A deficiency in CCR2\(^+\) monocytes: the hidden side of Alzheimer’s disease

Gaëlle Naert and Serge Rivest*

Neuroscience Laboratory, CHU de Québec Research Center and Department of Molecular Medicine, Faculty of Medicine, Laval University, 2705 Laurier Boulevard, Québec, QC, Canada G1V 4G2

* Correspondence to: Serge Rivest, E-mail: serge.rivest@crchuq.ulaval.ca

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by intracellular neurofibrillary tangle formation and extracellular amyloid-\(\beta\) (A\(\beta\)) deposition. To date, microglia seem to act as double-edged swords, being either beneficial (e.g. clearance of A\(\beta\)) or detrimental (e.g. secretion of neurotoxic factors) in AD. Following a rather intense debate on the question, a consensus has emerged that microglia can renew themselves via proliferation of already differentiated microglia as well as via the de novo recruitment of monocytes of mouse models of AD. However, recent advances suggest distinct function for resident and bone marrow-derived microglia (BMDM), and have emphasized the neuroprotective functions of BMDM. BMDM is the only subset of cells that restrict cerebral amyloidosis in the AD brain, which has been recently attributed to CCR2\(^+\) monocytes. Moreover, an impaired recruitment of CCR2\(^+\) monocytes has been reported in AD patients, as seen from the CCR2\(^+\) monocytopenia found in the bloodstream and BM. The present review summarizes the current knowledge on the roles and dysfunctions of CCR2\(^+\) monocytes in AD and their potential as key therapeutic targets.

Keywords: Alzheimer’s disease, bone marrow-derived microglia, monocytes, cytokines, CCR2, CX\(_3\)CR1, M-CSF

Introduction

Alzheimer’s disease (AD) is a heterogeneous and progressive age-related neurodegenerative disease characterized by a steady decline in cognitive and executive functions. AD prevalence in the elderly is currently increasing dramatically due to longer life expectancy. The pathological diagnosis of AD depends on the presence of both intraneuronal tangles consisting of phosphorylated tau proteins, and senile plaques of amyloid-\(\beta\) (A\(\beta\)). The A\(\beta\) peptides, predominantly A\(\beta\)\(_{40}\) and A\(\beta\)\(_{42}\), are derived from the amyloid precursor protein (APP) through proteolytic processing mediated by the sequential activity of \(\beta\)- and \(\gamma\)-secretases (Selkoe, 2001). Although A\(\beta\)\(_{40}\) is more abundant, A\(\beta\)\(_{42}\) tends to cluster faster into oligomers, forming A\(\beta\) fibrils that form deposit. Although A\(\beta\) accumulation in extracellular plaques is a defining characteristic of AD pathology, substantial evidence shows that toxic A\(\beta\) peptides play a critical role in the progress of this devastating disease. Senile plaques result from the accumulation of several other proteins and an inflammatory reaction around A\(\beta\) deposits. These plaques contain dystrophic neurites, activated microglia, and reactive astrocytes (Selkoe, 2002). Despite substantial progress in understanding AD pathogenesis, no treatment has yet been shown to be effective against AD. In recent years, the interplay between immunity and neurodegeneration has gained growing attention through studies designed to unravel disease mechanisms and identify novel therapeutic avenues. Since the action of immune cells can affect the peripheral as well as central nervous systems (CNS), age-related neurodegenerative diseases need to be assessed at both central and peripheral levels. In the CNS, immune cells have been viewed as having neuroprotective effects, whereas they can also participate in neurological disorders upon inflammation, aging, and neurodegenerative processes. Recent evidence supports the emerging hypothesis that in AD, the key pathogenic phenomenon consists in the long-term maladaptive activation of innate immunity (Eikelenboom et al., 2011). In this review, we address new evidence in support of the hypothesis that the immune system—especially via CCR2\(^+\) monocytes—is closely involved in AD pathogenesis, and appears to be affected in this disorder. The available evidence points toward promising strategies using CCR2\(^+\) monocytes as therapeutic targets.

The CCR2/CCL2 axis in the limelight

The C–C chemokine receptor type 2 (CCR2) or CD192 (cluster of differentiation 192) binds the five pro-inflammatory monocyte chemoattractant proteins (MCPs) MCP-1 (CCL2), MCP-2 (CCL8), MCP-3 (CCL7), MCP-4 (CCL13), and MCP-5 (CCL12) (Murphy et al., 2000). Chemokines are small (8–14 kDa) secreted proteins that regulate leukocyte trafficking through interaction with a subset of seven-transmembrane G-protein-coupled receptors (Luster, 1998; Charo and Ransohoff, 2006; Auffray et al., 2009a). CCR2 is mainly expressed on the surface of monocytes and a small
percentage of natural killer (NK) and T cells (Mack et al., 2001; Saederup et al., 2010), and mediates the migration of lymphocytes, blood-derived dendritic cells, and monocytes/macrophages (Murphy et al., 2000). CCL2, the main ligand of CCR2, binds the latter receptor only, and is the most potent activator of the signal transduction pathway leading to monocyte transmigration (Deshmane et al., 2009; Conductier et al., 2010). CCL2 is expressed by many cell types, including endothelial cells (Cushing et al., 1990; Rollins et al., 1990), astrocytes (Glabinski et al., 1996; Smits et al., 2002), and microglia (Meda et al., 1995; El Khoury et al., 2003; Deshmane et al., 2009).

Monocytes are incompletely differentiated BM-derived cells that have a lifetime of 1–3 days in blood circulation and enter peripheral tissues where they give rise to a heterogeneous lineage of monocytic phagocytes. These blood mononuclear cells are characterized by the expression of several clusters of differentiation (CD) such as CD11b, CD115, CD11c, CD14, and CD16 in humans, or CD11b and CD115 in mice (Geissmann et al., 2008). Three functional subsets of human monocytes and two subsets of mouse monocytes have been identified so far, based on the expression pattern of specific surface molecules (Auffray et al., 2010). Such subsets of human monocytes and two subsets of mouse monocytes—CX3CR1lowCCR2+GR1+Ly6Chigh, which are actively recruited to inflamed tissues, can be distinguished from resident monocytes—CX3CR1hiCCR2−GR1−Ly6Clo, which, in contrast, are characterized by a CX3CR1-dependent recruitment to non-inflamed tissue (Geissmann et al., 2003). Recently, a novel classification of mouse monocytes was proposed on the basis of Ly6C and CD43 expression, thus introducing a subdivision of CX3CR1lowCCR2+Gr1+Ly6Chigh monocytes according to their CD43 expression level (Ziegler-Heitbrock et al., 2010) (cf. Figure 1 for human and mouse monocyte correspondences). CX3CR1lowCCR2+GR1+Ly6Chigh monocytes migrate to different organs upon inflammation, whereas those expressing high CCR2 levels have been reported to infiltrate the brain in mouse models of multiple sclerosis (Izikson et al., 2000), at sites of axonal injury (Babcock et al., 2003), and in a mouse model of hepatic inflammation (D’Mello et al., 2009).

**CCL2: a novel player in AD pathogenesis**

The first evidence suggesting that CCL2 may be an important player in AD was related to its up-regulation in mature senile plaques, microglia (Ishizuka et al., 1997), and microvessels (Grammas and Ovase, 2001) of AD patients. Enhanced CCL2 expression was also observed in the brain of AD transgenic mice (Simard et al., 2006; El Khoury et al., 2007; Naert and Rivest, 2011a). Interestingly, CCL2 production is induced by Aβ in cultures of microglia (Meda et al., 1995; El Khoury et al., 2003) and astrocytes (Smits et al., 2002). Circulating CCL2 levels are also increased in the cerebrospinal fluid (CSF) (Galimberti et al., 2006b) and serum of patients with mild cognitive impairment (MCI) and AD, although CCL2 level decreases with AD progression (Galimberti et al., 2006a), resulting in an inverse correlation in CCL2 levels between CSF and plasma (Sun et al., 2003). Recently, CCL2 levels in CSF have been shown to correlate with a faster cognitive decline in the follow-up of patients with prodromal AD (Westin et al., 2012). Nevertheless, these data must be considered with caution since CSF levels of CCL2 increase with age in healthy controls (Blasko et al., 2006). Levels of CCL2 mRNA are also altered in peripheral blood mononuclear cells (PBMC) from AD patients (Galimberti et al., 2006a). In cultures of peripheral monocytes, Aβ induces the production of inflammatory cytokines such as CCL2 and stimulates the differentiation of monocytes into adherent macrophages in a dose-related fashion (Fiala et al., 1998). In addition to mononuclear CCL2 production, Aβ also induces CCL2 production in cultured human endothelial cells as well as in the brain of AD patients (Vukic et al., 2009). In the blood–brain barrier (BBB) model, Aβ and monocytes on the brain side stimulate monocyte transmigration from the blood to the brain compartments (Fiala et al., 1998), a process likely potentiated by the Aβ-induced production of CCL2. Indeed, a sustained increase in CCL2 level has been recently shown to disrupt the BBB and therefore facilitate recruitment of leukocytes into the CNS (Roberts et al., 2012). In AD, the increased Aβ production seems to contribute to the up-regulation of CCL2 production in both microglia and monocytes (Meda et al., 1996; Fiala et al., 1998) and therefore, to increase monocyte infiltration into the brain.

In view of mounting evidence supporting the importance of CCL2 in AD, several studies have been designed to better understand its role in AD, albeit with conflicting findings. In a Tg2576 mouse model overexpressing CCL2 in astrocytes (APP/CCL2), Aβ deposits and plaque-associated microglia are more abundant than in Tg2576 mice (Yamamoto et al., 2005). While chronic expression of CCL2 has an adverse effect on Aβ deposition in APP mice, CCL2 actually enhances microglia-mediated Aβ degradation both in vitro and in vivo (Yamamoto et al., 2007). Therefore, CCL2-enhanced Aβ aggregation appears to predominate over CCL2-enhanced Aβ degradation. Although CCL2 has no effect on Aβ production, it accelerates Aβ oligomer formation and the progression of neurocognitive dysfunction (Yamamoto et al., 2005, 2007; Kiyota et al., 2009b). Nevertheless, these results must be interpreted with caution since CCL2 expression levels in the APP/CCL2 mouse model were enhanced 10 000-fold compared with wild-type (WT) mice, and chemokines may have opposite effects depending on their concentration. On the other hand, CNS-targeted gene delivery of a dominant-negative CCL2 mutant, functioning as a CCL2 inhibitor, reduces microglial activation and lowers Aβ deposits in the brain of APP/PS1 mice (Kiyota et al., 2009a). Taken together, these data clearly point to an important role of CCL2 in AD and single out microglia as major players, notwithstanding the fact that the exact function of CCL2 has yet to be further clarified.

**Microglia: monocyte-like cells?**

Microglia are resident macrophages in the CNS and spinal cord (Perry and Gordon, 1988) and constitute 10%–15% of total cells in the brain, albeit with a heterogeneous distribution (Soulet and Rivest, 2008b). During brain development, microglia in the CNS...
are predominantly derived from the yolk sac (Ginhoux et al., 2010; Schulz et al., 2012; Kierdorf et al., 2013). In the adult, microglia are replenished via proliferation of microglial precursors (Cuadros and Navascues, 1998; Soulet and Rivest, 2008a). Under normal conditions, leukocyte infiltration is prevented by the BBB, resulting in only scarce penetration of monocytes and in a low level of monocyte-derived microglial precursors in regions devoid of BBB (Figure 2A). In contrast, the rate of leukocyte infiltration can be increased in the context of CNS disorders such as AD (Erickson et al., 2012). As mentioned previously, the increased CCL2 expression observed in the AD mouse model (Simard et al., 2006; El Khoury et al., 2007) and AD post-mortem brain (Ishizuka et al., 1997; Grammas and Ovase, 2001) might contribute to an enhanced infiltration of leukocytes. Quite notably, microgliosis, i.e. the focal accumulation of activated and proliferating microglia, is observed in the AD brain, which likely leads to stimulating both the renewal and infiltration processes, as well as microglial clustering around Aβ plaques (Figure 2B) (Malm et al., 2005; Stalder et al., 2005; Simard et al., 2006). Microglia share major characteristics with the monocyte populations. The two standard subsets of monocytes are able to infiltrate into the inflamed brain, with Ly6Chigh cells being the predominant subset involved in that respect (Saederup et al., 2010). However, the CX3CR1lowGR1CCR2+ Ly6Chigh subset is believed to be largely responsible for the production of blood-derived microglia under pathological conditions (Mildner et al., 2007, 2011; Getts et al., 2008; D’Mello et al., 2009; Naert and Rivest, 2012b).

Although we refer to monocyte-derived microglia as the second

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**Figure 1** Monocyte and lymphocyte frequency and CCR2 expression in human aging and AD. In human bloodstream, aging alters the frequency of PBMC by inducing an expansion of non-classical and intermediate monocytes. No data are yet available on either monocyte frequency or CCR2 status during AD. The effect of age on lymphocyte frequency has been extensively investigated using several markers that distribute CD4+ and CD8+ lymphocytes into multiple subsets whose complexity of organization cannot be properly described within the confines of the present review. However, the distribution of the two major lymphocyte subsets has been analyzed in AD. CD4+ lymphocytes were found to be more abundant than CD8+ lymphocytes. Despite a global increase in CCR2 expression among the lymphocyte populations, CCR2 was increased in CD4+ lymphocytes and decreased in the CD8+ subset.

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**Table 1**

<table>
<thead>
<tr>
<th>human subset</th>
<th>mouse counterpart</th>
<th>cell frequency in aging</th>
<th>CCR2 level in aging</th>
<th>cell frequency in AD</th>
<th>CCR2 level in AD</th>
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<tr>
<td>Classical</td>
<td>Classical</td>
<td>(2) (CD14low)</td>
<td>(3)</td>
<td>n.d.</td>
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<tr>
<td>Non-classical</td>
<td>Non-classical</td>
<td>(1)</td>
<td>no CCR2+ cells.</td>
<td>n.d.</td>
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n.d. = not determined; (1) Hearps et al., 2012; (2) Nyugen et al., 2010; (3) Seidler et al., 2010; (4) Zhang et al., 2013; (5) Lombardi et al., 1999; (6) Magaki et al., 2008; (7) Pellicano et al., 2010; (8), 2012; (9) Reale et al., 2008; (10) Richardts-Salzburg et al., 2007; (11) Lumnion et al., 2012; (12) Harries et al., 2012.
A proposed model for monocyte and BMDM in the AD mouse model. Two pools of microglia exist: the resident microglia and the newly differentiated microglia derived from BMC during brain injury and diseases. Precursors of BMDM are monocytes, which can be divided into two subsets: CX3CR1lowCCR2+GR1+Ly6Chigh and CX3CR1highCCR2+GR1−Ly6Chigh (A) In the healthy mouse, 60%–70% of monocytes are Ly6Chigh and CCR2 is involved in their egress from BM into the bloodstream. In the healthy brain, rare monocyte infiltration and a low level of monocyte-derived microglial precursors are observed due to a functional BBB. A weak infiltration of monocytes occurs after mice irradiation (represented by the dotted arrow). (B) In AD, global monocytopenia occurs in the bloodstream due to a decreased production of Ly6Chigh monocytes resulting in lower frequency of Ly6Chigh monocytes. Ly6Clow monocytes remain unaffected during aging in a context of AD. Despite monocytopenia, monocyte infiltration into the brain is facilitated by CCL2-induced BBB disruption. Both monocyte subsets (Ly6Chigh monocytes being the main species involved) then migrate into the brain and differentiate into subsets of microglia, which thus supplement resident microglia. This infiltration (represented by the solid arrow) is enhanced by the irradiation weakening the BBB. Both activated resident microglia and BMDM are attracted to Aβ deposits, but resident microglia are apparently inefficient for subsequent Aβ elimination. Only Ly6Chigh monocyte-derived microglia are able to phagocytize and eliminate soluble Aβ without changing plaque formation. Therefore, the decreased production of this microglial subset observed in AD could contribute to the etiology and progression of this disease.
population of microglia that exists under pathological conditions, there is a debate at the moment on whether they should be defined as being microglia or macrophages. We prefer using the term blood-derived microglia since they settle in the brain. In support of this terminology are the blood-derived Kupffer cells in the liver.

Monitoring CCR2 in the brain

Recently, CCR2-red fluorescent protein (RFP) knock-in mice (CCR2+/RFP) were generated and crossed with CX3CR1-GFP mice (CX3CR1+/GFP) to investigate trafficking of monocyte subsets (Saederup et al., 2010). These ‘two-color’ mice confirmed previous studies regarding the fact that CCR2 is expressed in all circulating blood monocytes. Ly6CChigh monocytes (~80% of the population) express cell surface RFP and CCR2 with only weak expression of CX3CR1GFP, whereas CCR2 surface-negative monocytes are predominantly Ly6Cclow and highly express CX3CR1GFP. Permeabilization process doubles CCR2 signal intensity in Ly6Cclow monocytes while having no effect on Ly6CChigh monocytes, demonstrating that ~50% of CCR2 is present in the cytoplasm of the Ly6CChigh subset. There is evidence that Ly6Cclow monocytes, as well as NK cells and T cells, do transcribe Ccr2, but fail to translate it into protein, as indicated by the lack of CCR2 immunoreactivity despite the presence of CCR2 mRNA (Saederup et al., 2010). Therefore, additional signals might initiate CCR2 translation in these monocytes, thus providing a mechanism for the rapid CCR2 up-regulation.

In the healthy brain of CCR2+/RFP, CX3CR1+/GFP mice, no RFP signal is detected, although a diffuse GFP signal is visible. As shown by colocalization with the microglial marker IBA-1, CX3CR1GFP is present in microglial cells throughout the brain in both healthy and diseased tissues. In contrast, microglia do not express CCR2 under naïve condition (Saederup et al., 2010). Ly6CChigh monocytes (and thus enriched for CCR2 expression) are known to be the main subset of monocytes that infiltrate the CNS of mouse models of multiple sclerosis (King et al., 2009; Mildner et al., 2009), indicating an overwhelming advantage for this subset in accessing the CNS under inflamed conditions, and suggesting that this process is CCR2-dependent. The well-defined characteristics of the mononuclear myeloid cell infiltration in experimental autoimmune encephalomyelitis (EAE) is mirrored by a population-specific pattern of CCR2 and CX3CR1 expression, suggesting that the CCR2+/Ly6CChigh and CX3CR1high/Ly6Cclow subsets retain functional specialization after invading the inflamed CNS (Saederup et al., 2010). Although Ly6CChigh monocytes are the major subset infiltrating the brain, CD45low microglial cells do not express CCR2 and display high CX3CR1 expression upon EAE-induced inflammation, suggesting the loss of CCR2 expression in microglia. Recently, CCR2 expression was shown to vanish during monocyte differentiation into microglia at embryonic stages in CCR2+/RFP, CX3CR1+/GFP mice (Mizutani et al., 2012). There is a lack of CCR2 expression not only in adult resident microglia, but also in all resident cell types. Moreover, CCR2RFP expression is not significantly up-regulated in microglia upon inflammation (Mizutani et al., 2012). This is consistent with a modification in CCR2 expression through cell differentiation. Indeed, there is a progressive increase in the fraction of cells expressing CCR2 as differentiation proceeded from self-renewing hematopoietic stem cells to multipotent progenitors, next to cells committed to the myeloid lineage, and finally, to fully differentiated monocytes (Si et al., 2010). These recent studies fully account for the previously noted absence of CCR2 staining in healthy and diseased adult brain. Recently, microglia-depleted brain regions of transgenic animals expressing the thymidine kinase (TK) gene under control of the CD11b promoter are repopulated with new IBA1-positive cells within 2 weeks (Varvel et al., 2012). The engrafted cells express high levels of CD45 and CCR2. Although more numerous and morphologically distinct from resident microglia, the engrafted cells exhibit a distribution remarkably similar to that of resident microglia. This provides the first evidence that circulating CCR2+ monocytes have the potential to occupy the adult CNS myeloid niche normally inhabited by microglia, and that a strong homeostatic drive exists to maintain the myeloid component cell population in the mature brain.

A dual role of microglia in AD

Microglia activation by Aβ may be associated with detrimental inflammation of the brain in AD patients (McGeer and McGeer, 1998). However, the exact role played by microglia in the pathogenesis of AD has only recently begun to be addressed (Liu and Hong, 2003). Several subsets of microglia exist in the brain and appear to play different roles (Hansich, 2013a, b; Lampron et al., 2013; London et al., 2013). In addition, a high level of plasticity enables microglia to rapidly change their phenotype and function depending on the environmental context. These brain immune cells may act as a double-edged sword in AD, with either beneficial or detrimental effects (Schwartz et al., 2006; Naert and Rivest, 2011b). Using chimeric APPswe/PS1 mice harboring GFP-labeled hematopoietic cells, cluster of microglia surrounding Aβ deposits are clearly shown to comprise not only resident cells, but also microglia derived from myeloid progenitors (Simard and Rivest, 2004, 2006). To investigate the function of each microglial subset, APPswe/PS1/TK mice were used to abolish the monocyte population infiltrating the brain (Simard et al., 2006). This selective ablation of proliferating myeloid cells requires a long period of ganciclovir administration, which leads to a rapid lethal effect (Heppner et al., 2005). Therefore, ganciclovir was delivered directly into the CNS of APPswe/PS1 mice, eliminating monocytes in the vicinity of the brain prior to their differentiation into microglia, while systemic macrophages remained intact. Preventing monocyte recruitment increased the abundance of Aβ deposits without affecting the number of plaque-associated microglia (Simard et al., 2006). These data support the beneficial role of infiltrating myeloid cells and the inability of resident microglia to prevent Aβ plaque formation. More recently, in order to restrict TK expression in APPswe/PS1 mice to resident microglia and to overcome ganciclovir-mediated myelotoxicity (Heppner et al., 2005; Simard et al., 2006), APPswe/PS1 chimeric mice with congenic GFP-labeled WT BM were generated and received systemic ganciclovir treatment (Grathwohl et al., 2009). Depletion of resident microglia had no effect on the amount of Aβ40, Aβ42, or Aβ deposits, despite a ~30% decrease in microglia number in most brain regions (Grathwohl et al., 2009). These data have to be interpreted cautiously, since APPswe/PS1 chimeric mice were transplanted with
WT bone marrow cells (BMC), which have been shown to be neuroprotective in AD and to have the ability to clear cerebral amyloidosis (Michaud et al., 2011; Naert and Rivest, 2012b). In addition, intracerebral administration of ganciclovir, which results in a 97% reduction of microglia number, had also no effect on either Aβ plaque formation or Aβ-associated neuritic dystrophy (Grathwohl et al., 2009). Although such a treatment likely depletes BMDM, no increase in amyloidosis was observed. Comparison of the latter data with our study suggests that resident microglia might have contributed to the increase in Aβ accumulation observed using our approach (Simard et al., 2006). Furthermore, the fact that resident microglia are apparently left intact in our model, despite infusing the drug using the same method and duration (Simard et al., 2006), could be explained by our use of a 50-fold lower dose of ganciclovir (50 vs. 1 mg/ml, administered centrally in both studies), which may have left the pool of resident microglia largely unaffected (Simard et al., 2006; Grathwohl et al., 2009). Overall, these studies clearly point to the incapacity of resident microglia to counteract Aβ pathology in the AD mouse model.

Microglia may therefore exert either beneficial or detrimental effects in the CNS, according to which subset is considered. There is now compelling evidence supporting a neuroprotective role for BMDM and the inability of resident microglia to afford significant neuroprotection. Depending on the frequency and the type of the stimulus, microglia can exhibit neuroprotective or detrimental functions. Their environment is drastically modified in pathological conditions and this might strongly contribute to their functions. Their environment is drastically modified in pathological conditions and this might strongly contribute to their functions.

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**CRR2+ monocytes and CRR2+ monocyte-derived microglia: neuroprotective cells in AD?**

BMDM preferentially originate from the recruitment of CCR2+ monocytes, suggesting that these immune cells are those responsible for exerting the above-mentioned neuroprotective effects. Indeed, CCR2 deficiency in two AD mouse models, namely the Tg2576 (El Khoury et al., 2007) and APPSwe/PS1 mice (Naert and Rivest, 2011a), exacerbates and accelerates mnesic deficit while enhancing amyloidosis in APPSwe/PS1/CCR2−/− mice. In addition, the number of plaque-associated microglia has been shown to increase (likely through the enhanced proliferation of resident cells and/or recruitment of blood progenitors) (Naert and Rivest, 2011a), which probably reflects an enhanced chemokine production around Aβ deposits upon CCR2 deficiency. Importantly, transplantation of WT BMC—i.e. CCR2-competent cells—restores cognitive capacity and significantly diminishes Aβ accumulation in APPSwe/PS1/CCR2−/− mice (Figure 3) (Naert and Rivest, 2012b). Furthermore, intrafemoral injection of a CCR2 transgene preferentially induces CCR2 expression in the monocytic population and prevents the onset of memory decline and Aβ oligomer

![Figure 3](attachment:image.png) Transplantation of WT BMC prevents the onset of memory decline and restricts Aβ pathology in APPSwe/PS1 and APPSwe/PS1/CCR2−/− mice. APPSwe/PS1 and APPSwe/PS1/CCR2−/− mice were transplanted with WT or CCR2−/− BMC after whole body irradiation. Cognitive impairments are rescued by WT BMC transplantation in APPSwe/PS1 and APPSwe/PS1/CCR2−/− mice. Although Aβ deposits are not affected by BMC transplantation, the level of soluble Aβ is decreased in APPSwe/PS1 and APPSwe/PS1/CCR2−/− mice transplanted with WT BMC. In addition, fewer microglia are attracted to Aβ plaques and these microglial cells exhibit a decreased expression of TGF-β1, TGF-β-R1, and TGF-β-R2 in APPSwe/PS1 and APPSwe/PS1/CCR2−/− mice transplanted with WT BMC. In contrast, CCR2−/− BMC transplantation induces the occurrence of mnesic deficits, increases accumulation of soluble Aβ, and enhances microglial expression of TGF-β1, TGF-β-R1, and TGF-β-R2 around Aβ plaques in APPSwe/PS1 mice.
accretion in APPswe/PS1/CCR2−/− mice (Naert and Rivest, 2012b). Even more interestingly, both transplantation of WT BMC and BMC transplanted with the CCR2 transgene also prevent mnesic impairment and Aβ accumulation in APPswe/PS1 mice, which do not exhibit any known defect in CCR2 expression or function (Mildner et al., 2011; Naert and Rivest, 2012b). Taken together, these results strongly suggest that the hematopoietic system is impaired in APPswe/PS1 mice.

In this regard, a defect in monocytopoiesis occurs in aging APPswe/PS1 mice (Naert and Rivest, 2012a). Despite similar monocyte level in 1- and 3-month-old WT and APPswe/PS1 mice (Michaud et al., 2011; Naert and Rivest, 2012a,b), a defect in monocyte production was observed at 6 months of age. This monocytopoiesis results specifically in a decreased level of CX3CR1lowCCR2+ GR1+Ly6Chigh cells in the bloodstream, since the CX3CR1high CCR2− GR1− Ly6Clow counterparts remains unaffected (Naert and Rivest, 2012a). Therefore, such monocytopoiesis could likely result from a defective CCR2-dependent process. The major function of CCR2 is to allow the migration of CCR2+ monocytes from BM to the blood (Serbina and Pamer, 2006; Engel et al., 2008) and to mediate the trafficking of hematopoietic stem cells, progenitors, and monocytes to sites of inflammation as well as monocyte infiltration into the brain (Izikson et al., 2000; Babcock et al., 2003; Si et al., 2010). A defect in CCR2 function is expected to cause the retention of CX3CR1lowCCR2+ GR1+Ly6Chigh monocytes in the BM, as observed in CCR2-deficient mice (Serbina and Pamer, 2006). On the other hand, there is a drop in the frequency of CX1CR1lowCCR2+ GR1+Ly6Chigh monocytes in the BM from 6-month-old APPswe/PS1 mice (Naert and Rivest, 2012a), consistent with an impaired production and/or differentiation of inflammatory monocytes (Figure 2B). In addition, CCR2 abundance in the plasma membrane of Ly6Chigh monocytes is similar between 6-month-old WT and APPswe/PS1 mice (unpublished data), suggesting that plasma membrane insertion of CCR2 and differentiation of the few remaining Ly6Chigh monocytes produced in APPswe/PS1 mice remain normal and functional. Therefore, the low blood frequency of Ly6Chigh monocytes observed in CCR2-deficient mice may likely result from a decreased production. Accordingly, the fact that 6-month-old APPswe/PS1 mice exhibit a decreased ratio of GR1+/GR1− monocytes leads to a strong reduction in the pool of CX1CR1lowGR1+ CCR2+ Ly6Chigh monocytes available not only to clear Aβ in the bloodstream, but also for their recruitment into the brain and removal of brain Aβ. Overall, these recent data support the concept that AD is accompanied by a marked alteration in the number, phenotype distribution and functionality of mononuclear cells. Nonetheless, further studies are needed to fully understand the mechanism underlying the changes observed in the BM during AD development.

Monocytes and CCR2 status in AD patients

In addition to changes in the abundance, distribution, and functions of microglia, peripheral immune cells also display notable alterations in patients with AD. However, PBMC analyses from AD patients have yielded conflicting results, thus precluding any consensus on how AD affects leukocyte characteristics. Nonetheless, there is a strong likelihood that AD inevitably entails modifications of the hematopoietic system.

First, a careful scrutiny of studies reporting modifications of the immune system during aging is essential for interpreting studies on AD-specific leukocyte populations. Aged healthy adults exhibit an expansion of the non-classical CD14+CD16+ subtype—i.e. the intermediate CD14++CD16+ subtype, whereas the population of CD14++CD16- monocytes, a subdivision of the classical CD14++ CD16- monocytes—i.e. the counterpart of mouse CX3CR1low GR1+ CCR2+ Ly6Chigh—are decreased (Figure 1) (Nyugen et al., 2010; Seidler et al., 2010; Hearps et al., 2012). In addition, age alters the phenotype of specific monocyte subsets, resulting in a lower expression of activation markers and chemokine receptors (Nyugen et al., 2010; Seidler et al., 2010; Hearps et al., 2012). CD14++CD16- monocytes display significant time-dependent variations in CCR2 expression, with highest levels in healthy adults between 30 and 50 years old and decreased expression in adults older than 50 years of age (Seidler et al., 2010). Moreover, monocytes from older individuals have been shown to exhibit impaired phagocytosis and shortened telomeres, suggesting a dysregulation of monocyte function with aging (Hearps et al., 2012). However, conflicting results have been reported from analyses of peripheral leukocyte populations (mainly lymphocytes and monocytes) in AD (Lombardi et al., 1999; Richartz-Salzburger et al., 2007; Magaki et al., 2008; Reale et al., 2008; Pellicano et al., 2010, 2012), although all studies suggested that AD may be associated with immune disruption.

Monocytes from AD patients exhibit poor in vitro differentiation and weak phagocytic properties and undergo apoptosis after Aβ application (Fiala et al., 2005). Recently, a decreased abundance of monocytes was found in MCI patients compared with AD patients, suggesting a transient decrease in monocyte numbers in the etiology of AD (Lunnon et al., 2012). When PBMC were analyzed in a large aged human population recently, a global increase in CCR2 expression was associated with lower mini-mental state examination (MMSE) scores (Harries et al., 2012). These data corroborate the increase in CCR2 expression previously reported in PBMC from AD patients (figure 1) (Reale et al., 2008; Pellicano et al., 2010). It must be noted, however, that these studies examine CCR2 expression levels in lymphocytes only, leaving CCR2 status in monocytes an open question (Reale et al., 2008; Pellicano et al., 2010). Interestingly, there are contrasting differences in the regulation of CCR2 expression among the lymphocyte populations, i.e., CCR2 expression is found to be globally up-regulated in CD4+ lymphocytes and down-regulated in CD8+ lymphocytes (figure 1) (Reale et al., 2008). The missing link to correlate data from the AD mouse model with AD patients is likely to be found in the CCR2 status of patient monocytes, for which data are unfortunately unavailable. Nonetheless, it may be inferred that due to the prevalence of lymphocytes in human PBMC, a modification in monocytic CCR2 levels would likely be masked by the CCR2 up-regulation found in lymphocytes. However, a recent study has found lower levels of absolute percent of CD14 cells in early AD patients and a decreased CCR2 expression on AD blood monocytes (Zhang et al., 2013). In addition to determining the full status of CCR2 expression in the PBMC population, it should be of great interest to assess the full distribution of monocyte subsets in blood. Therefore, further work will be required to better understand the role of CCR2 in monocytes of AD patients.
Therapeutic effect of hematopoietic cytokines

The beneficial effects of hematopoietic cytokines such as G-CSF or GM-CSF have been established in AD mouse models (Tsai et al., 2007; Volmar et al., 2008; Sanchez-Ramos et al., 2009; Boyd et al., 2010; Jiang et al., 2010; Li et al., 2011). Furthermore, G-CSF was found to decrease both brain Aβ deposition and soluble Aβ accumulation by directly activating microglia and enhancing the recruitment of BMDM in APP mice (Sanchez-Ramos et al., 2009). Similar systemic treatment with M-CSF prevented and restored cognitive impairments, increased the number of microglia, and decreased the number of Aβ deposits in APPswe/PS1 mice through enhancing Aβ phagocytosis by microglia (Boissonneault et al., 2009), a known effect of M-CSF (Mitrasinovic and Murphy, 2003; Mitrasinovic et al., 2003; Majumdar et al., 2007).

M-CSF (also known as Csf-1) is involved in the development and differentiation of blood monocytes (Ryan et al., 2001; Auffray et al., 2009b). Indeed, a defect in M-CSF and/or its receptor M-CSFR (CD115) drastically reduces monocyte frequency in bloodstream (Ryan et al., 2001; Dai et al., 2002). More recently, a 4-day systemic M-CSF treatment was able to prevent and rescue cognitive impairments in APPswe/PS1 mice and re-establish circulating levels of monocytes (Naert and Rivest, 2012a). These properties of M-CSF are especially important as APPswe/PS1 mice exhibit significant monocytopenia with aging (Naert and Rivest, 2012a). This defect in mononuclear cells is not dependent on CD115, since M-CSF treatment increased monocyte frequency in 4-month-old APPswe/PS1 mice to levels similar to those found in age-matched WT mice. Therefore, BMC and monocytes of APPswe/PS1 mice likely express a functional CSF1-R. However, we cannot exclude a defect in M-CSF production in the BM of AD mice. M-CSF treatment fails to increase monocyte populations in CCR2−/− and APPswe/PS1/CCR2−/− mice, suggesting that M-CSF exerts these effects via a CCR2-dependent mechanism (Naert and Rivest, 2012a). Thus, BMC and monocytes in APPswe/PS1 mice are sensitive to M-CSF and express a functional CCR2 receptor, suggesting that the observed monocytopenia is more likely due to an impairment in monocyte migration and/or differentiation. More work will be necessary to clearly identify the mechanism responsible for the defect in myeloid lineage observed in AD.

Conclusion

The past decade has brought major progress in our understanding of monocyte-mediated immune defense in AD. Although many questions remain unanswered, the neuroprotective effect of CCR2+ monocytes is now well established, and several lines of evidence show that this subset exhibits a major defect during aging in the context of AD. The latter demonstration is rather significant inasmuch as CCR2+ monocytes are critical to lymphocyte activation (Soudja et al., 2012). There is thus clear evidence supporting the beneficial properties of inflammatory monocytes, which are at the central crossroad in the insufficient activation of innate immunity that characterizes AD.

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References


CCR2\(^+\) monocyte defect in Alzheimer’s disease


