

Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification

Gilles J. Guillemin^{*,†,1} and Bruce J. Brew[‡]

^{*}Centre for Immunology, Neuroimmunology Department, and [‡]Department of Neurology, St. Vincent's Hospital, Sydney, NSW, Australia; and [†]University of New South Wales, Sydney, Australia

Abstract: The phenotypic differentiation of systemic macrophages that have infiltrated the central nervous system, pericytes, perivascular macrophages, and the “real” resident microglial cells is a major immunocytochemical and immunohistochemical concern for all users of cultures of brain cells and brain sections. It is not only important in assessing the purity of cell cultures; it is also of fundamental importance in the assessment of the pathogenetic significance of perivascular inflammatory phenomena within the brain. The lack of a single membranous and/or biochemical marker allowing conclusive identification of these cells is still a major problem in neurobiology. This review briefly discusses the functions of these cells and catalogs a large number of membranous and biochemical markers, which can assist in the identification of these cells. *J. Leukoc. Biol.* 75: 388–397; 2004.

Key Words: brain · monocyte cells · differentiation

INTRODUCTION

The phenotypic differentiation of systemic macrophages that have infiltrated the central nervous system (CNS), pericytes, perivascular macrophages, and the “real” resident microglial cells (**Fig. 1**) is a major immunocytochemical and immunohistochemical concern for all users of cultures of brain cells and brain sections.

This review will concentrate on discussion of the available markers for identification of each cell after a review of their ontogeny and function.

For the purpose of this review, we will use the term “brain macrophage” to encompass macrophages infiltrating the brain, pericytes, perivascular macrophages, and microglia. There is a considerable heterogeneity in the phenotype of brain macrophages even within the latter groups. For example, Perry and Gordon [1] have emphasized three different types of microglia: radially branched (found in the gray matter), longitudinally branched (found in the white matter), and compact microglia [found exclusively in those parts of the brain lacking a blood brain barrier (BBB)]. Other investigators have differentiated granular and agranular pericytes [2]. Nonetheless, there does not appear to be any fundamental, functional difference between these subtypes of brain macrophage, and so, this review

will only discuss the aforementioned four types of brain macrophage.

ONTOGENY

A controversial, although fundamental, issue in neurobiology concerns the nature and origin of brain macrophages. We agree with the most commonly accepted hypothesis, namely, that the most likely source for all or most brain macrophages is the monocyte. Amoeboid microglia in the developing brain, however, probably have an additional source—the pial macrophages, which in turn, are derived from mesenchymal progenitor cells in the yolk sac [3]. Monocytes appear to migrate into the brain from several sites during embryogenesis and may continue to enter, at least from blood vessels, in the adult state [4]. Once in the brain, monocytes differentiate into one of the four types of brain macrophage depending on the signals associated with the microenvironment. Although there is no definitive proof for this model, there is certainly evidence to support it. Thomas [2] cites work that has shown pericytes leaving the basal lamina and migrating to the perivascular space where they are indistinguishable from perivascular macrophages. These same cells have then been reported to migrate into the brain parenchyma, where they are indistinguishable from infiltrating macrophages [5]. Furthermore, the monocytes that enter the brain in the stab-wound model have been observed to “transform” into microglia. Finally, Perry and Gordon [1] have demonstrated that perivascular macrophages, pericytes, and probably microglia “turn over” from circulating monocytes. The rates of turnover vary considerably in mice: several months for perivascular macrophages and pericytes; years for microglia (although some may not turn over at all). Similar kinetics are not known for humans. It is also unknown whether particular diseases may affect these rates.

Pericytes

Pericytes are generally accepted as being of mesodermal origin [2, 4]. They appear to migrate into the tissue during the latter stages of vascularization and assume their characteristic loca-

¹ Correspondence: Centre for Immunology, Neuroimmunology Department, St. Vincent's Hospital, Boundary St., Darlinghurst, Sydney, NSW, 2010, Australia. E-mail: G.Guillemin@cfi.unsw.edu.au

Received March 19, 2003; revised August 17, 2003; accepted September 23, 2003; doi: 10.1189/jlb.0303114.

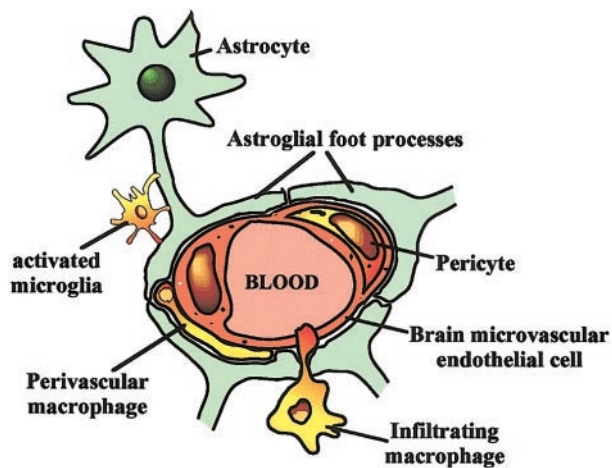


Fig. 1. Simplified schematic drawing of the myeloid cells in the brain perivascular area.

tion and properties. Mesenchymal precursor cells settle on newly formed capillary sprouts and differentiate into pericytes as they become enclosed within basal lamina [6].

Perivascular macrophages

Based on their morphology and immunophenotype, perivascular macrophages appear to be very similar to blood-derived macrophages (see Table 2 in ref. [7]). Moreover, using transplants of green fluorescent protein-transfected bone marrow cells in adult mice, Bechmann et al. [5] concluded that brain perivascular cells are a population of migratory macrophages and not resident histiocytes. These data essentially supplant those of earlier studies such as Kida et al. [8] and the idea that they are distinct from pericytes, microglia, and macrophages.

Microglia

The origin of microglia has been one of the most controversial issues in glial research [3, 9, 10]. The large majority of neurobiologists now believe that they are derived from monocytes and to a lesser extent, from mesenchymal progenitor cells. The alternative view—that they originate from the neuroepithelium, as do neurons and other brain glial cells—is becoming increasingly unlikely.

PHYSIOLOGIC AND PATHOLOGIC CONDITIONS

In physiologic conditions, the brain contains resting microglia, perivascular macrophages, and pericytes, as well as a few “patrolling” macrophages. In pathologic situations, all these cell types are activated. Microglia and pericytes may proliferate to a limited extent in contrast to macrophages, which cannot. Commonly, in pathologic conditions affecting the brain, there is BBB disruption, which will allow entry of more macrophages from the blood.

Pericytes represent the first line of immunologic defense of the brain [11]; indeed, pericytes can act as antigen-presenting cells. Pericytes can inhibit endothelial cell growth, promote

vessel constriction, and transform into smooth muscle cells. In essence, pericytes seem important as structural support in the microcirculation and in BBB function. Pericytes have been considered to be significant in the pathogenesis of tissue damage in hypoxia, hypertension, diabetic retinopathy, trauma, Alzheimer’s disease, multiple sclerosis, and in CNS tumor formation [7, 12–14]. In first the 2 h after injury, pericytes and perivascular macrophages are activated and start to migrate from their original location [5, 14]. Concomitantly, activated blood monocyte/macrophages and lymphocytes appear to migrate through the disrupted BBB in which the number of tight junctions has strongly decreased [15]. Chemoattractive molecules, particularly the chemokine monocyte chemoattractant protein-1 (MCP-1) produced by astrocytes, attract these blood monocytes [16]. At the same time, resting microglia become activated and change their phenotype to ameboid microglia capable of phagocytosis [17, 18]. The involvement of activated microglia and perivascular macrophages associated with disruption of the BBB is a recent novel hypothesis for one aspect of the pathogenesis of Alzheimer’s disease [19–21]. It should be emphasized that the above outlined sequence of events in inflammation is very much simplified. Recent studies have also ascribed a neuroprotective role to activated microglia [22]. The precise signals that determine whether microglia in inflammatory states are phagocytic or neurotrophic are still to be determined.

If there are common aspects of ontogeny and function, why is it important to differentiate the types of brain macrophages? The response to this is that although there are common facets, there are distinct differences in gene expression and function. For example, there are important differences in the production of quinolinic acid (QUIN), a neurotoxin derived from tryptophan catabolism within the brain [23]. We and others [24–26] have shown that QUIN is elevated in several brain diseases (e.g., AIDS dementia complex, Alzheimer diseases, trauma, meningitis). QUIN may also cause gross opening of the BBB to large molecules, including proteins and immune blood cells [27]. Monocytic cells almost exclusively produce QUIN [28–31]. In brain inflammatory conditions, there is evidence suggesting a major role for resident brain macrophages in the overproduction of QUIN [32], which can induce production of large quantities of MCP-1 by astrocytes [33]. As described above, MCP-1 is one of the most potent chemoattractants for blood monocytes. We and others [29] showed that *de novo* QUIN production by human interferon- γ -activated macrophages was 20- to 30-fold greater than microglial synthesis. Some transcriptional discrepancy might explain the lower microglial ability to produce QUIN in comparison with macrophage [31, 34]. Activated microglia may be the primary endogenous cell type responsible for QUIN synthesis within the CNS in inflammatory diseases. However, under pathological conditions in which the BBB is altered and/or leukocytic infiltration in the brain parenchyma, most of the intracerebral QUIN is derived from activated macrophages. The ability of the pericyte to produce QUIN is unknown. Another important example of a difference between brain macrophages relates to the contractile properties of the pericyte [35]. Perivascular macrophages do not have this capacity.

TABLE 1. Review of membranous, biochemical and morphological markers of activated microglial cells in comparison with blood derived macrophages

Markers	Activated microglia*	Macrophage [†]	References
Peroxidase activity	–	+	[38, 48]
Proliferation	+	–	[38, 41, 47, 49–51]
Cluster of differentiation (CD) 4 (L3T4; W3/25)	+/- (4%)	+	[39, 52, 53]
CD 9 (DRAP-27; p24)	+	+	[54]
CD 11a (αL integrin LFA-1)	+	+	[50, 55–57]
CD 11b [Mac1, αM integrin, C3b complement receptor, orexin (OX)42]	+	+	[40, 43, 47, 50, 52, 55, 57–66]
CD 11c (αX integrin)	+++	+++	[44, 45, 48, 52, 56, 67]
CD 13 (aminopeptidase N)	++	++	[68–70]
CD 14 [lipopolysaccharide (LPS) receptor]	+/-	+++	[45, 47, 52, 57, 67, 71–73]
CDw17 (IacCer)	++	+	[70, 74]
CD 18 (β2 integrin)	+	+	[75]
CD 26 (EC 3.4.14.5)	–	–	[52]
CD 36 (M5, GPIV)	+	++	[76]
CD 40 (Bp50, tumor necrosis factor receptor)	+	+	[72, 77]
CD 44 (Hermes cell adhesion molecule)	+	+	[56, 72]
CD 45 (LCA, Ly5)	+	+++	[50, 52, 58, 64, 65, 67, 72, 78, 79]
CD 53 (OX44)	+	++	[54]
CD 54 (intercellular adhesion molecule-1)	+	+	[50, 56, 72, 77, 80–82]
CD 56 (neuron/cell adhesion molecule isoform)	–	–	[83]
CD 58 (LFA-3)	+	+	[56]
CD 63 [Lysosomal-associated membrane Protein-3 (LAMP-3)]	+	++	[84]
CD 64 [Fc receptor for immunoglobulin G I (FcγI)]	++	++	[47, 66]
CD 66 a, c, d, e (neutrophil chemotactic activity)	+	? (No data)	[70]
CD 68 (macrosialin, gp110)	+++	+++	[41, 43–45, 47, 48, 79, 85–89]
CD 80 (B7-1)	+	–	[43, 65, 67, 72, 90–92]
CD 86 (B7-2, B70)	+	+/-	[43, 65–67, 72, 77, 90–92]
CD 87 (urokinase-type plasminogen activator receptor)	++	+++	[70]
CD 92 (CDw92)	++	++	[70, 93, 94]
CD 106 (VCAM-1)	+	+/-	[80, 95]
CD 107a (LAMP-1)	+	–	[54, 96]
CD 147 (neurothelin, OX47)	+	+	[54, 70]
CD 155 (poliovirus receptor)	++	+	[70, 97]
CD 162 (P-selectin glycoprotein ligand-1)	+	++	[54]
CD 163 (MI30)	+	++	[73, 98]
CD 171 (L1, LICAM)	–	+/-	[48, 99]
CD 200 (OX2)	+	+	[100, 101]
Major histocompatibility complex (MHC) class I (OX18) [human leukocyte antigen (HLA) A, B, C]	+	+	[50–53, 64, 66, 72, 75, 102]
MHC class II (OX6)	+ (Constitutive)	+	[39–41, 44, 50–52, 60, 63, 65, 73, 75, 77, 87, 102, 103]
Concanavalin A	+	+	[104]
F4/80	+	+	[58, 105]
Fibronectin	+	+	[47]
GD3	++	+/- (?)	[106, 107]
Glucose transporter (GLUT)-1	+	+	[108, 109]
GLUT-3	–	–	[108, 109]
GLUT-5	+	+	[59, 109, 110]
HLA-DR (human class II)	+ (Constitutive)	+	[40, 43, 66, 67, 79, 88, 111–114]
Ion-binding adaptor-1	+	+	[115]
Isolectin B4 (<i>Griffonia simplicifolia</i>)	++	++	[59, 114, 116, 117]
LN	+	–	[47, 118]
Lectin <i>Bandeiraea simplicifolia</i>	+	+	[104, 119–121]
Tomato lectin	+	+	[85]
Lectin <i>Ricinus communis</i> agglutinin-1	+	+	[40, 43, 66, 84, 85, 89, 104, 116, 122–124]
LN-1	+/-	–	[7, 125–127]
LN-3	+++	++	[7, 126]
LN-5	+	+++	[7, 126]
Lysozyme	+/-	+	[48, 89, 128]

TABLE 1. (Continued)

Markers	Activated microglia*	Macrophage†	References
Electronic microscopy	Spiky aspect	Rose aspect	[38, 46, 47]
NG2	–	++	[129]
Nonspecific esterase	+	++	[40, 48, 50, 57, 122, 128, 130, 131]
Phagocytosis of latex beads	++	++	[38, 43, 50, 66, 87, 132]
Acid phosphatase	+	++	[40, 50, 57, 122]
Production of quinolinic acid	+	+++	[29, 31]
Acetylated low-density lipoprotein receptor	+	+	[84, 87, 133, 134]
FcR	+	+++	[57, 78, 118, 131, 133]
RFD7	–	+++	[48, 135–137]
Substance P	+++	+	[138]
Vimentin	+	+	[118, 139]
α1-Chymotrypsin	–	++	[128, 140, 141]
α1-Trypsin	+	+	[128]
β2-Microglobulin	+	+	[51, 142]

* Activated microglial cells, whatever the species. † Macrophage (not monocyte). –, Not expressed; +/-, low or controversial expression; +, expressed; ++, strong expression; +++, highly expressed.

With this background, it is pertinent to review methods of identifying these different types of brain macrophage.

MICROGLIA VERSUS MACROPHAGE

Most of the scientific papers concerning the characterization of the microglial cell have been published between the late 1980s and early 1990s [36]. Moreover, a large majority of these publications pertain to the characterization of microglial cells obtained from animals, more particularly, rodents (for review, see refs. [37, 38]), and only a small proportion are concerned with human microglial cells [39–42]. Currently, none of these publications, even the most recent [42–45], describes the existence of a single, specific marker for the microglial cell, with one possible exception. Using scanning electronic microscopy (SEM), Giulian et al. [46] showed that microglia from postnatal rat brain are covered with spines (more than 20 per cell) in a distinctive manner, which contrasts with the smooth surfaces of bone marrow cells and the ruffled surfaces (“Rose aspect” in the tables) of tissue macrophages [47]. The spine-bearing surface of microglia appears to be a specific cell marker, which is not changed with age or a variety of immunostimulants. However, SEM is a complicated method, which cannot be technically used in many in vitro, ex vivo, and in vivo studies. It is still necessary to use several markers together to be able to accurately and easily differentiate macrophages from microglia.

Characterization of the microglial cells is even more difficult, as these cells share several antigens with different cell types (Table 1), including macrophages (CD11b, CD68), endothelial cells [vascular cell adhesion molecule 1 (VCAM-1)], lymphocytes [lymphocyte function-associated antigen (LFA), leukocyte common antigen (LCA), laminin 1 (LN-1)], and oligodendrocyte (GD3). Moreover, published studies about the expression of some microglial markers are occasionally contradictory (Table 1). It is important to highlight that in all these studies, the expression of biological and biochemical micro-

glial markers may vary according to parameters, such as the following examples.

The cell activation

As for monocytes and macrophages, there are marked variations in the expression of membranous markers and in the biochemical activities between the nonactivated microglial cell (ramified) and the activated microglial cell [67]. Changes in the cell morphology are, of course, very significant [38, 46, 143, 144].

The cell maturation

Differences in maturation between microglial cells derived from adult or fetal tissue are another major parameter influencing the expression of markers [145]. To our knowledge, there is no one study that has compared the phenotypic expression between adult and fetal microglial cells from the same species.

The interspecies variations

Even if the microglial markers are the same between species, some differences in function can, however, be found [130]. For example, murine microglial cells are not able to produce the neurotoxin quinolinic acid, whereas human cells can [31], and murine cells exhibit differences in the migratory response to chemokines [145].

The cell-culture conditions

To maintain healthy microglial cells in vitro, the culture medium has to be complemented with growth factors such as macrophage-colony stimulating factor (M-CSF) and granulocyte M-CSF (GM-CSF) [49, 146, 147], with cytokines such as interleukin-3 [49], or with commercial supplements such as B-27 [147, 148] or N2 [149]. The presence of such growth factors in the culture medium may also influence the expression of the microglial markers.

The phenotypic heterogeneity

Lastly, as for the astrocyte, different microglial subpopulations are present in the CNS and display a marked functional and phenotypic heterogeneity [85, 150–155].

Table 1 reviews, in a nonexhaustive manner, a large number of membranous, biochemical, and morphological markers of activated microglial cells (whatever the species) in comparison with blood-derived macrophage. Two major sources of information can be used to obtain complementary data on each single CD: *Leucocyte Typing VI* [70] and the NIH Website <http://www.ncbi.nlm.nih.gov/prow/guide/45277084.htm>.

Of note, a monoclonal antibody (5-D-4) directed against a surface epitope (hypersulfated keratane sulfate) is able to specifically recognize ramified microglial cells but not ameboid microglial cells, monocytes, or macrophages [156].

Characterization of the microglial cell versus the peripheral macrophage has been examined in only a limited number of studies [7, 38, 46, 48, 67, 135, 156–158]. As described above, the combined detection of three or four markers can lead to a quasi-certain identification of the microglial cell. Several studies using flow cytometry or classic immunocytochemistry or immunohistochemistry defined a profile of characterization of the microglial cell corresponding to the following phenotype: CD68+, CD45 low, CD11b+, CD11c high, MHC class II+ and CD14– [41, 45, 47, 48, 64, 159, 160]. Among all the myeloid cells, it seems that only the microglia cell appears spurred with spikes on SEM [46, 47]. This morphologic appearance is valid for more than 99% of the microglial cells, does not change with age, and is not modified by the cellular activation from cytokines. Finally, some other markers, such as the capacity to proliferate in vitro [38, 41, 47], the production of LN [47], the peroxidase activity [38, 48], as well as the RFD7 expression [48], can add important complementary information. **Table 2** summarizes the selection of these markers.

THE PERICYTE VERSUS THE MICROGLIA AND THE PERIVASCULAR MACROPHAGE

The pericyte is another myeloid cell type often located adjacent but distinct from the perivascular macrophage [161]. However,

TABLE 2. Selection of ten markers allowing the differentiation between microglial cells and macrophages

Markers	Microglia	Macrophage
Proliferation	+	–
Peroxidase activity	–	+
Electronic microscopy	Spiky aspect	Rose aspect
LN production	+	–
CD 68 (macrosialin, gp110)	+++	+++
CD 11b	+	+
CD 11c (αX integrin)	+++	+
CD 14 (LPS receptor)	+/-	+++
CD 45 (LCA, Ly5)	+	+++
RFD7	–	+++

The consensus profile of characterization for the microglial cell is: CD68+, CD45 low, CD11b+, CD11c high, MHC class II+, CD14–.

it is far easier to distinguish a microglial cell from a pericyte than from a macrophage. There are several parameters allowing relatively easy differentiation between pericyte and microglia:

The localization

The pericytes are associated with the brain microvasculature, where they are entirely contained within the basal lamina on the abluminal surface of endothelial cells. Pericyte cell processes are located over the endothelial cells tight-junction regions [162]. Microglia do not have direct contact with endothelial cells, as the latter is sheared by astroglial terminations (Fig. 1).

The morphology

CNS pericytes are polymorphs. They can have an oval-to-elongated cell body with branching processes, which encircle the blood vessel (Fig. 1) [2]. As for microglia, CNS pericytes can display a marked heterogeneity in vitro. When grown on plastic, pericytes can appear as large, irregularly shaped cells. Their morphology is more likely that of an astrocyte than a microglial cell [163, 164].

The capacity of proliferation

In culture, microglia and pericytes have a very slow doubling time, and they never reach confluence [165]. In addition, the pericyte has very poor, plated efficiency (<50%), whereas microglia have the capacity to strongly attach to plastic. Phosphatidylcholine (PC) and microglia appear to be relatively resistant to trypsin [47, 165].

The immunochemical markers

As for microglia, no single, specific marker has been identified for the pericyte yet. However, there are some markers that are much more likely to be associated with PC, such as RGS5, which is a member of the RGS family of GTPase-activating proteins [166], the cell-surface 3G5 ganglioside antigen [167, 168], the platelet-derived growth factor (PDGF)- β receptor [169], and the high molecular weight melanoma-associated antigen (HMW-MAA). The latter is specific for microvascular pericytes but is only detected in proliferating cells [170]. RGS7 is expressed by microglia [171] but not RGS5 [172]; 3G5 is present on neurons but not on microglia [173]; and the PDGF- β receptor can be expressed by microglia [174].

As for microglia, a simple association of one of the above markers together with one of the many macrophage markers expressed by the pericyte [2] such as MHC classes I and II molecules [175] would be enough to specifically identify brain (PC) from other brain cells (**Table 3**).

CONCLUSION

Although many questions remain unanswered in regard to the various types of brain macrophages, newly developed methodologies hold promise. Among these are probably laser capture microdissection and gene array. The former could be used to obtain the relevant type of brain macrophage, and the latter could be used to determine which genes are up- or down-

TABLE 3. Review of membranous, biochemical and morphological markers of activated microglial cells in comparison with pericytes

Markers	Microglia	Pericyte	References
α-Smooth muscle actin	–	+	[176]
HMW-MAA	–	+	[170]
RGS5	–	+	[171, 172]
3G5	–	+	[167, 168, 173]
γ-Glutamyltranspeptidase	–	+	[177, 178]
Alkaline phosphatase	–	+	[179]
Aminopeptidase A	–	+	[164]
Aminopeptidase N	–	+	[164, 180]
Butyryl cholinesterase	–	+	[181, 182]
Nestin	–	+	[164]
<i>Griffonia simplicifolia</i> lectin	+	–	[52, 183]
Adherence to plastic	Good	Poor	[165]
CD45	+	–	[52, 183]
ED-2	+	+/-	[183]
PDGF-β receptor	+/-	+	[169, 184]
FcR	+	+	[185]
CD4	+	+	[186, 187]
CD11b (CR3 receptor)	+	+	[183, 186, 187]
MHC class I	+	+	[175, 188]
MHC class II	+	+	[163, 175]
Vimentin	+	+	[189]
von Willebrand factor	–	–	[163]
Desmin	–	–	[163, 184]
Glial fibrillary acidic protein	–	–	[163]

regulated in each cell. With this knowledge, more specific and perhaps diagnostic markers could be developed.

ACKNOWLEDGMENTS

The St. Vincent's Clinic Foundation, the NHMRC, the NSW Health Department, and the UNSW have supported this work.

REFERENCES

- Perry, V. H., Gordon, S. (1997) Microglia and Macrophages. In *Immunology of the Nervous System* (R. W. Keane, W. F. Hickey, eds.), New York, NY, Oxford University Press, 155–172.
- Thomas, W. E. (1999) Brain macrophages: on the role of pericytes and perivascular cells. *Brain Res. Brain Res. Rev.* **31**, 42–57.
- Kaur, C., Hao, A. J., Wu, C. H., Ling, E. A. (2001) Origin of microglia. *Microsc. Res. Tech.* **54**, 2–9.
- Jordan, F. L., Thomas, W. E. (1988) Brain macrophages: questions of origin and interrelationship. *Brain Res.* **472**, 165–178.
- Bechmann, I., Priller, J., Kovac, A., Bontert, M., Wehner, T., Klett, F. F., Bohsung, J., Stuschke, M., Dirnagl, U., Nitsch, R. (2001) Immune surveillance of mouse brain perivascular spaces by blood-borne macrophages. *Eur. J. Neurosci.* **14**, 1651–1658.
- Rhodin, J. A. G., Fujita, H. (1989) Capillary growth in the mesentery of normal young rats. Intravital video and electron microscope analyses. *J. Submicrosc. Cytol. Pathol.* **21**, 1–34.
- Sasaki, A., Nakazato, Y., Ogawa, A., Sugihara, S. (1996) The immunophenotype of perivascular cells in the human brain. *Pathol. Int.* **46**, 15–23.
- Kida, S., Steart, P. V., Zhang, E. T., Weller, R. O. (1993) Perivascular cells act as scavengers in the cerebral perivascular spaces and remain distinct from pericytes, microglia and macrophages. *Acta Neuropathol. (Berl.)* **85**, 646–652.
- Theele, D. P., Streit, W. J. (1993) A chronicle of microglial ontogeny. *Glia* **7**, 5–9.
- Cuadros, M. A., Navascues, J. (1998) The origin and differentiation of microglial cells during development. *Prog. Neurobiol.* **56**, 173–189.
- Balabanov, R., Beaumont, T., Dore-Duffy, P. (1999) Role of central nervous system microvascular pericytes in activation of antigen-primed splenic T-lymphocytes. *J. Neurosci. Res.* **55**, 578–587.
- Yamagishi, S., Yonekura, H., Yamamoto, Y., Fujimori, H., Sakurai, S., Tanaka, N., Yamamoto, H. (1999) Vascular endothelial growth factor acts as a pericyte mitogen under hypoxic conditions. *Lab. Invest.* **79**, 501–509.
- Dore-Duffy, P., Owen, C., Balabanov, R., Murphy, S., Beaumont, T., Rafols, J. A. (2000) Pericyte migration from the vascular wall in response to traumatic brain injury. *Microvasc. Res.* **60**, 55–69.
- Allt, G., Lawrenson, J. G. (2001) Pericytes: cell biology and pathology. *Cells Tissues Organs* **169**, 1–11.
- Fiala, M., Liu, Q. N., Sayre, J., Pop, V., Brahmandam, V., Graves, M. C., Vinters, H. V. (2002) Cyclooxygenase-2-positive macrophages infiltrate the Alzheimer's disease brain and damage the blood-brain barrier. *Eur. J. Clin. Invest.* **32**, 360–371.
- Chen, Y., Hallenbeck, J. M., Ruetzler, C., Bol, D., Thomas, K., Berman, N. E., Vogel, S. N. (2003) Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischemic brain injury and is associated with recruitment of inflammatory cells. *J. Cereb. Blood Flow Metab.* **23**, 748–755.
- Streit, W. J., Walter, S. A., Pennell, N. A. (1999) Reactive microgliosis. *Prog. Neurobiol.* **57**, 563–581.
- Nakamura, Y. (2002) Regulating factors for microglial activation. *Biol. Pharm. Bull.* **25**, 945–953.
- Grammas, P., Yamada, M., Zlokovic, B. (2002) The cerebrovasculature: a key player in the pathogenesis of Alzheimer's disease. *J. Alzheimers Dis.* **4**, 217–223.
- de la Torre, J. C. (2002) Vascular basis of Alzheimer's pathogenesis. *Ann. N. Y. Acad. Sci.* **977**, 196–215.
- Cullen, K. M. (1997) Perivascular astrocytes within Alzheimer's disease plaques. *Neuroreport* **8**, 1961–1966.
- Streit, W. J. (2002) Microglia as neuroprotective, immunocompetent cells of the CNS. *Glia* **40**, 133–139.
- Stone, T. W., Perkins, M. N. (1981) Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS. *Eur. J. Pharmacol.* **72**, 411–412.
- Stone, T. W. (1993) Neuropharmacology of quinolinic and kynurenic acids. *Pharmacol. Rev.* **45**, 309–379.
- Brew, B. J., Pemberton, L., Evans, L., Saito, K., Penny, R., Cooper, D. A., Heyes, M. P. (1995) Quinolinic acid production is related to macrophage tropic isolates of HIV-1. *J. Neurovirol.* **1**, 369–374.
- Guillemin, G. J., Brew, B. J., Noonan, C., Cullen, K. (2003) Indoleamine dioxygenase and quinolinic acid in plaque and neurons in post-mortem brain tissue from Alzheimer's disease and control cases. *Neuroreport*, in press.
- Owe-Young, R., Guillemin, G. J., Mukhtar, M., Pomerantz, R. J., Stins, M., Kim, K. S., Armati, P. J., Brew, B. J. (2003) Kynurenine pathway metabolism in human blood-brain barrier endothelial cells. Vth International Conference of Cerebral Vascular Biology; 2003 June 15–19; Amarillo, TX. In *Cerebral Vascular Biology*, Texas Tech University.
- Heyes, M. P., Achim, C. L., Wiley, C. A., Major, E. O., Saito, K., Markey, S. P. (1996) Human microglia convert L-tryptophan into the neurotoxin quinolinic acid. *Biochem. J.* **320**, 595–597.
- Espey, M. G., Chernyshev, O. N., Reinhard, J. J., Nambodiri, M. A., Colton, C. A. (1997) Activated human microglia produce the excitotoxin quinolinic acid. *Neuroreport* **8**, 431–434.
- Guillemin, G. J., Kerr, S. J., Smythe, G. A., Smith, D. G., Kapoor, V., Armati, P. J., Croitoru, J., Brew, B. J. (2001) Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection. *J. Neurochem.* **78**, 842–853.
- Guillemin, G. J., Smith, D. G., Armati, P. J., Brew, B. J. (2003) Expression kynurenine pathway enzymes in human macrophages and microglia. *Adv. Exp. Med. Biol.*, in press.
- Stone, T. W. (2001) Endogenous neurotoxins from tryptophan. *Toxicol.* **39**, 61–73.
- Guillemin, G. J., Croitoru-Lamourey, J., Dormont, D., Armati, P. J., Brew, B. J. (2003) Quinolinic acid upregulates chemokine production and chemokine receptor expression in astrocytes. *Glia* **41**, 371–381.
- Alberati-Giani, D., Ricciardi-Castagnoli, P., Kohler, C., Cesura, A. M. (1996) Regulation of the kynurenine metabolic pathway by interferon-γ in murine cloned macrophages and microglial cells. *J. Neurochem.* **66**, 996–1004.
- Bandopadhyay, R., Orte, C., Lawrenson, J. G., Reid, A. R., De Silva, S., Allt, G. (2001) Contractile proteins in pericytes at the blood-brain and blood-retinal barriers. *J. Neurocytol.* **30**, 35–44.

36. Hanisch, U. K., Kohsaka, S., Moller, T. (2002) Editorial. *Glia* **40**, 131–132.
37. Moore, S., Thanos, S. (1996) The concept of microglia in relation to central nervous system disease and regeneration. *Prog. Neurobiol.* **48**, 441–460.
38. Giulian, D., Baker, T. J. (1986) Characterization of amoeboid microglia isolated from developing mammalian brain. *J. Neurosci.* **6**, 2163–2178.
39. Peudenier, S., Hery, C., Montagnier, L., Tardieu, M. (1991) Human microglial cells: characterization in cerebral tissue and in primary culture, and study of their susceptibility to HIV-1 infection. *Ann. Neurol.* **29**, 152–161.
40. Hassan, N. F., Campbell, D. E., Rifat, S., Douglas, S. D. (1991) Isolation and characterization of human fetal brain-derived microglia in vitro culture. *Neuroscience* **41**, 149–158.
41. Lee, S. C., Liu, W., Brosnan, C. F., Dickson, D. W. (1992) Characterization of primary human fetal dissociated central nervous system cultures with an emphasis on microglia. *Lab. Invest.* **67**, 465–476.
42. De Groot, C. J., Montagne, L., Janssen, I., Ravid, R., Van Der Valk, P., Veerhuis, R. (2000) Isolation and characterization of adult microglial cells and oligodendrocytes derived from postmortem human brain tissue. *Brain Res. Brain Res. Protoc.* **5**, 85–94.
43. Nagai, A., Nakagawa, E., Hatori, K., Choi, H. B., McLarnon, J. G., Lee, M. A., Kim, S. U. (2001) Generation and characterization of immortalized human microglial cell lines: expression of cytokines and chemokines. *Neurobiol. Dis.* **8**, 1057–1068.
44. de Groot, C. J., Hulshof, S., Hoozemans, J. J., Veerhuis, R. (2001) Establishment of microglial cell cultures derived from postmortem human adult brain tissue: immunophenotypic and functional characterization. *Microsc. Res. Tech.* **54**, 34–39.
45. Albright, A. V., Shieh, J. T., O'Connor, M. J., Gonzalez-Scarano, F. (2000) Characterization of cultured microglia that can be infected by HIV-1. *J. Neurovirol.* **6**, S53–S60.
46. Giulian, D., Li, J., Bartel, S., Broker, J., Li, X., Kirkpatrick, J. B. (1995) Cell surface morphology identifies microglia as a distinct class of mononuclear phagocyte. *J. Neurosci.* **15**, 7712–7726.
47. Guillemain, G., Boussin, F. D., Croitoru, J., Franck-Duchenne, M., Le Grand, R., Lazarini, F., Dormont, D. (1997) Obtention and characterization of primary astrocyte and microglial cultures from adult monkey brains. *J. Neurosci. Res.* **49**, 576–591.
48. Ulvestad, E., Williams, K., Mork, S., Antel, J., Nyland, H. (1994) Phenotypic differences between human monocytes/macrophages and microglial cells studied in situ and in vitro. *J. Neuropathol. Exp. Neurol.* **53**, 492–501.
49. Kloss, C. U., Kreutzberg, G. W., Raivich, G. (1997) Proliferation of ramified microglia on an astrocyte monolayer: characterization of stimulatory and inhibitory cytokines. *J. Neurosci. Res.* **49**, 248–254.
50. Ohsawa, K., Imai, Y., Nakajima, K., Kohsaka, S. (1997) Generation and characterization of a microglial cell line, MG5, derived from a p53-deficient mouse. *Glia* **21**, 285–298.
51. Lauro, G. M., Babiloni, D., Buttarelli, F. R., Starace, G., Cocchia, D., Ennas, M. G., Sogos, V., Gremo, F. (1995) Human microglia cultures: a powerful model to study their origin and immunoreactive capacity. *Int. J. Dev. Neurosci.* **13**, 739–752.
52. Ford, A. L., Goodsall, A. L., Hickey, W. F., Sedgwick, J. D. (1995) Normal adult ramified microglia separated from other central nervous system macrophages by flow cytometric sorting. Phenotypic differences defined and direct ex vivo antigen presentation to myelin basic protein-reactive CD4+ T cells compared. *J. Immunol.* **154**, 4309–4321.
53. Hassan, N. F., Rifat, S., Campbell, D. E., McCawley, L. J., Douglas, S. D. (1991) Isolation and flow cytometric characterization of newborn mouse brain-derived microglia maintained in vitro. *J. Leukoc. Biol.* **50**, 86–92.
54. Inoue, H., Sawada, M., Ryo, A., Tanahashi, H., Wakatsuki, T., Hada, A., Kondoh, N., Nakagaki, K., Takahashi, K., Suzumura, A., Yamamoto, M., Tabira, T. (1999) Serial analysis of gene expression in a microglial cell line. *Glia* **28**, 265–271.
55. Jeetle, J. K., Hagger, G. N., Topps, S. S., Male, D. K., Rezaie, P. (2002) Microglial colonization of the developing mouse brain: the effect of CD11b deletion. *Neuropathol. Appl. Neurobiol.* **28**, 164.
56. Le Naour, R., Lussiez, C., Raoul, H., Mabondzo, A., Dormont, D. (1997) Expression of cell adhesion molecules at the surface of in vitro human immunodeficiency virus type 1-infected human monocytes: relationships with tumor necrosis factor α , interleukin 1β , and interleukin 6 syntheses. *AIDS Res. Hum. Retroviruses* **13**, 841–855.
57. Olivier, M., Berthon, P., Chastang, J., Cordier, G., Lantier, F. (2001) Establishment and characterization of ovine blood monocyte-derived cell lines. *Vet. Immunol. Immunopathol.* **82**, 139–151.
58. Williams, A. E., Lawson, L. J., Perry, V. H., Fraser, H. (1994) Characterization of the microglial response in murine scrapie. *Neuropathol. Appl. Neurobiol.* **20**, 47–55.
59. Cheepsunthorn, P., Radov, L., Menzies, S., Reid, J., Connor, J. R. (2001) Characterization of a novel brain-derived microglial cell line isolated from neonatal rat brain. *Glia* **35**, 53–62.
60. Csuka, E., Hans, V. H., Ammann, E., Trentz, O., Kossmann, T., Morganti-Kossmann, M. C. (2000) Cell activation and inflammatory response following traumatic axonal injury in the rat. *Neuroreport* **11**, 2587–2590.
61. Elkabes, S., DiCicco-Bloom, E. M., Black, I. B. (1996) Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J. Neurosci.* **16**, 2508–2521.
62. Merz, G. S., Schwenk, V., Schuller-Levis, G., Gruca, S., Wisniewski, H. M. (1987) Isolation and characterization of macrophages from scrapie-infected mouse brain. *Acta Neuropathol. (Berl.)* **72**, 240–247.
63. Pedersen, E. B., Fox, L. M., Castro, A. J., McNulty, J. A. (1993) Immunocytochemical and electron-microscopic characterization of macrophage/microglia cells and expression of class II major histocompatibility complex in the pineal gland of the rat. *Cell Tissue Res.* **272**, 257–265.
64. Sedgwick, J. D., Schwender, S., Imrich, H., Dorries, R., Butcher, G. W., ter Meulen, V. (1991) Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system. *Proc. Natl. Acad. Sci. USA* **88**, 7438–7442.
65. Zhang, G. X., Li, J., Ventura, E., Rostami, A. (2002) Parenchymal microglia of naive adult C57BL/6J mice express high levels of B7.1, B7.2, and MHC class II. *Exp. Mol. Pathol.* **73**, 35–45.
66. Lee, Y. B., Nagai, A., Kim, S. U. (2002) Cytokines, chemokines, and cytokine receptors in human microglia. *J. Neurosci. Res.* **69**, 94–103.
67. Becher, B., Antel, J. P. (1996) Comparison of phenotypic and functional properties of immediately ex vivo and cultured human adult microglia. *Glia* **18**, 1–10.
68. Look, A. T., Ashmun, R. A., Shapiro, L. H., Peiper, S. C. (1989) Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. *J. Clin. Invest.* **83**, 1299–1307.
69. Ziaber, J., Baj, Z., Pasnik, J., Chmielewski, H., Tchorzewski, H. (2000) Expression of aminopeptidase N (APN) on peripheral blood mononuclear cells' surface as a marker of these cells' transendothelial migration properties in the course of multiple sclerosis. *Mediators Inflamm.* **9**, 45–48.
70. Kishimoto, T., Kikutani, H., von dem Borne, A., Goyert, S. M., Mason, D. Y., Miyasaka, M., Moretta, M., Okumura, K., Shaw, S. J., Springer, T. A., Sugamura, K., Zola, H. (1997) *Leucocyte Typing VI*, New York, NY, Garland Publishing.
71. Beschoner, R., Schluesener, H. J., Gozalan, F., Meyermann, R., Schwab, J. M. (2002) Infiltrating CD14+ monocytes and expression of CD14 by activated parenchymal microglia/macrophages contribute to the pool of CD14+ cells in ischemic brain lesions. *J. Neuroimmunol.* **126**, 107–115.
72. Havenith, C. E., Askew, D., Walker, W. S. (1998) Mouse resident microglia: isolation and characterization of immunoregulatory properties with naive CD4+ and CD8+ T-cells. *Glia* **22**, 348–359.
73. Yang, P., Chen, L., Zwart, R., Kijlstra, A. (2002) Immune cells in the porcine retina: distribution, characterization and morphological features. *Invest. Ophthalmol. Vis. Sci.* **43**, 1488–1492.
74. Lund-Johansen, F., Olweus, J., Horejsi, V., Skubitz, K. M., Thompson, J. S., Vilella, R., Symington, F. W. (1992) Activation of human phagocytes through carbohydrate antigens (CD15, sialyl-CD15, CDw17, and CDw65). *J. Immunol.* **148**, 3221–3229.
75. Grau, V., Herbst, B., van der Meide, P. H., Steiniger, B. (1997) Activation of microglial and endothelial cells in the rat brain after treatment with interferon- γ in vivo. *Glia* **19**, 181–189.
76. Coraci, I. S., Husemann, J., Berman, J. W., Hulette, C., Dufour, J. H., Campanella, G. K., Luster, A. D., Silverstein, S. C., El-Khoury, J. B. (2002) CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to β -amyloid fibrils. *Am. J. Pathol.* **160**, 101–112.
77. Aloisi, F., De Simone, R., Columba-Cabezas, S., Penna, G., Adorini, L. (2000) Functional maturation of adult mouse resting microglia into an APC is promoted by granulocyte-macrophage colony-stimulating factor and interaction with Th1 cells. *J. Immunol.* **164**, 1705–1712.
78. Carson, M. J., Reilly, C. R., Sutcliffe, J. G., Lo, D. (1998) Mature microglia resemble immature antigen-presenting cells. *Glia* **22**, 72–85.
79. Yang, P., Das, P. K., Kijlstra, A. (2000) Localization and characterization of immunocompetent cells in the human retina. *Ocul. Immunol. Inflamm.* **8**, 149–157.

80. Lee, S. J., Benveniste, E. N. (1999) Adhesion molecule expression and regulation on cells of the central nervous system. *J. Neuroimmunol.* **98**, 77–88.
81. Sebire, G., Hery, C., Peudener, S., Tardieu, M. (1993) Adhesion proteins on human microglial cells and modulation of their expression by IL1 α and TNF α . *Res. Virol.* **144**, 47–52.
82. Cotman, C. W., Hailer, N. P., Pfister, K. K., Soltesz, I., Schachner, M. (1998) Cell adhesion molecules in neural plasticity and pathology: similar mechanisms, distinct organizations? *Prog. Neurobiol.* **55**, 659–669.
83. Brevig, T., Kristensen, T., Zimmer, J. (1999) Expression of major histocompatibility complex antigens and induction of human T-lymphocyte proliferation by astrocytes and macrophages from porcine fetal brain. *Exp. Neurol.* **159**, 474–483.
84. Briers, T. W., Desmaretz, C., Vanmechelen, E. (1994) Generation and characterization of mouse microglial cell lines. *J. Neuroimmunol.* **52**, 153–164.
85. Andjelkovic, A. V., Nikolic, B., Pachter, J. S., Zecevic, N. (1998) Macrophages/microglial cells in human central nervous system during development: an immunohistochemical study. *Brain Res.* **814**, 13–25.
86. Langosch, J. M., Gebicke-Haerter, P. J., Norenberg, W., Illes, P. (1994) Characterization and transduction mechanisms of purinoceptors in activated rat microglia. *Br. J. Pharmacol.* **113**, 29–34.
87. Lue, L. F., Brachova, L., Walker, D. G., Rogers, J. (1996) Characterization of glial cultures from rapid autopsies of Alzheimer's and control patients. *Neurobiol. Aging* **17**, 421–429.
88. Roggendorf, W., Strupp, S., Paulus, W. (1996) Distribution and characterization of microglia/macrophages in human brain tumors. *Acta Neuropathol. (Berl.)* **92**, 288–293.
89. Hulette, C. M., Downey, B. T., Burger, P. C. (1992) Macrophage markers in diagnostic neuropathology. *Am. J. Surg. Pathol.* **16**, 493–499.
90. De Simone, R., Giampaolo, A., Giometto, B., Gallo, P., Levi, G., Peschle, C., Aloisi, F. (1995) The costimulatory molecule B7 is expressed on human microglia in culture and in multiple sclerosis acute lesions. *J. Neuropathol. Exp. Neurol.* **54**, 175–187.
91. Menendez, I. B., Cerase, J., Ceracchini, C., Levi, G., Aloisi, F. (1997) Analysis of B7-1 and B7-2 costimulatory ligands in cultured mouse microglia: upregulation by interferon- γ and lipopolysaccharide and downregulation by interleukin-10, prostaglandin E2 and cyclic AMP-elevating agents. *J. Neuroimmunol.* **72**, 83–93.
92. Matsubara, T., Pararajasegaram, G., Wu, G. S., Rao, N. A. (1999) Retinal microglia differentially express phenotypic markers of antigen-presenting cells in vitro. *Invest. Ophthalmol. Vis. Sci.* **40**, 3186–3193.
93. Pickl, W. F., Majdic, O., Kohl, P., Stockl, J., Riedl, E., Scheinecker, C., Bello-Fernandez, C., Knapp, W. (1996) Molecular and functional characteristics of dendritic cells generated from highly purified CD14+ peripheral blood monocytes. *J. Immunol.* **157**, 3850–3859.
94. Wille, S., Szekeres, A., Majdic, O., Prager, E., Staffler, G., Stockl, J., Kunthaler, D., Prieschl, E. E., Baumruker, T., Burtscher, H., Zlabinger, G. J., Knapp, W., Stockinger, H. (2001) Characterization of CDw92 as a member of the choline transporter-like protein family regulated specifically on dendritic cells. *J. Immunol.* **167**, 5795–5804.
95. Rosner, K., Ross, C., Karlsmark, T., Skovgaard, G. L. (2001) Role of LFA-1/ICAM-1, CLA/E-selectin and VLA-4/VCAM-1 pathways in recruiting leukocytes to the various regions of the chronic leg ulcer. *Acta Derm. Venereol.* **81**, 334–339.
96. Sakai, E., Miyamoto, H., Okamoto, K., Kato, Y., Yamamoto, K., Sakai, H. (2001) Characterization of phagosomal subpopulations along endocytic routes in osteoclasts and macrophages. *J. Biochem. (Tokyo)* **130**, 823–831.
97. Freistadt, M. S., Eberle, K. E. (2000) Hematopoietic cells from CD155-transgenic mice express CD155 and support poliovirus replication *in vivo*. *Microb. Pathog.* **29**, 203–212.
98. Sanchez, C., Domenech, N., Vazquez, J., Alonso, F., Ezquerro, A., Dominguez, J. (1999) The porcine 2A10 antigen is homologous to human CD163 and related to macrophage differentiation. *J. Immunol.* **162**, 5230–5237.
99. Pancook, J. D., Reisfeld, R. A., Varki, N., Vitiello, A., Fox, R. I., Montgomery, A. M. (1997) Expression and regulation of the neural cell adhesion molecule L1 on human cells of myelomonocytic and lymphoid origin. *J. Immunol.* **158**, 4413–4421.
100. Neumann, H. (2001) Control of glial immune function by neurons. *Glia* **36**, 191–199.
101. Hoek, R. M., Ruuls, S. R., Murphy, C. A., Wright, G. J., Goddard, R., Zurawski, S. M., Blom, B., Homola, M. E., Streit, W. J., Brown, M. H., Barclay, A. N., Sedgwick, J. D. (2000) Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* **290**, 1768–1771.
102. Grenier, Y., Ruijs, T. C., Robitaille, Y., Olivier, A., Antel, J. P. (1989) Immunohistochemical studies of adult human glial cells. *J. Neuroimmunol.* **21**, 103–115.
103. Bo, L., Mork, S., Kong, P. A., Nyland, H., Pardo, C. A., Trapp, B. D. (1994) Detection of MHC class II-antigens on macrophages and microglia, but not on astrocytes and endothelia in active multiple sclerosis lesions. *J. Neuroimmunol.* **51**, 135–146.
104. Kaur, C., Ling, E. A., Wong, W. C. (1990) Lectin labelling of amoeboid microglial cells in the brain of postnatal rats. *J. Anat.* **173**, 151–160.
105. 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.
106. Ellison, J. A., de Vellis, J. (1995) Amoeboid microglia expressing GD3 ganglioside are concentrated in regions of oligodendrogenesis during development of the rat corpus callosum. *Glia* **14**, 123–132.
107. Wolswijk, G. (1995) Strongly GD3+ cells in the developing and adult rat cerebellum belong to the microglial lineage rather than to the oligodendrocyte lineage. *Glia* **13**, 13–26.
108. Maher, F. (1995) Immunolocalization of GLUT1 and GLUT3 glucose transporters in primary cultured neurons and glia. *J. Neurosci. Res.* **42**, 459–469.
109. Malide, D., Davies-Hill, T. M., Levine, M., Simpson, I. A. (1998) Distinct localization of GLUT-1, -3, and -5 in human monocyte-derived macrophages: effects of cell activation. *Am. J. Physiol.* **274**, E516–E526.
110. Payne, J., Maher, F., Simpson, I., Mattice, L., Davies, P. (1997) Glucose transporter Glut 5 expression in microglial cells. *Glia* **21**, 327–331.
111. Esiri, M. M., al Izzi, M. S., Reading, M. C. (1991) Macrophages, microglial cells, and HLA-DR antigens in fetal and infant brain. *J. Clin. Pathol.* **44**, 102–106.
112. Dandrea, M. R., Reiser, P. A., Gumula, N. A., Hertzog, B. M., Andrade-Gordon, P. (2001) Application of triple immunohistochemistry to characterize amyloid plaque-associated inflammation in brains with Alzheimer's disease. *Biotech. Histochem.* **76**, 97–106.
113. Styren, S. D., Civin, W. H., Rogers, J. (1990) Molecular, cellular, and pathologic characterization of HLA-DR immunoreactivity in normal elderly and Alzheimer's disease brain. *Exp. Neurol.* **110**, 93–104.
114. Baldus, S. E., Wickenhauser, C., Stefanovic, A., Schmitz, B., Thiele, J., Fischer, R. (1998) Enrichment of human bone marrow mononuclear phagocytes and characterization of macrophage subpopulations by immunoenzymatic double staining. *Histochem. J.* **30**, 285–291.
115. Imai, Y., Ibat, I., Ito, D., Ohsawa, K., Kohsaka, S. (1996) A novel gene *iba1* in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. *Biochem. Biophys. Res. Commun.* **224**, 855–862.
116. Franceschini, V., Ciani, F. (1992) A histochemical study of the microglial cells in the brain of Salamandra salamandra by lectin binding. *Eur. J. Histochem.* **36**, 215–222.
117. Ridoux, V., Valin, A., Synguelakis, M., Le Gal La Salle, G. (1994) *Ex vivo* culture of adult microglial cells from previously lesioned rat brains. *C. R. Acad. Sci. III* **317**, 217–224.
118. Rieske, E., Graeber, M. B., Tetzlaff, W., Czlonkowska, A., Streit, W. J., Kreutzberg, G. W. (1989) Microglia and microglia-derived brain macrophages in culture: generation from axotomized rat facial nuclei, identification and characterization *in vitro*. *Brain Res.* **492**, 1–14.
119. Mertsch, K., Hanisch, U. K., Kettenmann, H., Schnitzer, J. (2001) Characterization of microglial cells and their response to stimulation in an organotypic retinal culture system. *J. Comp. Neurol.* **431**, 217–227.
120. Strauchen, J. A. (1984) Lectin receptors as markers of lymphoid cells. I. Demonstration in tissue section by peroxidase technique. *Am. J. Pathol.* **116**, 297–304.
121. Chapes, S. K. (1989) Formalin-fixed macrophages bind tumor targets similarly to viable macrophages. *J. Leukoc. Biol.* **45**, 322–328.
122. Hassan, N. F., Prakash, K., Chehimi, J., McCawley, L. J., Douglas, S. D. (1991) Isolation and characterization of newborn rabbit brain-derived microglia. *Clin. Immunol. Immunopathol.* **59**, 426–435.
123. Hutchins, K. D., Dickson, D. W., Rashbaum, W. K., Lyman, W. D. (1990) Localization of morphologically distinct microglial populations in the developing human fetal brain: implications for ontogeny. *Brain Res. Dev. Brain Res.* **55**, 95–102.
124. Mannoji, H., Yeager, H., Becker, L. E. (1986) A specific histochemical marker (lectin Ricinus communis agglutinin-I) for normal human microglia, and application to routine histopathology. *Acta Neuropathol. (Berl.)* **71**, 341–343.
125. Miles, J. M., Chou, S. M. (1988) A new immunoperoxidase marker for microglia in paraffin section. *J. Neuropathol. Exp. Neurol.* **47**, 579–587.
126. Sasaki, A., Nakanishi, Y., Nakazato, Y., Yamaguchi, H. (1991) Application of lectin and B-lymphocyte-specific monoclonal antibodies for the

- demonstration of human microglia in formalin-fixed, paraffin-embedded brain tissue. *Virchows Arch. A Pathol. Anat. Histopathol.* **419**, 291–299.
127. Dickson, D. W., Mattiace, L. A. (1989) Astrocytes and microglia in human brain share an epitope recognized by a B-lymphocyte-specific monoclonal antibody (LN-1). *Am. J. Pathol.* **135**, 135–147.
 128. Esiri, M. M., Booss, J. (1984) Comparison of methods to identify microglial cells and macrophages in the human central nervous system. *J. Clin. Pathol.* **37**, 150–156.
 129. Jones, L. L., Yamaguchi, Y., Stallcup, W. B., Tuszynski, M. H. (2002) NG2 is a major chondroitin sulfate proteoglycan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. *J. Neurosci.* **22**, 2792–2803.
 130. Hayes, G. M., Woodroffe, M. N., Cuzner, M. L. (1988) Characterisation of microglia isolated from adult human and rat brain. *J. Neuroimmunol.* **19**, 177–189.
 131. Jordan, F. L., Thomas, W. E. (1987) Identification of microglia in primary cultures of mixed cerebral cortical cells. *Brain Res. Bull.* **19**, 153–159.
 132. Ward, S. A., Ransom, P. A., Booth, P. L., Thomas, W. E. (1991) Characterization of ramified microglia in tissue culture: pinocytosis and motility. *J. Neurosci. Res.* **29**, 13–28.
 133. Brockhaus, J., Ilschner, S., Banati, R. B., Kettenmann, H. (1993) Membrane properties of amoeboid microglial cells in the corpus callosum slice from early postnatal mice. *J. Neurosci.* **13**, 4412–4421.
 134. Ottnad, E., Parthasarathy, S., Sambrano, G. R., Ramprasad, M. P., Quehenberger, O., Kondratenko, N., Green, S., Steinberg, D. (1995) A macrophage receptor for oxidized low density lipoprotein distinct from the receptor for acetyl low density lipoprotein: partial purification and role in recognition of oxidatively damaged cells. *Proc. Natl. Acad. Sci. USA* **92**, 1391–1395.
 135. Ulvestad, E., Williams, K., Bjerkvig, R., Tiekotter, K., Antel, J., Matre, R. (1994) Human microglial cells have phenotypic and functional characteristics in common with both macrophages and dendritic antigen-presenting cells. *J. Leukoc. Biol.* **56**, 732–740.
 136. Li, H., Newcombe, J., Groome, N. P., Cuzner, M. L. (1993) Characterization and distribution of phagocytic macrophages in multiple sclerosis plaques. *Neuropathol. Appl. Neurobiol.* **19**, 214–223.
 137. Spiteri, M. A., Poulter, L. W. (1991) Characterization of immune inducer and suppressor macrophages from the normal human lung. *Clin. Exp. Immunol.* **83**, 157–162.
 138. Lai, J. P., Zhan, G. X., Campbell, D. E., Douglas, S. D., Ho, W. Z. (2000) Detection of substance P and its receptor in human fetal microglia. *Neuroscience* **101**, 1137–1144.
 139. Graeber, M. B., Streit, W. J., Kreutzberg, G. W. (1988) The microglial cytoskeleton: vimentin is localized within activated cells in situ. *J. Neurocytol.* **17**, 573–580.
 140. Esiri, M. M., McGee, J. O. (1986) Monoclonal antibody to macrophages (EMB/11) labels macrophages and microglial cells in human brain. *J. Clin. Pathol.* **39**, 615–621.
 141. Gould, S. J., Howard, S. (1991) An immunohistological study of macrophages in the human fetal brain. *Neuropathol. Appl. Neurobiol.* **17**, 383–390.
 142. Carozzi, S., Nasini, G., Schelotto, C., Caviglia, P. M., Canepa, M., Zanin, T., Cantaluppi, A., Salit, M. (1990) Peritoneal macrophage β -2 microglobulin production and bacterial peritonitis in CAPD patients. *ASAI Trans.* **36**, M369–M371.
 143. Davis, E. J., Foster, T. D., Thomas, W. E. (1994) Cellular forms and functions of brain microglia. *Brain Res. Bull.* **34**, 73–78.
 144. Toku, K., Tanaka, J., Fujikata, S., Hamamoto, Y., Horikawa, Y., Miyoshi, K., Tateishi, N., Suzuki, Y., Maeda, N. (1999) Distinctions between microglial cells and peripheral macrophages with regard to adhesive activities and morphology. *J. Neurosci. Res.* **57**, 855–865.
 145. Cross, A. K., Woodroffe, M. N. (1999) Chemokines induce migration and changes in actin polymerization in adult rat brain microglia and a human fetal microglial cell line in vitro. *J. Neurosci. Res.* **55**, 17–23.
 146. Lee, S. C., Liu, W., Brosnan, C. F., Dickson, D. W. (1994) GM-CSF promotes proliferation of human fetal and adult microglia in primary cultures. *Glia* **12**, 309–318.
 147. Fujita, H., Tanaka, J., Toku, K., Tateishi, N., Suzuki, Y., Matsuda, S., Sakanaka, M., Maeda, N. (1996) Effects of GM-CSF and ordinary supplements on the ramification of microglia in culture: a morphometrical study. *Glia* **18**, 269–281.
 148. Brewer, G. J. (1995) Serum-free B27/neurobasal medium supports differentiated growth of neurons from the striatum, substantia nigra, septum, cerebral cortex, cerebellum, and dentate gyrus. *J. Neurosci. Res.* **42**, 674–683.
 149. Slepko, N., Levi, G. (1996) Progressive activation of adult microglial cells in vitro. *Glia* **16**, 241–246.
 150. Lawson, L. J., Perry, V. H., Dri, P., Gordon, S. (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**, 151–170.
 151. Mander, T. H., Morris, J. F. (1995) Immunophenotypic evidence for distinct populations of microglia in the rat hypothalamo-neurohypophysial system. *Cell Tissue Res.* **280**, 665–673.
 152. Amat, J. A., Ishiguro, H., Nakamura, K., Norton, W. T. (1996) Phenotypic diversity and kinetics of proliferating microglia and astrocytes following cortical stab wounds. *Glia* **16**, 368–382.
 153. Streit, W. J., Graeber, M. B. (1991) Perivascular location and phenotypic heterogeneity of microglial cells in the rat brain. *J. Neuroimmunol.* **33**, 87.
 154. Streit, W. J., Graeber, M. B. (1993) Heterogeneity of microglial and perivascular cell populations: insights gained from the facial nucleus paradigm. *Glia* **7**, 68–74.
 155. Rezaie, P., Patel, K., Male, D. K. (1999) Microglia in the human fetal spinal cord—patterns of distribution, morphology and phenotype. *Brain Res. Dev. Brain Res.* **115**, 71–81.
 156. Wilms, H., Wollmer, M. A., Sievers, J. (1999) In vitro-staining specificity of the antibody 5-D-4 for microglia but not for monocytes and macrophages indicates that microglia are a unique subgroup of the myelomonocytic lineage. *J. Neuroimmunol.* **98**, 89–95.
 157. Kettenmann, H., Hoppe, D., Gottmann, K., Banati, R., Kreutzberg, G. (1990) Cultured microglial cells have a distinct pattern of membrane channels different from peritoneal macrophages. *J. Neurosci. Res.* **26**, 278–287.
 158. Flaris, N. A., Densmore, T. L., Molleston, M. C., 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.
 159. Milligan, C. E., Levitt, P., Cunningham, T. J. (1991) Brain macrophages and microglia respond differently to lesions of the developing and adult visual system. *J. Comp. Neurol.* **314**, 136–146.
 160. Dick, A. D., Pell, M., Brew, B. J., Foulcher, E., Sedgwick, J. D. (1997) Direct ex vivo flow cytometric analysis of human microglial cell CD4 expression: examination of central nervous system biopsy specimens from HIV-seropositive patients and patients with other neurological disease. *AIDS* **11**, 1699–1708.
 161. Owe-Young, R. (2003) The pericyte. In *Microglia (in French)*, vol. 7 [A.p.l.n.p.p. (ANPP), ed.], Paris, ANPP, in press.
 162. Abbott, N. J. (2000) Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell. Mol. Neurobiol.* **20**, 131–147.
 163. Balabanov, R., Dore-Duffy, P. (1998) Role of the CNS microvascular pericyte in the blood-brain barrier. *J. Neurosci. Res.* **53**, 637–644.
 164. Alliot, F., Rutin, J., Leenen, P. J., Pessac, B. (1999) Pericytes and periendothelial cells of brain parenchyma vessels co-express aminopeptidase N, aminopeptidase A, and nestin. *J. Neurosci. Res.* **58**, 367–378.
 165. D'Amore, P. A. (1990) Culture and study of pericytes. In *Cell Culture Techniques in Heart and Vessel Research* (E. H. M. Piper, ed.), Berlin, Springer-Verlag, 299–314.
 166. Bondjers, C., Kalen, M., Hellstrom, M., Scheidl, S. J., Abramsson, A., Renner, O., Lindahl, P., Cho, H., Kehr, J., Betscholtz, C. (2003) Transcription profiling of platelet-derived growth factor- β -deficient mouse embryos identifies RGS5 as a novel marker for pericytes and vascular smooth muscle cells. *Am. J. Pathol.* **162**, 721–729.
 167. Andreeva, E. R., Pugach, I. M., Gordon, D., Orekhov, A. N. (1998) Continuous subendothelial network formed by pericyte-like cells in human vascular bed. *Tissue Cell* **30**, 127–135.
 168. Helmbold, P., Nayak, R. C., Marsch, W. C., Herman, I. M. (2001) Isolation and in vitro characterization of human dermal microvascular pericytes. *Microvasc. Res.* **61**, 160–165.
 169. Gerhardt, H., Betscholtz, C. (2003) Endothelial-pericyte interactions in angiogenesis. *Cell Tissue Res.* **314**, 15–23.
 170. Schlingemann, R. O., Rietveld, F. J., de Waal, R. M., Ferrone, S., Ruitter, D. J. (1990) Expression of the high molecular weight melanoma-associated antigen by pericytes during angiogenesis in tumors and in healing wounds. *Am. J. Pathol.* **136**, 1393–1405.
 171. Hausmann, O. N., Hu, W. H., Keren-Raifman, T., Witherow, D. S., Wang, Q., Levay, K., Frydel, B., Slepak, V., Bethea, J. (2002) Spinal cord injury induces expression of RGS7 in microglia/macrophages in rats. *Eur. J. Neurosci.* **15**, 602–612.
 172. Betscholtz, C. (2003) Microglia do not express RGS5. Personal communication.
 173. Powers, A. C., Rabizadeh, A., Akeson, R., Eisenbarth, G. S. (1984) Characterization of monoclonal antibody 3G5 and utilization of this antibody to immobilize pancreatic islet cell gangliosides in a solid phase radioassay. *Endocrinology* **114**, 1338–1343.

174. Lafuente, J. V., Adan, B., Alkiza, K., Garibi, J. M., Rossi, M., Cruz-Sanchez, F. F. (1999) Expression of vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptor- β (PDGFR- β) in human gliomas. *J. Mol. Neurosci.* **13**, 177–185.
175. Graeber, M. B., Streit, W. J., Buringer, D., Sparks, D. L., Kreutzberg, G. W. (1992) Ultrastructural location of major histocompatibility complex (MHC) class II positive perivascular cells in histologically normal human brain. *J. Neuropathol. Exp. Neurol.* **51**, 303–311.
176. Herman, I. M., D'Amore, P. A. (1985) Microvascular pericytes contain muscle and nonmuscle actins. *J. Cell Biol.* **101**, 43–52.
177. Frey, A., Mecklein, B., Weiler-Guttler, H., Mockel, B., Flach, R., Gassen, H. G. (1991) Pericytes of the brain microvasculature express γ -glutamyl transpeptidase. *Eur. J. Biochem.* **202**, 421–429.
178. Risau, W., Dingler, A., Albrecht, U., Dehouck, M. P., Cecchelli, R. (1992) Blood-brain barrier pericytes are the main source of γ -glutamyl-transpeptidase activity in brain capillaries. *J. Neurochem.* **58**, 667–672.
179. Lawrenson, J. G., Reid, A. R., Finn, T. M., Orte, C., Allt, G. (1999) Cerebral and pial microvessels: differential expression of γ -glutamyl transpeptidase and alkaline phosphatase. *Anat. Embryol. (Berl.)* **199**, 29–34.
180. Kunz, J., Krause, D., Gehrman, J., Dermietzel, R. J. (1995) Changes in the expression pattern of blood-brain barrier-associated pericytic aminopeptidase N (pAP N) in the course of acute experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **59**, 41–55.
181. Sims, D. E. (1991) Recent advances in pericyte biology—implications for health and disease. *Can. J. Cardiol.* **7**, 431–443.
182. Gerhart, D. Z., Drewes, L. R. (1987) Butyrylcholinesterase in pericytes associated with canine brain capillaries. *Cell Tissue Res.* **247**, 533–536.
183. Balabanov, R., Washington, R., Wagnerova, J., Dore-Duffy, P. (1996) CNS microvascular pericytes express macrophage-like function, cell surface integrin α M, and macrophage marker ED-2. *Microvasc. Res.* **52**, 127–142.
184. Hellstrom, M., Kaln, M., Lindahl, P., Abramsson, A., Betsholtz, C. (1999) Role of PDGF-B and PDGFR- β in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* **126**, 3047–3055.
185. Mato, M., Ookawara, S., Saito-Taki, T. (1986) Serological determinants of fluorescent granular perithelial cells along small cerebral blood vessels in rodent. *Acta Neuropathol. (Berl.)* **72**, 117–123.
186. Hickey, W. F., Vass, K., 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.
187. Graeber, M. B., Streit, W. J., Kiefer, R., Schoen, S. W., Kreutzberg, G. W. (1990) New expression of myelomonocytic antigens by microglia and perivascular cells following lethal motor neuron injury. *J. Neuroimmunol.* **27**, 121–132.
188. Streit, W. J., Graeber, M. B., Kreutzberg, G. W. (1989) Expression of Ia antigen on perivascular and microglial cells after sublethal and lethal motor neuron injury. *Exp. Neurol.* **105**, 115–126.
189. Diaz-Flores, L., Gutierrez, R., Varela, H., Rancel, N., Valladares, F. (1991) Microvascular pericytes: a review of their morphological and functional characteristics. *Histol. Histopathol.* **6**, 269–286.