japan) for earlier communications and discussions. This work was partially supported by research grants from the Open University and the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. CUHK4412/06M).

REFERENCES

Albini, T.A., Wang, R.C., Reiser, B., Zamir, E., Wu, G.S., Rao, N.A., 2005. Microglial stability and repopulation in the retina. Br. J. Ophthalmol. 89, 901–903.

Alliot, F., Godin, I., Pessac, B., 1999. Microglia derive from progenitors originating from the yolk sac, and which proliferate in the brain. Dev. Brain Res. 117, 145–152.

Anderson, K.L., Perkin, H., Surh, C.D., Venturini, S., Maki, R.A., Torbett, B.E., 2000. Transcription factor PU.1 is necessary for development of thymic and myeloid progenitor-derived dendritic cells. J. Immunol. 164, 1855–1861.

Asheuer, M., Pflumio, F., Benhamida, S., Dubart-Kupperschmitt, A., Fouquet, F., Imai, Y., Aubourg, P., Cartier, N., 2004. Human CD34+ cells differentiate into microglia and express recombinant therapeutic protein. Proc. Natl. Acad. Sci. U. S. A. 101, 3557–3562.

Bakri, Y., Sarrazin, S., Mayer, U.P., Tillmanns, S., Nerlov, C., Boned, A., Sieweke, M.H., 2005. Balance of MafB and PU.1 specifies alternative macrophage or dendritic cell fate. Blood 105, 2707–2716.

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

Beck, H., Voswinckel, R., Wagner, S., Ziegelhoeffer, T., Heil, M., Helisch, A., Schaper, W., Acker, T., Hatzopoulos, A.K., Plate, K.H., 2003. Participation of bone marrow-derived cells in long-term repair processes after experimental stroke. J. Cereb. Blood Flow Metab. 23, 709–717.

Biffi, A., De Palma, M., Quattrini, A., Del Carro, U., Amadio, S., Visigalli, I., Sessa, M., Fasano, S., Brambilla, R., Marchesini, S., Bordignon, C., Naldini, L., 2004. Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells. J. Clin. Invest. 113, 1118–1129.

Corbisiero, V., Hagger, G., Topps, S., Kohsaka, S., Imai, Y., Male, D., Rezaie, P., 2003. Colonization of the developing mouse

brain by microglial progenitors. Neuroembryology 2, 181–182.
Cuadros, M.A., Navascues, J., 1998. The origin and differentiation of microglial cells during development. Prog. Neurobiol. 56, 173–189.
Cucchiarini, M., Ren, X.L., Perides, G., Terwilliger, E.F., 2003. Selective gene expression in brain microglia mediated via adeno-associated virus type 2 and type 5 vectors. Gene Ther. 10, 657–667.
Dobrenis, K., 1998. Microglia in cell culture and in transplantation therapy for central nervous system disease. Methods 16, 320–344.
Fathallah-Shakh, H.M., Kafrouni, A.I., Zhao, L.J., Smith, G.M., Forman, J., 2000. Gene transfer into brain parenchyma elicits antitumour effects. Cancer Res. 60, 1797–1799.
Faust, N., Huber, M.C., Sippel, A.E., Bonifer, C., 1997. Different macrophage populations develop from embryonic/fetal and adult hematopoietic tissues. Exp. Hematol. 25, 432–444.
Fisher, R.C., Scott, E.W., 1998. Role of PU.1 in hematopoiesis. Stem Cells 16, 25–37.
Flügel, A., Bradl, M., Kreutzberg, G.W., Graeber, M.B., 2001. Transformation of donor-derived bone marrow precursors into host microglia during autoimmune CNS inflammation and during the retrograde response to axotomy. J. Neurosci. Res. 66, 74–82.
Guerriero, A., Langmuir, P.B., Spain, L.M., Scott, E.W., 2000. PU.1 is required for myeloid-derived but not lymphoid-derived dendritic cells. Blood 95, 879–885.
Hamada, M., Moriguchi, T., Yokomizo, T., Morito, N., Zhang, C., Takahashi, S., 2003. The mouse mafB 5′-upstream fragment directs gene expression in myelomonocytic cells, differentiated macrophages and the ventral spinal cord in transgenic mice. J. Biochem. 134, 203–210.
Hess, D.C., Abe, T., Hill, W.D., Studdard, A.M., Carothers, J., Masuya, M., Fleming, P.A., Drake, C.J., Ogawa, M., 2004. Hematopoietic origin of microglial and perivascular cells in brain. Exp. Neurol. 186, 134–144.
Huang, K., Serria, M.S., Nakabayashi, H., Nishi, S., Sakai, M., 2000. Molecular cloning and functional characterization of the mouse *mafB* gene. Gene 242, 419–426.
Imai, F., Sawada, M., Suzuki, H., Kiya, N., Hayakawa, M., Nagatsu, T., Marunouchi, T., Kanno, T., 1997. Migration activity of microglia and macrophages into rat brain. Neurosci. Lett. 237, 49–52.
Imai, F., Sawada, M., Suzuki, H., Zlokovic, B.V., Kojima, J., Kuno, S., Nagatsu, T., Nitatori, T., Uchiyama, Y., Kanno, T., 1999. Exogenous microglia enter the brain and migrate into ischemic hippocampal lesions. Neurosci. Lett. 272, 127–130.
Jeetle, J., Hagger, G., Topps, S., Male, D., Rezaie, P., 2002. Microglia in the CD11b knockout mouse. Neuroembryology 1, 191.
Kaur, C., Hao, A.J., Wu, C.H., Ling, E.A., 2001. Origin of microglia. Microsc. Res. Tech. 54, 2–9.
Kelly, L.M., Englmeier, U., Lafon, I., Sieweke, M.H., Graf, T., 2000. MafB is an inducer of monocytic differentiation. EMBO J. 19, 1987–1997.
Kennedy, D.W., Abkowitz, J.L., 1997. Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. Blood 90, 986–993.
Krivit, W., Sung, J.H., Shapiro, E.G., Lockman, L.A., 1995. Microglia: the effector cell for reconstitution of the central nervous system following bone marrow transplantation for lysosomal and peroxisomal storage diseases. Cell Transplant 4, 385–392.
Lassmann, H., Hickey, W.F., 1993. Radiation bone marrow chimeras as a tool to study microglia turnover in normal brain and inflammation. Clin. Neuropathol. 12, 284–285.
Morris, L., Graham, C.F., Gordon, S., 1991. Macrophages in hematopoietic and other tissues of the developing mouse detected by the monoclonal antibody F4/80. Development 112, 517–526.
Naito, M., Umeda, S., Yamamoto, T., Moriyama, H., Umezu, H., Hasegawa, G., Usuda, H., Shultz, L.D., Takahashi, K., 1996. Development, differentiation, and phenotypic heterogeneity of murine tissue macrophages. J. Leukoc. Biol. 59, 133–138.
Perry, V.H., Hume, D.A., Gordon, S., 1985. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience 15, 313–326.
Priller, J., Flügel, A., Wehner, T., Boentert, M., Haas, C.A., Prinz, M., Fernandez-Klett, F., Prass, K., Bechmann, I., De Boer, B.A., Frotscher, M., Kreutzberg, G.W., Persons, D.A., Dirnagl, U., 2001. Targeting gen-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. Nat. Med. 7, 1356–1361.
Reddy, V.A., Iwama, A., Iotzova, G., Schulz, M., Elsasser, A., Vangala, R.K., Tenen, D.G., Hiddemann, W., Behre, G., 2002. Granulocyte inducer C/EBPα inactivates the myeloid master regulator PU.1: possible role in lineage commitment decisions. Blood 100, 483–490.
Rezaie, P., 2003. Microglia in the human nervous system during development. Neuroembryology 2, 18–31.
Rezaie, P., Male, D., 2002a. Differentiation, ramification and distribution of microglia within the central nervous system examined. Neuroembryology 1, 29–43.
Rezaie, P., Male, D., 2002b. Mesoglia and microglia—a historical review of the concept of mononuclear phagocytes within the central nervous system. J. Hist. Neurosci. 11, 325–374.
Rezaie, P., Corbisiero, V., Male, D., 2005a. Transient expression of MIDC-8 in the normal mouse brain. Neurosci. Lett. 377, 189–194.
Rezaie, P., Dean, A., Male, D., Ulfig, N., 2005b. Microglia in the cerebral wall of the human telencephalon at second trimester. Cereb. Cortex 15, 938–949.
Sawada, M., Imai, F., Suzuki, H., Hayakawa, M., Kanno, T., Nagatsu, T., 1998. Brain-specific gene expression by immortalized microglial cell-mediated gene transfer in the mammalian brain. FEBS Lett. 433, 37–40.
Servet-Delprat, C., Arnaud, S., Jurdic, P., Nataf, S., Grasset, M-F., Soulas, C., Domenget, C., Destaing, O., Rivollier, A., Perret, M., Dumontel, C., Hanau, D., Gilmore, G.L., Belin, M-F., Rabourdin-Combe, C., Mouchiroud, G., 2002. Flt3+ macrophage precursors commit sequentially to osteoclasts, dendritic cells and microglia. BMC Immunol. 3, 15.
Shepard, J.L., Zon, L.I., 2000. Developmental derivation of embryonic and adult macrophages. Curr. Opin. Hematol. 7, 3–8.
Simard, A.R., Rivest, S., 2004. Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. FASEB J. 18, 998–1000.
Streit, W.J., 2001. Microglia and macrophages in the developing CNS. Neurotoxicology 22, 619–624.
Streit, W.J., 2002. Microglia in the Regenerating and Degenerating Central Nervous System. Springer-Verlag, New York.
Takahashi, K., 2001. Development and differentiation of macrophages and related cells: historical review and current concepts. J. Clin. Exp. Hematop. 41, 1–33.
Takahashi, K., Naito, M., Takeya, M., 1996. Development and heterogeneity of macrophages and their related cells through their differentiation pathways. Pathol. Int. 46, 473–485.
Tsuchiya, T., Park, K.C., Toyonaga, S., Yamada, S.M., Nakabayashi, H., Nakai, E., Ikawa, N., Furuya, M., Tominaga, A., Shimizu, K., 2005. Characterization of microglia induced from mouse embryonic stem cells and their migration into the brain parenchyma. J. Neuroimmunol. 160, 210–218.
Unger, E.R., Sung, K.H., Manivel, J.C., Chenggis, M.L., Blazar, B.R., 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.

Vallieres, L., Sawchenko, P.E., 2003. Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. J. Neurosci. 23, 5197–5207.

Vitry, S., Bertrand, J.Y., Cumano, A., Dubois-Dalcq, M., 2003. Primordial hematopoietic stem cells generate microglia but not myelin-forming cells in a neural environment. J. Neurosci. 23, 10724–10731.

Walton, M.R., Gibbons, H., MacGibbon, G.A., Sirimanne, E., Saura, J., Gluckman, P.D., Dragunow, M., 2000. PU.1 expression in microglia. J. Neuroimmunol. 104, 109–115.

Watanabe, R., Takase-Yoden, S., Fukumitsu, H., Nakajima, K., 2002. Cell transplantation to the brain with microglia labeled by neuropathogenic retroviral vector system. Cell Transplant 11, 471–473.