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Review

Microglia – insights into immune system structure, function, and reactivity in the central nervous system

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Summary. Microglia are essential cellular components of a well-functioning central nervous system (CNS). The development and establishment of the microglial population differs from the other major cell populations in the CNS i.e. neurons and macroglia (astrocytes and oligodendrocytes). This different ontogeny gives microglia unique properties. In recent years detailed studies of the microglial population have been greatly facilitated by the use of bone marrow (BM) chimeric animals. Experimental BM transplants have provided the opportunity to trace and investigate how BM cells migrate into the CNS and settle to become microglia. Furthermore various functional properties of microglia in the normal and pathological CNS are now being revealed because of combinations of BM transplantations and experimental disease models. Here, we describe some of the latest findings in microglial biology and discuss the potential for using microglia in therapeutic interventions.

Key words: Bone marrow transplantation, Chimera, Reactive microgliosis

The embryonic CNS and its colonization by microglia

Microglial precursors begin to colonize the embryonic human CNS during the first trimester, around 6.5 to 8 weeks of gestation, leading to a well-established microglial population in the second trimester (Hutchins et al., 1990; Esiri et al., 1991; Geny et al., 1995; Rezaie and Male, 1999). The developing CNS is colonized by microglia from specific sites of predilection, described as "fountains of microglia" by del Rio-Hortega. These include the superior tela choroidea and the pia mater covering the cerebral peduncules. From here, the cells migrate into the corpus callosum, the cerebral trigonum (fornix) and the optic thalami (thalami), and then into additional areas of the brain. In the cerebellum, the primary site for microglial entry is the inferior tela choroidea of the bulbocerebellar fold of the pia mater (del Rio-Hortega, 1932, 1939).

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Ontogenetic origin of microglia

The mechanism of microglial ontogenesis is an important subject in microglial biology, and has been debated for many years. When del Rio Hortega first used silver carbonate staining to distinguish microglia from oligodendrocytes in 1919, which identified microglia in Cajal's "third element", he proposed that microglia were of mesodermal origin (Cajal, 1913; del Rio-Hortega, 1919a,b, 1932). Today, his description of the process whereby the microglial population becomes established from cells originating in the mesodermal germ layer is well accepted. However, other mechanisms have been suggested and investigated.

Early studies tracing ink- and carbon-labeled monocytes injected into the bloodstream demonstrated that blood-borne monocytes could enter the brain and become microglia in newborn rats (Ling, 1979, 1981; Ling et al., 1980), suggesting monocytes as possible progenitors of microglia. However, macrophages or microglial-like cells can be observed in the CNS prior to the development of vasculature in the brain (Ashwell, 1991; Sorokin et al., 1992; Cuadros et al., 1993; Wang et

al., 1996), indicating that alternate mechanisms must occur. Intraparenchymal microglial precursor cells capable of becoming microglia have also been observed in the developing and adult mouse CNS, independent of the presence of meninges and circulating cells in the blood (Alliot et al., 1991). Some results have been interpreted as support for a neuroectodermal origin of microglia (Kitamura et al., 1984; Hao et al., 1991). However, the bulk of evidence supporting mesodermal precursors as cells of origin for microglia seems convincing (Lawson et al., 1992; Priller et al., 2001; Bechmann et al., 2005; Wirenfeldt et al., 2007).

Microglia in the mature CNS

In the fully developed CNS, microglia are numerous and distributed throughout the parenchyma. In the healthy, undisturbed CNS, microglia are commonly referred to as "resting microglia". The function of microglia in this state has for many years been fairly unclear. Recently, however, novel information has been acquired that clarifies part of their function under normal conditions. By two-photon microscopy, microglia in this "resting state" *in vivo* were shown to constantly probe their immediate surroundings (Nimmerjahn et al., 2005). Because of this constant probing activity, it has been suggested that the designation of "resting microglia" should be changed to "surveying microglia" (Hanisch and Kettenmann, 2007; Kettenmann and Verkhratsky, 2008). Microglial reactivity is a transition from this highly active surveying state towards an even more reactive state, where microglia focus their activity on a pathological event and respond with morphological and functional changes to the disturbance present. Microglia exhibit "functional plasticity", meaning that they adapt their level of reactivity to the activating stimulus and in that way are able to respond properly to pathological processes in the CNS parenchyma. Intercellular distances between "surveying microglia" are generally 50-60 µm under healthy conditions, with their somata remaining in a fairly fixed position. In contrast, microglial processes are very dynamic and highly motile with new processes constantly being formed, and others being retracted (Nimmerjahn et al., 2005). Therefore, the well known image of a microglial cell with a small elongated soma and long slender processes extending from it should be viewed as a snapshot of the appearance of that cell at that particular moment, a morphology which could change within a matter of a few minutes. The fate of microglia after activation and after the activating stimulus has subsided is not clear.

The surveying activity of microglia in the normal brain as described above has been very nicely demonstrated with two-photon microscopy (Davalos et al., 2005; Nimmerjahn et al., 2005). Microglia can, however, also be visualized *in vivo* in humans by PET-imaging. In the normal CNS a very low level of a receptor, the peripheral benzodiazepine binding site (PBBS), is expressed. The synthetic ligand PK11195 can bind to this receptor and be used as a PET ligand when labeled with the ^{11}C isotope. Activated microglia up-regulate PBBS and therefore increase their binding of PK11195. *In vivo* activated microglia are the primary source of PK11195 binding, hence this increased PK11195 binding can be utilized for visualizing microglial reactivity *in vivo* in humans by PET-scanning, e.g. in dementia (Banati et al., 1997; Cagnin et al., 2001). The intensity of PK11195 may be related to the level of microglial activation (as demonstrated in a rat model) rather than the number of activated microglia (Ito et al., 2010).

Activating stimuli for microglia can be very diverse. Two interesting regulatory mechanisms known to influence microglial activity are adenosine triphosphate (ATP) and the CD200-CD200R complex. ATP is a potent regulator of microglial surveying activity and microglial processes display strong chemotaxis towards extracellular ATP (Honda et al., 2001; Davalos et al., 2005). Two-photon microscopy imaging studies have emphasized how highly responsive microglia are to injury and pathology in the CNS, directing their processes towards damage in the parenchyma within minutes (Davalos et al., 2005). This rapid and directed response by microglial processes adds to the traditional panel of criteria used to classify microglial activation, including changes in morphology, proliferation, antigen expression, and cytokine production (Streit et al., 1999; Ladeby et al., 2005). For microglia to react as swiftly as has been demonstrated in two-photon microscopy studies, these cells must accordingly be very closely regulated. In the CNS CD200 is expressed on neurons and the CD200 receptor CD200R is expressed on microglia (Webb and Barclay, 1984; Hoek et al., 2000; Wright et al., 2000). The CD200-CD200R complex is involved in regulation of myeloid cell activation, and microglia spontaneously display a more activated phenotype in mice lacking the CD200 molecule (Hoek et al., 2000). Increased expression of CD200 has, on the contrary, been demonstrated to protect neurons against microglial-induced damage (Chitnis et al., 2007). These results demonstrate a key role for the CD200-CD200R complex in regulating microglial activity in the CNS.

New microglia in the mature CNS

Microglia are very sensitive to any disturbance or imbalance in the CNS parenchyma and react swiftly to such events. As part of this process, the resident microglial population can be supplemented with bone marrow derived cells that migrate into the CNS from the blood to become new microglia (Fig. 1; Priller et al., 2001; Bechmann et al., 2005; Massengale et al., 2005; Wirenfeldt et al., 2007). This remarkable phenomenon in the mature CNS, which happens in the event of a parenchymal lesion, in certain ways resembles the colonization by microglia that occurs in the developing CNS. Novel microglia migrating into the CNS from the blood join the resident microglia in the process of

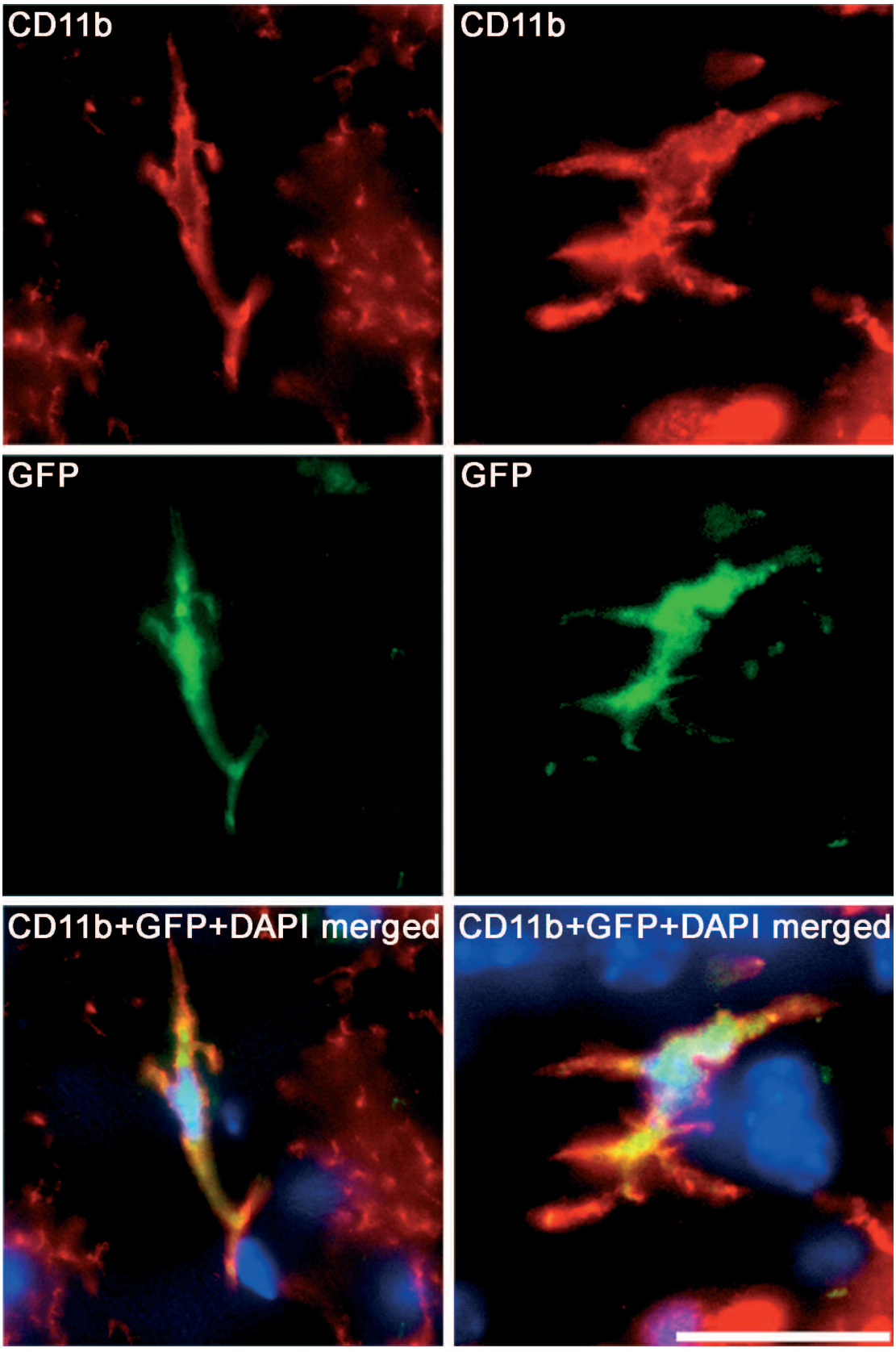

Fig. 1. BM derived microglia enter the dentate gyrus of GFP-BM chimeric mice, seven days after perforant pathway–lesion. This experimental lesion in the entorhinal cortex causes anterograde axonal and terminal degeneration of perforant pathway presynaptic elements and reactive microgliosis in affected areas of the hippocampus and dentate gyrus. Microglial cells are visualized by immunofluorescence staining with anti-CD11b (red) and expression of GFP (green). Co-localization of CD11b, GFP, and DAPI (blue) nuclear staining is demonstrated in the bottom two images. Scale bar: 20µm. Modified and reprinted from Am. J. Pathol. (2007, 171:617–631) with permission from the American Society for Investigative Pathology (Wirenfeldt et al., 2007).

reactive microgliosis. This has been demonstrated in various models of acute CNS injury (Priller et al., 2001; Wirenfeldt et al., 2005, 2007; Clausen et al., 2008; Lambertsen et al., 2009) as well as chronic inflammation (Simard et al., 2006; Remington et al., 2007). Supplementation of the microglial population can also occur in the normal adult CNS, though at a much lower rate. Experiments in mice have shown that the microglial population turns over about once in the lifespan of a mouse (Lawson et al., 1992; Kennedy and Abkowitz, 1997; Massengale et al., 2005; Wirenfeldt et al., 2005). These results portray microglia as a very adaptive, dynamic, and constantly changing population of cells.

Bone marrow chimeric mice

Studies of microglial turnover, such as those described above, are generally carried out using bone marrow chimeric animals (animals that have cells of genetically different constitution as a result of bone marrow transplantation). Bone marrow chimeras are created based on the premise that CNS-resident cells, including microglia, are resistant to doses of irradiation that are sufficient to ablate the hematopoietic compartment. This is usually accomplished using a lethal dose of whole body irradiation. Reconstituting irradiated recipients with labelled bone marrow cells then allows circulating cells that enter the CNS to be tracked and identified. Initial studies relied on MHC haplotypes for distinction, or on detection of donor cells from congenic strains. In the latter case, different antigen specificities of the CD45 molecule have been used, since monoclonal antibodies that can distinguish between CD45.1 (expressed by SJL mice) and CD45.2 (expressed by C57BL/6 mice) are available (Morse et al., 1987; Morse, 1992; Lai et al., 1998). These initial studies concluded that perivascular cells were rapidly replaced by circulating bone marrow derived cells, but suggested there was little or no replenishment or turnover of the parenchymal microglial population under steady-state or pathological conditions (Matsumoto and Fujiwara, 1987; Hickey and Kimura, 1988; Lassmann et al., 1993). Since MHC molecules are expressed at low levels in the brain, it is possible that immigrating cells may have been below the threshold of detection.

Over the past decade, however, bone marrow cells expressing green fluorescent protein (GFP) induced by retroviral vectors or isolated from GFP transgenic mice have been widely used as donor cells in chimeric mice. Unlike MHC and CD45, GFP can easily be detected in tissue sections by immunofluorescence or by use of anti-GFP specific antibodies. Priller and colleagues were the first to combine GFP and bone marrow chimera technology for the purpose of studying myeloid response in the CNS (Priller et al., 2001), and by doing so, created a very valuable resource. They, like subsequent studies, reported that replacement of the microglial population does occur, and this is accelerated by injury to the brain (Priller et al., 2001; Simard and Rivest, 2004; Bechmann et al., 2005; Massengale et al., 2005; Wirenfeldt et al., 2005).

Bone marrow chimerism in humans

Human bone marrow chimerism sometimes occurs when bone marrow transplantation is used as therapy, which often occurs in the treatment of leukemia or lymphoma. When allogeneic bone marrow transplantation is performed, the patient becomes a bone marrow chimera, as the donor cells are genetically different from the recipient's cells (with the exception of transplantation between monozygotic twins). When a female patient receives bone marrow from a male donor, the donor cells can be traced by labeling the Y-chromosome with fluorescence in situ hybridization (FISH). This has been utilized to determine whether bone marrow cells in humans also have the potential to differentiate into microglia. In one study bone marrow derived cells were observed in various compartments of the CNS, but microglia were not unambiguously defined (Unger et al., 1993). Donor bone marrow cells in another study have been shown to actually differentiate into neuronal cells (Mezey et al., 2003). Transgender microglia do, however, occur in female bone marrow transplant patients as demonstrated by Cogle et al. in tissue from three out of three patients examined (Cogle et al., 2004). These findings indicate that a differentiation of bone marrow cells into microglia occurs in humans, and this phenomenon could therefore have a therapeutic potential.

Irradiation and infiltration of bone marrow derived cells into the CNS

Recently it has been debated whether CNS infiltration of circulating blood cells in bone marrow chimeric animals is a normal part of the body's reaction to pathological events in the CNS, or whether colonization of the CNS by bone marrow derived cells is an artefact precipitated by radiation induced damage to the blood brain barrier or the brain itself (Massengale et al., 2005; Ajami et al., 2007; Mildner et al., 2007). Irradiation causes microglial cell loss and changes in proliferative and apoptotic responses - although normal cytokine responses appear to be preserved (Turrin et al., 2007; Wirenfeldt et al., 2007; Clausen et al., 2008; Lambertsen et al., 2009). Irradiation of the brain has, however, also been shown to induce acute increased permeability of the blood brain barrier. Wilson et al. demonstrated that a 20Gy dose of radiation applied locally to the brain resulted in an acute increase in blood brain barrier permeability both 24hrs and 48hrs after irradiation (Wilson et al., 2009). A lower dose of 4.5Gy has also been demonstrated to induce modifications of blood brain barrier permeability within the first 1-2 days (Diserbo et al., 2002). However, using horseradish peroxidase as a tracer McMahon et al. did not observe differences in blood brain barrier leakage between

control and irradiated and bone marrow transplanted mice (Mcmahon et al., 2002). It is likely that damage to the blood brain barrier caused by irradiation would depend on factors such as amount of radiation applied to the brain as well as length of time between radiation and examination of blood brain barrier damage.

Shielding the head of a mouse during irradiation is one method that has been used to investigate immigration of BM derived cells into the brain without potential radiation-induced damage to the brain parenchyma or blood brain barrier (Furuya et al., 2003). Reports suggest that it is advantageous to reduce the amount of irradiation applied to the head of a recipient mouse prior to bone marrow transplantation. A reduction from 10Gy to 5Gy applied to the head, while maintaining 10Gy to the rest of the body, significantly reduced the microglial burden in the olfactory bulb and the substantia nigra three weeks after irradiation (Furuya et al., 2003). Interestingly Mildner et al. found that postnatal microglial engraftment in the brain enhanced by CNS pathology requires brain conditioning e.g. by irradiation when the blood brain barrier is intact. BM-derived cells could only be detected in the brains of chimeric mice subjected to whole body irradiation, and not in chimeric mice with head shielding (Mildner et al., 2007).

Parabiosis models have also been applied to investigate possible confounding effects of irradiation in non-irradiated animals. In parabiosis two animals are joined surgically to create a common vascular compartment. One such study employed parabiotic chimeric mice in which GFP was used as a marker in bone marrow cells. In parabiotic normal unlesioned/ untreated C57BL/6 mice, facial nerve axotomy treated, and mSOD Tg mice, no evidence of bone marrow derived cells was found in the brain (Ajami et al., 2007). However, in a comprehensively engineered study by Massengale et al., it was demonstrated that BM cells do indeed infiltrate the brain in parabiotic mice, albeit at low numbers (up to 1.38% of brain microglia) (Massengale et al., 2005). Still, BM-derived microglia were not increased following kainic acid lesion induced injury to the hippocampus, at least at early time points (Massengale et al., 2005).

The dye 6-carboxylfluorescein diacetate (CFDA) has also been used to track entry of BM-derived cells, to avoid application of radiation to the brain (Bechmann et al., 2005). CFDA is an intracellular long-lasting fluorescent tracer, however, the fluorescence intensity of cells labeled with dyes such as CFDA halves each time the cells divide (Lyons and Parish, 1994; Samms et al., 2001). Proliferating cells will therefore gradually lose their fluorescence and hence their tracing marker. However CFDA, when injected into spleens of mice, allows blood-derived cells to be traced without irradiation and bone marrow transplantation. Combining intrasplenic CFDA injections and subsequent entorhinal cortex lesions, Bechmann et al. elegantly demonstrated that circulating cells can enter areas of anterograde axonal degeneration in the hippocampus after perforant pathway lesion without prior radiation conditioning of the brain (Bechmann et al., 2005).

Thus, there is compelling evidence that novel BM derived microglia can enter the CNS without prior irradiation, even though the proportion of cells is probably relatively small and may sometimes not even be measurable (Bechmann et al., 2005; Massengale et al., 2005). Nonetheless, sufficient numbers of BM-derived cells infiltrated the CNS in a mouse model of Alzheimer's disease to phagocytose amyloid deposits and restrict the formation of Aß plaques, which the resident microglia were unable to do (Simard et al., 2006). It therefore seems an oversimplification to assume that infiltration of bone marrow derived cells into the CNS in BM chimeric animals is solely an artifact of CNS-irradiation. However, there is no doubt that the extent of BM derived cells that turn into microglia varies greatly depending on a number of factors, including the experimental model used, the amount of radiation applied to the brain, and the period of time between the bone marrow transplant and the lesion and the death of the animal (Priller et al., 2001; Furuya et al., 2003; Wirenfeldt et al., 2005, 2007; Mildner et al., 2007). Thus, although irradiation clearly impacts the brain and has potential to affect migration of BM derived cells into the brain, infiltration of BM cells appears to be a naturally occurring phenomenon.

Macrophages versus microglia

Criteria typically used to identify immigrating BM cells as "microglia" in the CNS parenchyma of BM chimeric mice are expression of a BM marker (such as GFP) and myeloid markers (such as CD11b or Iba1). These cells exhibit morphologies ranging from round amoeboid to ramified cells (Vallieres and Sawchenko, 2003; Wirenfeldt et al., 2007). *In vitro* studies have shown that macrophages can develop a ramified morphology and microglia an amoeboid morphology (Sievers et al., 1994; Wilms et al., 1997; Bohatschek et al., 2001), suggesting that morphology alone may not discriminate these populations. In 1991, Sedgwick and colleagues demonstrated that the resident $CD11b^{+}$ microglial population in unmanipulated rat brain expressed low levels of CD45 ($CD45^{dim}$) using flow cytometry (Sedgwick et al., 1991). Analysis of CD45 levels in bone marrow-chimeric rats confirmed that $CD45^{dim}$ cells were radiation-resistant parenchymal microglial cells, and that $CD45^{high}$ cells were other CNS macrophages and some T lymphocytes (Ford et al., 1995). CD45 levels have also been used to discriminate resident from infiltrating myeloid cells in mouse brain (Renno et al., 1995; Carson et al., 1998). Flow cytometric evaluation of CD45 levels as a means of discriminating macrophages from microglia has been widely employed in CNS studies ranging from autoimmune inflammation and viral infection to injury and ischemia (Sedgwick et al., 1991; Renno et al., 1995;

Katz-Levy et al., 1999; Mcmahon et al., 2002; Babcock et al., 2003, 2006, 2008; Greter et al., 2005; Wirenfeldt et al., 2005; Lambertsen et al., 2009).

Although BM chimeras are widely used for histological analyses, and CD45 levels are commonly used for evaluation by flow cytometry, few studies have compared macrophage/microglial immigration by combining CD45 analysis with bone marrow chimera technology. Overall, these studies demonstrate significant increases in BM-derived $CD45^{high}$ macrophages and $CD45^{dim}$ microglia in experimental models. The proportion of $CD45^{high}$ cells expressing BM markers (e.g. GFP) generally matches the degree of reconstitution, which indicates that this does reflect an immigrating population. The proportion of $CD45^{dim}$ cells expressing BM markers is also significant, but generally much lower, especially after an acute insult to the CNS (Fig. 2; Wirenfeldt et al., 2005, 2007; Remington et al., 2007; Clausen et al., 2008; Lambertsen et al., 2009). In acute models e.g. perforant pathway transection, this can reach 0.6-13% of $CD45^{dim}$ microglia depending on the length of time between lesion and death and post transplantation survival time (Wirenfeldt et al., 2005, 2007). Higher proportions (up to 29%) of BM-derived microglia have been reported in mice with cuprizone-induced demyelination, a model in which there is relatively little involvement of $CD45^{high}$ macrophages (Remington et al., 2007), but in which a previous histological study, has demonstrated immigrating BM-derived cells as peripheral macrophages (Mcmahon et al., 2002).

Taken together, the data indicate that the majority of $CD45^{high}$ cells are reliably infiltrating macrophages, and that the majority of $CD45^{dim}$ cells form the resident microglial population, although a significant subset of $CD45^{dim}$ microglia are recently derived from the BM. It is not known whether these cells enter the CNS with intermediate CD45 levels, or as $CD45^{high}$ cells that then downregulate CD45 expression.

The exact nature of the bone marrow derived precursor cells that differentiate into microglia and macrophages is not entirely known. Recent studies, however, suggest precursors of adult murine microglia to be a subpopulation of monocytes that display a Ly-$6C^{hi}Gr\text{-}1^{+}CCR2^{+}CX3CR1^{lo}$ phenotype. Ly-$6C^{hi}CCR2^{+}$ cells have been demonstrated to accumulate in CNS lesions and differentiate into microglia (Mildner et al., 2007).

Microglial immune functions

The actual function of microglia, particularly in the absence of pathology, has to a large extent been unknown. Recently, however, interesting new data has provided insights into a number of important tasks that microglia perform, which contribute to maintaining a healthy homeostasis in the CNS.

Microglial involvement in human diseases

Any pathology in or damage to the CNS parenchyma is likely to elicit a reactive microglial response. In certain diseases in the human brain, however, microglia seem to play a central role in the pathogenesis of the disease itself. Microglia are the primary target cells for HIV in the CNS. Microglia express CD4 receptors and chemokine receptors such as CCR3 and CCR5 that enables HIV to bind to and infect microglia (He et al., 1997). Monocytes infected with HIV outside the CNS can act as Trojan horses and carry the virus through the blood brain barrier into the parenchyma, where the infection can spread to microglia (reviewed by Williams and Blakemore, 1990). HIV infection of the brain can eventually lead to HIV dementia (Navia et al., 1986a, b; Garden, 2002).

In Alzheimer's disease (AD) microglia are also know to have a central role in the pathogenesis. The exact way in which microglia are involved in the pathology of AD and to what extent the reactive microgliosis observed in AD is beneficial or may even be detrimental to the brain is still not resolved. Fibrillar Aß1-42 plays an important role in activating microglia through TLR2 in AD (Jana et al., 2008). It has been hypothesized that the subsequent microglial production of potential neurotoxic molecules, such as proinflammatory cytokines and reactive oxygen species,

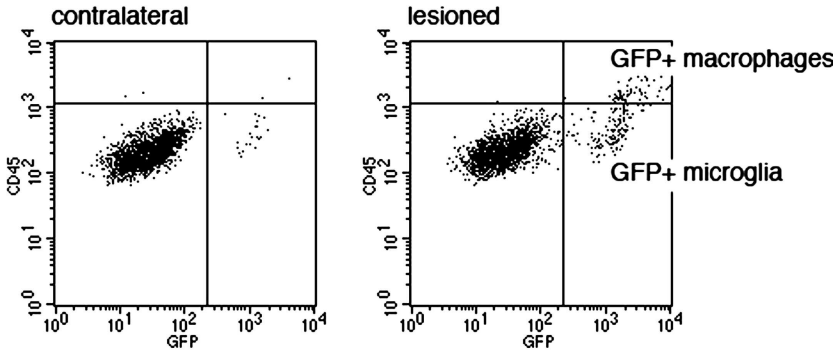

Fig. 2. Flow cytometry plots showing all $CD11b^{+}$ myeloid cells ($CD45^{dim}$ microglia and $CD45^{high}$ macrophages) in the lesioned and contralateral hippocampi of a GFP-BM chimeric mouse seven days after PP–lesion. Cells (events) in the lower and upper right quadrants represent immigrating GFP^{+} BM-derived microglia and macrophages, respectively. While most $CD45^{high}$ macrophages are GFP^{+}, only a subset of the $CD45^{dim}$ microglia is GFP^{+} and thus recently immigrated from the circulation.

could mediate some of the neurodegenerative pathology observed in AD (Akiyama et al., 2000). This hypothesis is supported by accumulation of activated microglia around brain amyloid deposits (Itagaki et al., 1989; Perlmutter et al., 1990) and observations that Aß can activate microglia (Jana et al., 2008). However, an alternative hypothesis has been suggested, namely that microglial senescence and dysfunction might be involved in the pathogenesis and progression of AD (Streit, 2004; Flanary et al., 2007). If microglia become dysfunctional and lose their normal homeostatic and neuroprotective functions, then their ability to clear Aß at a sufficient rate would be diminished and the development of plaques accelerated. Furthermore, activation may shorten the lifespan of microglia resulting in increased microglial cell death and concomitant release of neurotoxic substances from microglia, facilitating neurodegeneration (Streit, 2004).

A rare but very disabling disease, Rasmussen's encephalitis (RE), causes intractable epilepsy in childhood (Rasmussen et al., 1958). The etiology of RE is unknown, but very striking neuropathological signs can be observed in the brains of these patients including almost exclusive unilateral involvement of the brain, and often severe inflammatory changes with T-cell infiltration and microglial activation in the involved cerebral hemisphere (Vining et al., 1993; Pardo et al., 2004). Microglial reactivity is often scattered but can be very evident (Fig. 3; Wirenfeldt et al., 2009). Despite intense research it is still unresolved whether the reactive microgliosis observed in RE is a primary phenomenon or secondary to a yet undiscovered etiology.

Microglial reactivity is apparent in multiple sclerosis (MS) as well. The exact role of microglia in the complex pathogenesis of MS is not well known. There are, however, well described microglial functions known to be involved in MS pathology. For example, when activated, microglia can acquire the ability to stimulate Th1 and Th2 $CD4^+$ T-cell lines and present antigens, as has been demonstrated *in vitro* (Aloisi et al., 1999; Aloisi, 2001). Based on the autoimmune nature of MS, bone marrow transplantation has been attempted as treatment for this disease, based on the rationale that autoimmunity could be attenuated by myeloablative treatment and immune function restored by subsequent bone marrow transplantation (Burt et al., 1997). This treatment has, however, thus far not been effective in patients with progressive disease and high pretransplantation disability scores (Burt et al., 2003; Samijn et al., 2006).

Phagocytosis of apoptotic neurons by microglia without inflammation

Investigation of a very rare disease named polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL) or Nasu-Hakola disease has helped provide a unique insight into how microglia function in the normal CNS (Takahashi et al., 2005; Thrash et al., 2009). Loss of function mutations in the gene coding for TYRO protein tyrosine kinase binding protein (TYROBP, formerly DAP12) has devastating consequences resulting in PLOSL (Paloneva et al., 2000). In this disease dysfunction of osteoclasts and microglia seems to account for the ankle and wrist bone cysts and presenile dementia observed, two of the cardinal signs and symptoms of the disease (Paloneva et al., 2000, 2003; Cella et al., 2003; Takahashi et al., 2005). PLOSL unfortunately often leads to early death in the fifth decade of life (Bianchin et al., 2004). Microglia constitutively express the triggering receptor expressed on myeloid cells-2 (TREM-2), and this receptor and knock out mouse models as well as loss of function mutations in humans in the TREM-2/DAP12 receptor complex have provided knowledge about the functions

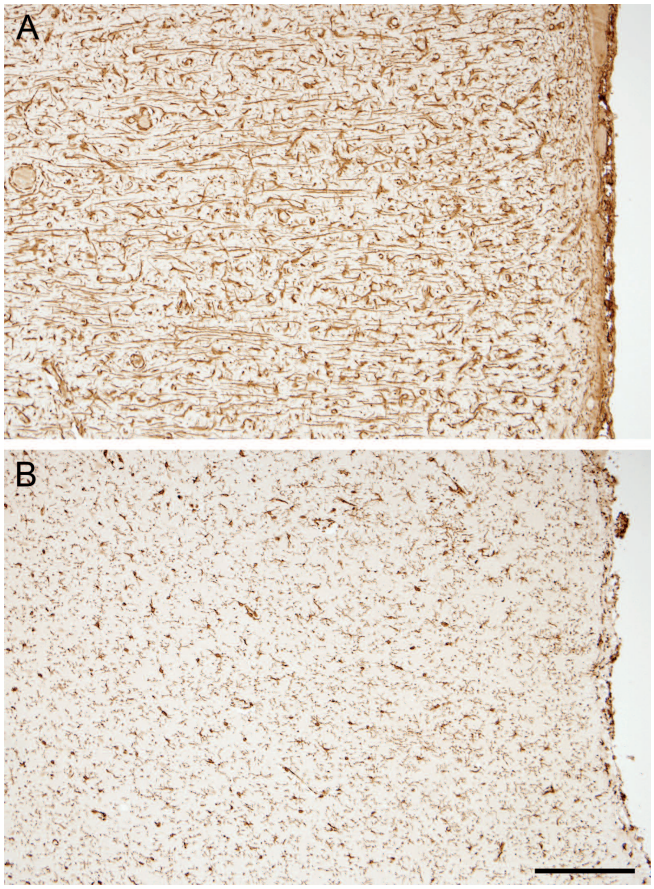

Fig. 3. Photomicrographs showing diverse microglial responses in an Iba1-immunolabeled section from the cerebral cortex of a patient with RE. Both images are from the same histological section. Marked microglial activation (including rod cell formation) occurs in one area **(A)**, whereas only slight microglial reactivity is found in an adjacent area **(B)**. This illustrates the striking microgliosis and patchy distribution of microglial activation often observed in the brains of RE patients. Scale bar: 250 µm.

of microglia in the normal CNS. In the CNS TREM-2 expressed on microglia is involved in the phagocytosis of apoptotic neurons without inflammation. A deficiency in TREM-2 results in impaired microglial phagocytosis of apoptotic neurons and enhanced expression of TREM-2 increases phagocytosis by microglia and reduces the gene expression of TNF-α, IL-1ß and NOS2 (Takahashi et al., 2005). A dysfunction of microglial ability to phagocytose apoptotic neurons therefore appears to cause presenile dementia. Furthermore in DAP12 deficient mice significantly fewer microglia have been observed in the basal ganglia and spinal cord compared to controls in 10 months old mice indicating DAP12 as important in preserving microglia (Otero et al., 2009). As part of their "functional plasticity", microglia have been termed "facultative macrophages" as microglia were initially thought to phagocytose only when neuronal death occured (Streit et al., 1988, 1999; Pennell and Streit, 1998). That still appears to be the case but in a broader sense, as microglia also are phagocytic in the normal CNS clearing out neurons dying by apoptosis, and not just neurons that have died as a result of a pathological or traumatic events in the CNS where actual inflammatory processes are involved. Microglia may also phagocytose myelin after axonal injury and beta-amyloid in mouse models of AD, neither of which involves neuronal death itself (Simard et al., 2006; Nielsen et al., 2009).

Grooming behavior and microglia in mice

It has been well known for years that microglia are an integral part of the immune system. New results have, however, now broadened the concept of microglia as immune active cells. In mice, the *Hoxb8* gene is involved in controlling grooming behavior, and within the brain regions that regulate grooming behavior, the cells derived from the *Hoxb8* cell lineage are microglia (Chen et al., 2010). By way of bone marrow transplantation it has been demonstrated that the excessive grooming behavior observed in the *Hoxb8* mutant mice can be reduced by normal bone marrow transplantation. This indicates microglial immune function in a very different perspective than usually depicted. Chen et al. conclude that grooming could be seen as a way of reducing the amount of pathogens present on the body, which fits perfectly with the function of the rest of the immune system, and in that regard it seems only natural that microglia would be involved in regulating grooming behavior (Chen et al., 2010).

Microglia in therapy

The close relationship between microglia and hematopoietic stem cells could potentially be utilized for therapeutic purposes. In a mouse model of metachromatic leukodystrophy (MLD) caused by lack of the enzyme arylsulphatase A (ARSA) the ARSA enzyme activity has been restored by transplanting hematopoietic stem cells that had been ex vivo transduced with the ARSA gene using lentivirus. In these transplanted MLD mice the ARSA enzyme activity in the hematopoietic system was reconstituted and symptoms and signs typical of MLD were prevented (Biffi et al., 2004). Also overexpression of the homeobox B4 gene in BM cells transplanted into MLD mice has been demonstrated to result in efficient detection of these cells in the brain in these mice and improvement of their motor skills (Miyake et al., 2010). The prospect of treating diseases in the CNS by introducing transgenic proteins via cells immigrating from the bone marrow appears as a possible way to target certain diseases in the CNS. In other studies human umbilical cord blood or mobilized peripheral blood $CD34^+$ cells were transduced with human adrenoleukodystrophy or EGFP genes and these cells were able to migrate into the CNS and develop into both perivascular and parenchymal microglia. These cells allowed for the expression of new transgenic proteins in the brain. These interesting data of human $CD34^+$ cells xenotransplanted into mice and developing into microglia in the CNS, show highly promising results of using a bone marrow graft for gaining access to delivering a protein to the CNS parenchyma (Asheuer et al., 2004).

Roles for BM-derived versus resident microglial cells

Very interesting data have been presented the last few years displaying microglia as unique cells in the CNS in the sense that during ontogenesis they arise from BM precursors and that the microglial population again in the mature brain can be supplemented with new BM-derived cells. Determining whether engraftment with novel microglia from BM-derived precursor cells occurs in the adult brain, as a naturally occurring phenomenon, is a complicated matter. Such engraftment is most likely dependent on several factors such as absence or presence of pathology in the CNS, the nature of such pathology, and the species investigated. Age is likely also to influence the possibility for blood borne precursor cells to migrate into the brain and settle there as novel microglia. Studies using bone marrow chimeras have demonstrated how many diverse functions these cells may have. Microglia may play a key role in neurodegenerative diseases, in particular AD, where especially the cells migrating into the brain from the bone marrow are interesting granted their unique ability to target amyloid plaques (Simard et al., 2006). Combined with the possibility of introducing new microglia into the CNS engineered for a specific purpose, these cells suggest a promising opportunity for therapy (Asheuer et al., 2004; Biffi et al., 2004).

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