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Review

Myeloid cell distribution and activity in multiple sclerosis

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Summary. Multiple sclerosis (MS) is a demyelinating disease in which an exacerbated immune response provokes oligodendrocyte loss and demyelination, the hallmarks of this neurological disease. The destruction of myelin due to the uncontrolled activity of the invading immune cells leads to the formation of MS plaques. Among the different leukocytes that participate in the immune response associated with MS, the role of myeloid cells has been analyzed extensively (i.e. macrophages, dendritic cells -DCs- and neutrophils). Hence, in this review we will summarize what is known about the distribution, expression and markers available to study myeloid cells, and their histopathology, not only in a standard animal model of MS (autoimmune experimental encephalomyelitis -EAE) but also in MS tissue. In this review, we will not only refer to mature myeloid cells but also to the undifferentiated and almost unexplored myeloid-derived suppressor cells (MDSCs). The active role of MDSCs in the prompt resolution of an immune episode is gaining importance, yet is still the subject of some debate. Finally, the similarities and differences between MS and EAE are discussed, particularly in terms of myeloid cell phenotype, activity and the markers used.

Key words: Dendritic cells, MDSCs, Neutrophils, Macrophages, EAE, Demyelinaion, Remyelination, Myelin, Functionality

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Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the human central nervous system (CNS) and a major cause of neurological disability among young adults. Most MS patients (85-90%) begin their clinical evolution with so-called relapsing-remitting (RR)-MS (Fig. 1), which is characterized by episodic symptoms that produce residual deficits or that are followed by full recovery within a few weeks (Inglese, 2006). The relapses in RR-MS are believed to be the consequence of focal inflammatory demyelinating lesions associated with a loss of oligodendrocytes, a histopathological hallmark of MS. A large percentage of RR-MS patients (50-60%) subsequently progress to a secondary progressive (SP) disease course, which is dominated by diffuse white matter (WM) abnormalities, grey matter atrophy, a high degree of axonal damage and cortical demyelination not associated with new actively demyelinating lesions (Lassmann et al., 2012). Only about 10-15% of patients develop the primary progressive form of multiple sclerosis (PP-MS), which is characterized by continuous deterioration without distinct relapses (Sellner et al., 2011).

Surprisingly, there are no important histopathological differences between the different types of MS patients (Breij et al., 2008; Frischer et al., 2009; Bramow et al., 2010; Lassmann, 2013). Hence, the neuropathological events associated with the clinical course of MS in each patient may involve different extents of leukocyte infiltration into the white and grey matter, partial remyelination, axonal transection and/or degeneration, neuronal cell death and glial proliferation (Noseworthy et al., 2000; Compston and Coles, 2008;

Henderson et al., 2009). Demyelinating lesions are classified as active, chronic-active and chronic-inactive lesions, depending on their histopathological characteristics and their intrinsic capability for spontaneous remyelination, which generates partially repaired shadow plaques. Indeed, all three types of lesion can be observed in the CNS of MS patients, independently of their clinical evolution and phenotype (Breij et al., 2008; Frischer et al., 2009; Bramow et al., 2010). This implies that during the development of a demyelinating lesion, not only CNS resident microglia and astrocytes, but also infiltrated inflammatory cells, can experiment changes in their activity and distribution that affect plaque evolution through different mechanisms.

Different subsets of immune cells are present in the inflamed tissue associated with demyelinating lesions, of which myeloid-derived cells are the most abundant. Myeloid cells arise from multipotent bone marrow haematopoietic stem cells that develop into mature myeloid cells (monocytes, dendritic cells -DCs- and granulocytes) essential for the normal activity of the innate and adaptive immune systems (Gabrilovich et al., 2012). In fact, myeloid cells critically shape the inflammatory environment during CNS autoimmunity, not only by producing pro-inflammatory mediators (Barnett et al., 2006; Mildner et al., 2009) but also by supporting remyelination (Miron et al., 2013). Myeloid cells within the CNS represent a prominent component of local inflammatory infiltrates that adopt different spatio-temporal distributions and phenotypes in different phases of demyelination in MS, as well as during the clinical course of experimental autoimmune encephalomyelitis (EAE), the most commonly used experimental model for MS (Constantinescu et al., 2011; Moreno et al., 2012).

Distribution of myeloid cells in EAE

Since its first description in 1933, EAE has become the most commonly accepted animal model of MS used to study the pathophysiology of the disease (Rivers et al., 1933). Immunization with self-antigenic epitopes of myelin actively induces an autoimmune response in the CNS of rodents (Duffy et al., 2014). The most widely evoked type of EAE is the monophasic variant induced with the encephalitogenic myelin oligodendrocyte glycoprotein peptide (MOG_{35-55}: CP-EAE). This model is carried out in C57BL/6J mice and its clinical course progresses through different consecutive phases. After an asymptomatic period (sensitization), the first clinical signs involve ascending paralysis that initially affects the tail and that progresses upwards to the forelimbs (effector phase). The maximum clinical score develops

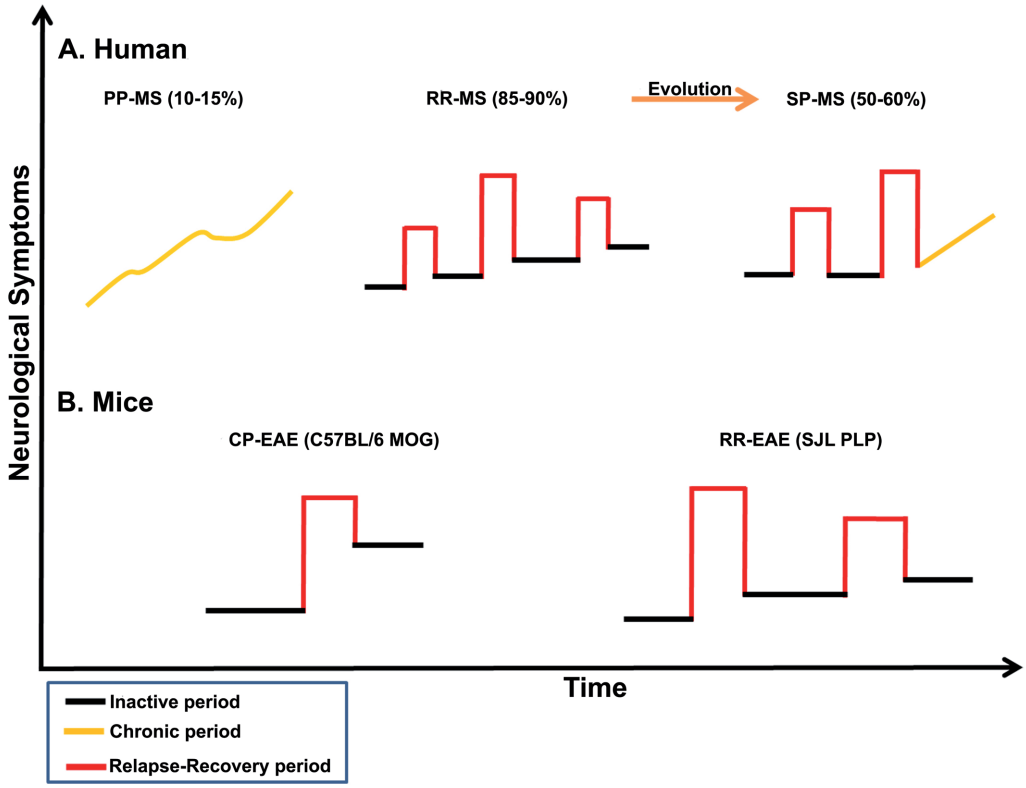

Fig. 1. Schematic representation of the different clinical variants in MS and EAE: **A.** Disease courses in MS (from left to right): primary progressive (continuous symptom accumulation without recoveries), relapsing-remitting (consecutive periods of relapse followed by remitting periods with total or partial recovery) and secondary progressive (continuous neurological disability accumulation after a previous relapsing remitting phase). **B.** In EAE, the clinical course may present unique relapse and peak phases followed by a modest recovery phase which ends in a chronic phase from which mice do not recover. This is called Chronic Progressive-EAE (CP-EAE). In other cases, EAE mice can show a relapsing remitting clinical course (RR-EAE) with consecutive relapses followed by moderate recoveries. Black lines represent inactive periods between relapses; red lines represent relapsing-remitting periods; yellow lines represent progressive phases.

after 1-3 days (disease peak), after which the animals enter into partial remission (a clinical score between 0.5 and 1) for an undefined number of days before the chronic phase commences from which the mice do not recover (Berard et al., 2010; Moline-Velazquez et al., 2011, 2014; Moreno et al., 2012; Duffy et al., 2014). In the SJL mouse strain, EAE can be induced by immunization with encephalitogenic epitopes of the proteolipid protein ($PLP_{139–151}$, $PLP_{178–191}$) or $MOG_{92–106}$. In these mice, after a similar sensitization phase, the disease is characterized by a relapsing-remitting course of paralysis (RR-EAE), which allows disease mechanisms or immunomodulatory strategies to be studied in such a relapsing autoimmune disease setting.

EAE shares many pathological features with MS, such as chronic neuroinflammation, demyelination and neuronal damage, and it is generated by autoimmune attack on the CNS (Baxter, 2007; Steinman and Zamvil, 2006). In addition, with the breakdown of the blood-brain barrier (BBB), multifocal infiltration of activated immune cells into the CNS can drive the attack on the myelin sheath (Guo and Schluesener, 2005). In this review, we will describe the distribution and activity of myeloid-derived infiltrating cells in the CNS during the course of EAE, i.e. that of macrophages, DCs and neutrophils (Figs. 2, 3). Moreover, we will pay particular attention to a new and interesting cell type that has an important influence on the control of the immune attack, myeloid-derived suppressor cells (MDSCs: Table 1 summarizes the main phenotypic markers used to study myeloid cells in the EAE model).

Macrophages

Numerous studies have focused on the distribution of macrophages in the different phases of EAE. Massive infiltration of peripheral macrophages has been reported in the CNS, at the onset and the peak phase, with a decrease in their density during the resolution and recovery phases of the disease (Jiang et al., 2014). Moreover, an important role has been ascribed for infiltrating macrophages during the relapsing phase of CP-EAE (Heppner et al., 2005; Denney et al., 2012; Sosa et al., 2013). The distribution of macrophages in the demyelinated spinal cord is not uniform, although a large number of $CD68^+$-macrophages were found in the WM of the lateral and ventral spinal cord at the peak of the disease (Martínez-Gómez et al., 2012). Moreover, inflammatory monocytes with the $CCR2^+/Ly\text{-}6C^{hi}$ phenotype cannot only be detected in the parenchyma but also in the perivascular areas of the CNS, associated with the promotion of EAE pathogenesis by their differentiation into mature macrophages and DCs (King et al., 2009; Mildner et al., 2009; Saederup et al., 2010).

Despite their different origins (resident microglia

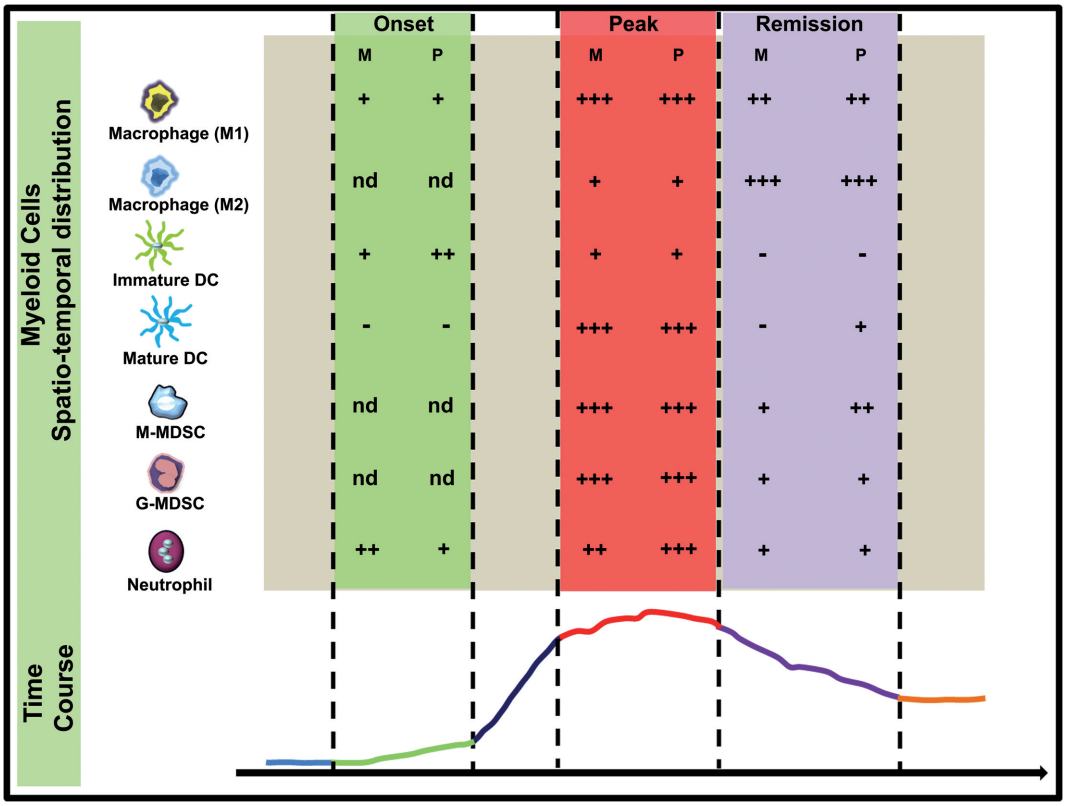

Fig. 2. Schematic spatial-temporal distribution of myeloid cells within the CNS of CP-EAE mice. The density of the myeloid cells within the meninge (M) and the parenchyma (P) of the CNS run parallel to the clinical impairment in all myeloid cells except in M2 macrophages that are maximal during the remitting phase. +: low density; ++: moderate density; +++: high density; -: absence. nd: not determined.

originate in the embryo from the yolk sac, whereas inflammatory macrophages are produced in the bone marrow at different developmental periods and in adulthood), the distinction between blood-derived macrophages and resident microglia has practical limitations in EAE (Ransohoff, 2007; Ajami et al., 2011; Michell-Robinson et al., 2015). Recently, resident microglia were described as lacking the chemokine receptor CCR2 and as expressing CX3CR1 (Mizutani et al., 2012), although inflammation up-regulates CCR2 expression by infiltrating macrophages. Very recently, a double-heterozygous *Ccr2*^{rfp}::*Cx3cr1*^{gfp} mouse, in which microglia (CD45^{dim}/Ly-6C^{-}) express GFP and macrophages (CD45^{high}/Ly-6C^{+}) express RFP, was used to study the roles of microglia-derived and monocyte-derived macrophages in CP-EAE (Yamasaki et al., 2014). At disease onset, monocyte-derived macrophages are in close contact with axoglial units and they have intracellular inclusions of myelin, suggesting that these monocyte-derived macrophages trigger demyelination at the initial phases of EAE. Moreover, a genomic analysis of inflammatory cells at the onset of EAE indicated that monocyte-derived macrophages up-regulate genes related to phagocytosis, migration, and prostanoid and nitric oxide production, whereas microglia-derived macrophages are best involved in clearing debris and suppressing cell activity (Yamasaki et al., 2014).

Two activity states have been described for macrophages in EAE, each fulfilling a different role: classically activated or M1 macrophages that shift towards a pro-inflammatory microbicidal function; and alternatively activated or M2 macrophages that are

Table 1. Summary of the main phenotypic markers of myeloid cells in EAE.

Marker	Macrophages		Dendritic	MDSCs		Neutrophils	Bibliography
	M1	M2	Cells	M-MDSCs	G-MDSCs		
CD11b	++	++	++	++	++	++	Ransohoff, 2007; Zhu et al., 2007, 2011; Moliné-Velázquez et al., 2011, 2014
F4/80	++	+	-	+	-	-	Ransohoff, 2007; Peranzoni et al., 2010
CD68	++	+	-	+	nd	-	Martínez Gómez et al., 2012
CCR2	+	nd	+	+	nd	nd	Saerderup, 2010; Yamasaki et al., 2014; Clarkson et al., 2015
CD11c	-/+	-	++	low	-	-	Sagar et al., 2012; Colton, 2013; Sosa et al., 2013; Moliné-Velázquez et al., 2014
Ly-6C	++	+	-	++	+	+	Peranzoni et al., 2010; Moliné-Velázquez et al., 2011; 2014; Zhu et al., 2007
Ly-6G	-	-	-	-/low	++	++	Peranzoni et al., 2010; Moliné-Velázquez et al., 2011; 2014; Zhu et al., 2007
Ly-6B.2	-	-	-	-	nd	++	Rosas et al., 2010; Moliné-Velazquez et al., 2011; Lee et al. 2013; Aubé et al. 2014
CD115	+	+	-	+	-	-	Peranzoni et al., 2010; Moliné-Velazquez et al., 2014
CD206	-	+	-	-	-	-	Mikita et al., 2011
CD124	-	+	-	+	-	-	Peranzoni et al., 2010; Moliné-velazquez et al., 2014
CD40	++	+	++				Mikita et al., 2011
MHC-II	++	+	++	low	-	-	Almolda et al., 2011; Mikita et al., 2011; Moliné-Velázquez et al., 2014
CD80	++	+	++	-	-	-	Almolda et al., 2011; Mikita et al., 2011
CD86	++	+	++	nd	nd	-	Almolda et al., 2011; Mikita et al., 2011
iNOS	++	-	-	+	-	-	Zhu et al., 2011; Jiang et al., 2014
Arg-1	-	++	-	+	+	+	Jiang et al., 2014; Kalyan and Kabelitz, 2014

G-MDSCs, granulocytic-myeloid-derived suppressor cells; M-MDSC, monocytic-myeloid-derived suppressor cells; nd, Not determined; ++, strong expression; +, moderate expression; -, no expression.

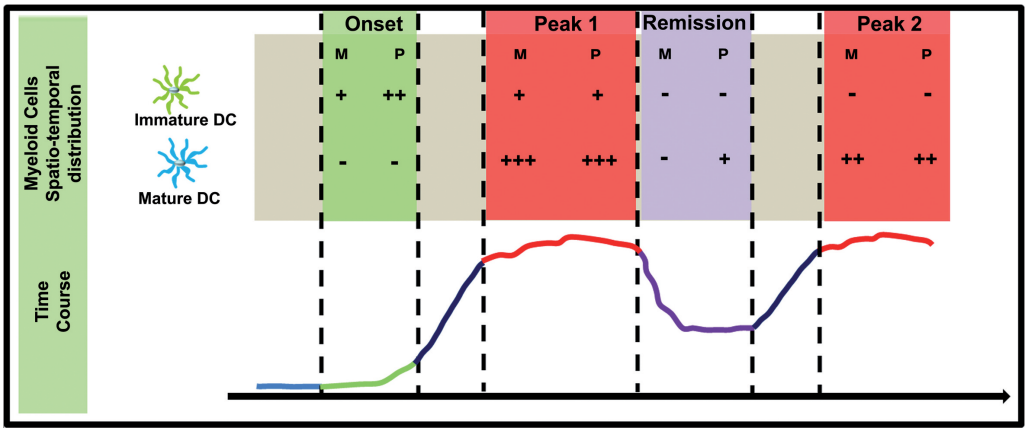

Fig. 3. Schematic spatial-temporal distribution of myeloid DCs within the CNS during the two first relapses of the typical RR-EAE. The distribution and density of DCs in the first relapse is very similar to the CP-EAE course. The scarce available data about the second relapse indicate that the DC infiltrate is less prominent at the peak than in the first one. There is a lack of morphological data about the distribution of the rest of myeloid cells in this EAE variant. P: CNS parenchyma; M: Meninge; +: low density; ++: moderate density; +++: high density; -: absence.

associated with wound healing and immune resolution (Sica and Mantovani, 2012; London et al., 2013). Both subtypes of macrophages express specific markers or different levels of the same marker (Table 1; Almolda et al., 2011; Mikita et al., 2011; Jiang et al., 2014). M1 macrophages produce several pro-inflammatory cytokines and they accumulate gradually until the disease reaches its peak, thereafter diminishing as the resolution and recovery phases follow. On the other hand, M2 macrophages are involved in the resolution of the inflammatory response in EAE, producing anti-inflammatory cytokines, as well as in the clearance of the cell debris that negatively affects remyelination and tissue repair (Batoulis et al., 2010; Mikita et al., 2011; Jiang et al., 2014).

Both types of activated macrophages can be found together during the course of EAE, yet they are regulated in a dynamic manner (Fig. 2). In fact, the imbalance between M1/M2 macrophages appears to be a key factor defining the severity of the inflammatory episode. Thus, a deficiency of SOCS3 on myeloid cells in CP-EAE produces stronger infiltration of activated M1 macrophages than in the classical EAE, with the consequent shift to an atypical clinical course of EAE that develops into a more severe disease with an earlier onset (Qin et al., 2012). Conversely, M2 macrophages are protective in CP-EAE (Ponomarev et al., 2005; Weber et al., 2007) and a pharmacological increase of the M2/M1 ratio during the asymptomatic period of EAE delays the onset and reduces the maximum clinical score of the immunized mice (Liu et al., 2013). In accordance with this shift from the M1 to M2 phenotype, enhancing the anti-inflammatory phenotype of these myeloid cells may represent an interesting therapeutic approach.

Dendritic cells

DCs are professional antigen presenting cells (APCs) that interact with naïve T lymphocytes to trigger T cell responses. Two types of DCs have been described: i) classical or conventional DCs (cDCs), which are migratory and with a $CD11c^{hi}$ $B220^{-}$ phenotype, resident in peripheral tissues and lymphoid organs; and ii) plasmacytoid DCs (pDCs) that fulfill clear protective roles and that are characterized by the expression of $CD11c^{int}$ $B220^{+}$ and PDCA-1^{+}, and which can be detected mainly in the blood and secondary lymphoid organs (Hesske et al., 2010; Irla et al., 2010; Colton, 2013). In this review, we will focus on cDCs as part of the myeloid cell infiltrate in EAE.

Interestingly, in EAE DCs adopt a clear spatial-temporal distribution within the CNS, with variations in their state of maturation (Figs. 2, 3) (Serafini et al., 2000). Thus, during the preclinical asymptomatic stage, immature DCs are the main subtype of infiltrating immune cells, with no lymphocytes present in the CNS. These immature DCs are recruited to the meninges and choroid plexus, as well as to discrete areas of the spinal cord WM, predominantly migrating from the pial surface (Serafini et al., 2000). Furthermore, at the peak of the first relapse, DCs adopt a more mature phenotype and while they show a dispersed pattern, they are denser in the spinal cord parenchyma than at the previous disease stages, and closely associated to $CD4^{+}$ T cells. By contrast, rare mature DCs are detected within the residual inflammatory infiltrates, persisting within the spinal cord during the early remission phase of the first relapse and disappearing thereafter (Serafini et al., 2000). However, in SJL mice that develop a second relapse, or in those with a chronic course of EAE, mature DCs are detected in the residual inflammatory infiltrate within the spinal cord parenchyma and in the perivascular region of the meninges (Serafini et al., 2000). Conversely, mature DCs can be detected in CP-EAE with CD4 T cells and macrophages, forming part of the inflammatory infiltrates of the spinal cord during the preclinical stage (Suter et al., 2000). More recently, it was demonstrated that DCs are an important factor involved in the aggressiveness of EAE. Thus, in CP-EAE a strong correlation was described between the severity of the neurological impairment in each mouse, and the abundance and widespread distribution of mature DCs in the CNS (Sagar et al., 2012).

The DC population in the CNS of EAE mice is not as homogeneous as was thought in the past. Accordingly, in bone marrow-chimeric CD45.1→CD45.2 CP-EAE, CNS DCs present phenotypic and functional variations that depend on their position (Hesske et al., 2010). Periphery myeloid $F4/80^{-}$ $CD45.1^{+}$ DCs are found in perivascular cuffs in the WM and in meningeal infiltrates, presenting variations in their ability to stimulate naïve T cells. By contrast, peripheral inflammatory $F4/80^{+}$ $CD45.1^{+}$ DCs are found exclusively within the CNS parenchyma and they may drive the efficient re-stimulation of encephalitogenic infiltrating T cells (Hesske et al., 2010).

As mentioned above, DCs have traditionally been identified in the CNS by the expression of CD11c and MHC-II (Table 1), although both markers are also expressed in other cell types like macrophages or microglia (Ponomarev et al., 2005; Bulloch et al., 2008). It is believed that during EAE, APCs in the CNS re-activate self-antigen-reactive T cells as they enter the CNS (Colton, 2013). Although several APCs have been proposed to be responsible for antigen presentation, based on their expression of MHC II and of the co-stimulatory molecules CD80, CD86 and CD40 (Ponomarev et al., 2005; Goverman, 2009), microglia and DCs are thought to be the most "professional" APCs in the CNS in the context of the EAE model (Greter et al., 2005; Sosa et al., 2013; Clarkson et al., 2015). The importance of DCs as APCs in EAE has been highlighted by using transgenic *H2-Ab1*$^{-/-}$ C57 mice in which the APC capacity was restricted to this myeloid cell type. Indeed, it is noteworthy that DCs are sufficient to present antigen to autoreactive transferred T cells in order to mediate CNS inflammation and clinical disease development (Greter et al., 2005). By contrast, DC

ablation prior to the onset of EAE, produced a delay in clinical progression and ameliorated the disease severity by blocking the recruitment of encephalitogenic T cells to the CNS in a CCR2-dependent manner (Clarkson et al., 2015). Alternatively, when DC ablation is performed at the peak of disease or later, mice show a more severe clinical course than in control EAE mice (Clarkson et al., 2015).

Although both CD11c$^+$-microglia and infiltrating CD11c$^+$-cells express co-stimulatory molecules (Table 1), and they induce the same degree of myelin-specific T cell proliferation, only infiltrating CD11c$^+$ cells produce Th1 and Th17-inducing cytokines. This suggests that microglia can induce Th1 and Th17 responses through a different route, which probably involves the microenvironment elicited by other cell types present during neuroinflammation (Wlodarczyk et al., 2014). In addition, CNS CD11c$^+$ cells in close association to CD3$^+$ T cells have MBP inclusions, suggesting that DCs could take-up MBP antigen for its presentation to T cells (Sagar et al., 2012). Interestingly, during the first 5 days after immunization, while DCs are present in the CNS, only microglia contain MBP, suggesting that microglia initially reactivate encephalitogenic T cells in the CNS. By contrast, most of the DCs within the CNS parenchyma contain MBP from 7 dpi onwards. These data suggest that microglia first and together with DCs afterwards, present myelin antigens locally within the CNS (Sosa et al., 2013).

Overall, these studies indicate that the trafficking of DCs to CNS lesions follows the degree of inflammation, and that DC recruitment and maturation within the CNS may be pivotal in local T cell activation, as well as in maintaining the cellular and humoral autoimmune responses leading to EAE progression.

Myeloid-derived suppressor cells

MDSCs are a heterogeneous group of undifferentiated myeloid cells present in the bone marrow that under normal conditions are capable of differentiating into granulocytes, monocytes or DCs (Gabrilovich and Nagaraj, 2009). During an inflammatory event (sepsis, traumatic stress, infection, cancer) MDSCs expand and acquire the ability to suppress T cell functions (Goni et al., 2002; Makarenkova et al., 2006; Delano et al., 2007; Talmadge, 2007; Gabrilovich et al., 2012).

In EAE, MDSCs have a CD11b^{high} Gr1^{int} phenotype (Zhu et al., 2007), or more specifically they can be split into two main subpopulations: G-MDSCs with granulocytic characteristics, defined as CD11b$^+$ Ly-6G^{high} Ly-6C^{low}; and M-MDSCs with a monocyte-like phenotype, CD11b$^+$ Ly-6G$^{-/low}$ Ly-6C^{high} (Zhu et al., 2007; Peranzoni et al., 2010; Moline-Velazquez et al., 2011; Ioannou et al., 2012). Yet once infiltrated, M-MDSCs present the typical markers of M1 and/or M2 macrophages (Table 1). Both M-MDSCs and G-MDSCs have been reported to regulate autoimmunity and to control the generation or perpetuation of EAE (Cripps and Gorham, 2011; Moline-Velazquez et al., 2011; Ioannou et al., 2012). Indeed, CD11b$^+$ Ly-6G$^+$ cells (G-MDSCs or a subset of neutrophils) are located within the inflammatory infiltrates of the spinal cord at the peak of EAE (Fig. 2), and the adoptive transfer of these G-MDSCs dampens demyelination and inhibits the encephalitogenic Th1 and Th17 responses. This latter effect occurs via immune suppression induced by PD-L1/PD-1 interactions (Ioannou et al., 2012). However, other reports demonstrate that M-MDSCs are the main MDSC subset in this mouse model of MS (Zhu et al., 2007, 2011; Moline-Velazquez et al., 2011), with the abundance of M-MDSCs paralleling the clinical course of the disease: M-MDSC accumulation reaches its highest level as the disease peaks and decreases during the late remission phase, disappearing completely at early and late chronic phases (Moline-Velazquez et al., 2011).

In EAE, M-MDSCs are thought to participate in the transition from the peak to the chronic phase of the disease by suppressing T cell function (Zhu et al., 2007). Moreover, MDSCs establish a close morpho-functional relationship with apoptotic T cells in the spinal cord of EAE mice (Moline-Velazquez et al., 2011). The mechanisms by which MDSCs suppress T cell function vary from the production of nitric oxide and reactive oxygen species, to arginine depletion mediated by Arg-I activity (Angulo et al., 2000; Goni et al., 2002; Rodriguez et al., 2004; Bronte and Zanovello, 2005; Zhu et al., 2007; Gabrilovich and Nagaraj, 2009). On the other hand, MDSCs were shown to have certain plasticity in response to the CNS microenvironment. Indeed, they behave as APCs when isolated at the onset of EAE, whereas they are clear MDSCs when studied at the peak EAE (Zhu et al., 2011). Furthermore, MDSC manipulation causes variations in the disease course since exposure to the retinoic acid analogue, Am80, delayed clinical recovery due to MDSC maturation (Moline-Velazquez et al., 2014). Indeed, activation of MDSC with the IFNγ/GM-CSF/LPS cocktail induces their polarization to a more suppressive phenotype and when transferred, these MDSCs markedly suppress the clinical course of EAE (Zhu et al., 2011).

In summary, the spatio-temporal distribution of MDSCs within the CNS parallels the clinical course of EAE (Fig. 2). These cells adopt different phenotypes and stages of maturation along the course of the disease. This is indicative of MDSCs fulfilling distinct biological activities depending on the microenvironment they encounter in the inflamed CNS, and at the different pathological stages of autoimmune diseases. As a result, the disease stage must be taken into consideration when MDSCs are to be exploited for disease interventions, undifferentiated MDSCs representing cells that may be interesting to target in future disease-modifying treatments for MS.

Neutrophils

Neutrophils are an essential component of the innate immune response known to play an important role in the first line of immune defense (von Vietinghoff and Ley, 2008). Evidence of the heterogeneity in the neutrophil repertoire is accumulating, identifying different subpopulations expressing different surface markers and fulfilling distinct functions (Amulic et al., 2012). The phenotype of neutrophils during EAE is mainly characterized by the expression of CD11b, Gr-1 (namely Ly-6G) and the specific neutrophil marker Ly-6B.2 (Rosas et al., 2010; Moline-Velazquez et al., 2011; Lee et al., 2013; Aube et al., 2014). The importance of their participation in EAE varies in function of the mouse strain and the antigen used for immunization. Neutrophils are the main component of CNS infiltrates when EAE is induced in BALB/c mice (Maatta et al., 1996), yet lesions contain only a small number of neutrophils when EAE develops in the sensitive SJL and Biozzi AB/H mice strains (Allen et al., 1993). By contrast, the severity of the clinical course of EAE is closely related to the importance of neutrophils within the inflammatory infiltrate. Thus, in the monophasic MOG-induced EAE in SJL/J mice, neutrophils are scarce within the small areas of demyelination that is associated with perivascular and meningeal cell infiltration in the CNS. Conversely, when animals receive a single dose of UV irradiation, a secondary progressive-like EAE course develops in one third of the mice, with large plaque-like demyelinating lesions formation that contain large numbers of macrophages and neutrophils (Tsunoda et al., 2005).

The target preference for neutrophil infiltration in EAE differs in function of the Th cell subset dominating EAE induction. In fact, it is well known that in C57/BL6 mice, adoptive Th1 or Th17 transfer produces a massive CNS invasion of immune cell infiltrates with a monocyte cell derived or neutrophil preponderance, respectively (Herges et al., 2012). Additionally, the spatial-temporal infiltration of neutrophils into the CNS differs during the clinical course of EAE (Fig. 2). Ly-6G$^+$-neutrophils appear in the perivascular meningeal and subpial spaces of the spinal cord in CP-EAE mice at the asymptomatic stage, mainly located in the ventral and lateral spinal cord WM, and rarely in the dorsal funiculi (Soulika et al., 2009). However, at onset and at later time points, Ly-6G$^+$-neutrophils are a much less prominent component of the spinal cord inflammatory infiltrates, which are increasingly dominated by Iba1$^+$ monocyte-derived cells (Soulika et al., 2009). In the same model, Ly6B.2$^+$-neutrophils are mainly localized in the meninges of the spinal cord during the pre-onset and onset stages, whereas they invade the spinal cord parenchyma at peak EAE. At the end of the spontaneous recovery period and in the chronic stage, the abundance of neutrophils decreases dramatically, and they become restricted to the sub-pial zone and the lateral funiculi of the spinal cord (Wu et al., 2010).

The close proximity of areas with prominent axonal degeneration and areas containing infiltrated neutrophils during the early and acute phases of EAE suggests that these granulocytes are one of the factors contributing to the acute damage of myelin and axons (Soulika et al., 2009; Wu et al., 2010). As neutrophil depletion delays or prevents the development of the disease (McColl et al., 1998), they would appear to participate in the pathogenesis of EAE possibly in two ways: by directing T-cell responses through the secretion of chemokines that attract DCs or T cells to the CNS (Kalyan and Kabelitz, 2014); and by disrupting the BBB through the activation of MMPs, proteases and gelatinases (Wojkowska et al., 2014). In this respect, by following the permeability of fluorescent vascular tracers using two-photon intra-vital imaging, it became evident that BBB disruption in EAE was correlated with the infiltration of mature granulomyelomonocytic cells, at least in the spinal cord (Aube et al., 2014). Alternatively, a role for neutrophils in the suppression of T cell responses cannot be ruled out, since they can release Arg-I from gelatinase-containing granules, thereby depleting the extracellular L-arginine that is essential for T-cell activation (Kalyan and Kabelitz, 2014).

Distribution of Myeloid Cells in the CNS of MS patients

The distribution, phenotype and activation state of the different myeloid cell types have been studied in the context of MS, albeit in a much less profound manner than in the animal models of the disease. Here, we summarize the main findings obtained from the histopathological analysis of autopsy or biopsy tissue, but not from the analysis of cerebrospinal fluid (CSF) from living or autopsied MS patients and control subjects (a summary of the main markers and the distributions of the different myeloid-derived cells is presented in Table 2).

Macrophages

Interest has been renewed in the histopathology of MS plaques in recent years. MS plaques can be subdivided into different histopathological subtypes according to features such as their myeloid cell distribution, especially HLA-DR$^+$-macrophages (Trapp et al., 1999; Chang et al., 2002; Frohman et al., 2006; Benito et al., 2007; Koning et al., 2007; Breij et al., 2008; Chang et al., 2008; Young et al., 2008; Clemente et al., 2011). Initially, active plaques exhibit abundant and evenly distributed HLA-DR$^+$-cells (mostly large, round, lipid-laden macrophages), although when phagocytotic macrophages contain myelin protein debris the plaques are considered to be more recently formed. Chronic-active plaques are characterized by a much lower cell density in the almost completely demyelinated inner area of the lesion, but with an enrichment of HLA-DR$^+$-lipid-laden macrophages at the border, or what is

commonly denominated the periplaque or rim. Chronic-inactive lesions have a clear, sharp edge, and they contain very few HLA-DR$^+$-cells. This macrophage distribution, or that defined by other common macrophage and microglia markers (F4/80, CD68), does not unravel the origin of the macrophages detected within the different types of demyelinated plaques. However, it was estimated that 30-50% of this intermingled population is derived from the microglia in active demyelinated plaques (Trebst et al., 2001). Similar studies have yet to be performed on chronic lesions.

The HLA-DR immunoreactivity of macrophages within MS lesions indicates these cells adopt APC activity in the inflamed CNS (Fabriek et al., 2005; Zhang et al., 2011). However, to be considered "professional" APCs, macrophages must express all the machinery needed for antigen presentation, i.e. the co-stimulatory molecules CD40, CD80 and CD86. Perivascular and foamy CD163$^+$-M2 macrophages within active and chronic-active lesions present signs of antigen presenting activity since they contain DC-SIGN, a marker of antigen capture by DCs, as well as CD40, CD80, CD86 and HLA-DR (Fabriek et al., 2005; Zhang et al., 2011; Vogel et al., 2013). In MS tissue, several attempts have been made to identify M1/M2 activity states similar to those described in mice. The most commonly used M1 markers of human macrophages include CD40, CD86, FcγRI (CD64) and FcγRII (CD32), while the mannose receptor (MR; CD206) and CD163 have been used to identify human M2 macrophages (Verreck et al., 2004, 2006; Zeyda et al., 2007; Mosser and Edwards, 2008; Ambarus et al., 2012; Durafourt et al., 2012; Miron et al., 2013). Accordingly, macrophages in inflammatory MS lesions express specific M1 markers, such as iNOS and CD40 (Gerritse et al., 1996; De Groot et al., 1997; Miron et al., 2013), being iNOS- and MR-containing macrophages most prevalent in active lesions and in the periplaque or chronic-active lesions, while MR macrophages are sparse in areas of complete remyelination (Miron et al., 2013). Furthermore, the presence of macrophages within active lesions and in the periplaque of chronic-active MS lesions is associated with that of the most important anti-inflammatory cytokines (IL-4 and IL-10) and their receptors (IL-4R and IL-10R, respectively: Cannella and Raine, 1995; Hulshof et al., 2002). This confusion regarding the presence of M1/M2 activation markers on macrophages and the observation of antigen-presenting activity on CD163$^+$-M2 macrophages (Fabriek et al., 2005; Zhang et al., 2011) has recently been clarified (Vogel et al., 2013). While foamy macrophages and microglia in active and chronic active lesions predominantly express M1 markers, the majority (approximately 70%) of CD40-positive macrophages also express the typical M2 marker, MR. This clearly indicates that the M1 and M2 activation states are dynamic and that they can fluctuate during plaque evolution. Moreover, it also appears that the information derived regarding the M1/M2 activation states in mouse EAE models will be difficult to translate to human macrophages in MS tissue.

Dendritic cells, MDSCs and neutrophils

In the healthy human CNS, DCs commonly

Table 2. Myeloid cell distribution and histological markers in multiple sclerosis tissue.

Cell type	Tissue localization	Abundance	Tissue markers	Bibliography
Macrophages	Meninges	+++	Phenotypic markers: CD68, F4/80 Activity state markers (1) HLA-DR, CD40, CD80, CD86, iNOS (M1), MR (M2), IL-4, IL-4R, IL-10, IL-10R, CD163	Breij et al., 2008; Cannella and Raine, 1995 de Groot et al., 1997; Clemente et al., 2011 Fabriek et al., 2005; Fhroman et al., 2006 Gerritse et al., 1996; Hulhsof et al., 2002 Trapp et al., 1999; Miron et al., 2013 Trebst et al., 2001; Vogel et al., 2013 Young et al., 2008; Zhang et al., 2011
	Perivascular cuffs	+++		
	Active lesion	+++		
	Chronic-active lesion (periplaque)	+++		
	Chronic-active lesion (plaque)	+		
	Chronic-inactive lesion	-		
Dendritic cells	Meninges	++	Immature: CD1c Intermediate: CD209 Mature: fascin, CD83	Plumb et al., 2003 Greter et al., 2005 Seraffini et al., 2006 Henderson et al., 2009
	Perivascular cuffs	++		
	Parenchyma	+		
G-MDSCs	ND	ND	HLA-DR2-CD14-CD33+CD15+(2)	Gabrilovich et al., 2012
M-MDSCs			HLA-DR2-/lowCD14+CD33+CD15-(2)	
Neutrophils	Absent (3)	-	Elastase	Aubé et al., 2014

G-MDSCs: granulocytic-myeloid-derived suppressor cells; HLA-DR: human leukocyte antigen-DR (MHC-II); M-MDSC: monocytic-myeloid-derived suppressor cells; MR: Mannose receptor; ND: Not determined; (1) Markers of M1/M2 activity are not well defined on MS macrophages; (2): markers for MDSCs in cancer; (3): Aubé et al. (2014) described very scarce cells in perivascular cuffs of one particular MS patient: +++, high abundance; ++, moderate abundance; +, low abundance; -, absence.

considered as the "professional" APCs of the immune system, can only be found in the meninges and choroid plexus (Matyszak and Perry, 1996; McMenamin, 1999). In contrast to all other body tissues, the brain parenchyma is devoid of typical DCs (Plumb et al., 2003). A few resident DCs have been reported within the perivascular space of non-inflamed tissue, leading to the idea that they may be the initial APCs recognized by invading encephalitogenic T cells (Greter et al., 2005). In a potent inflammatory response, such as that which occurs in MS, DCs can be detected in the CNS, either associated to discrete rare perivascular cuffs or in the meningeal space (Plumb et al., 2003; Greter et al., 2005). The maturation state of DCs in the different types of demyelinating plaques has also been explored in MS tissue, and whereas $CD1c^+$-immature DCs are almost absent in MS lesions, the intermediate differentiation state marker DC-SIGN (CD209) can be observed in cells containing myelin components in the perivascular cuffs of early active and chronic (both active and inactive) MS lesions. Mature DC markers, such as fascin or CD83, are detected less often in perivascular cuffs or in the leptomeningeal spaces of MS tissue (Plumb et al., 2003; Greter et al., 2005). To function as APCs in the MS brain, DCs should be in close contact with T cells in the same perivascular cuffs and intermingled with MHC-II immunoexpressing cells, something that has been described at distinct stages of MS disease evolution (Serafini et al., 2006) and in newly forming demyelinating plaques (Henderson et al., 2009). In summary, in the normal brain it appears that DCs lie close to blood vessels, yet in MS lesions, when self-antigens are released due to continuous myelin destruction, DCs proliferate or are recruited to the CNS. Once these DCs mature, they may contribute to the local activation and expansion of presumably pathogenic T cells.

To date, there is no data at all about the presence of MDSCs in MS tissue. G-MDSCs with T cell suppressive activity have been detected in the blood of RR-MS patients, being more abundant during the active rather than the recovery phase of the relapse (Ioannou et al., 2012). However, it remains unclear whether MDSCs migrate towards the CNS parenchyma and exert similar roles as in EAE. In the blood of MS patients and in many cancer studies, human G-MDSCs are classified as $HLA\text{-}DR2^{-/low}CD14^-CD33^+CD15^+$ (Gabrilovich et al., 2012), which probably implies that a new set of cell markers should be considered for MDSC studies in human CNS tissue.

Compared to other innate immune cells, such as DCs and monocytes, and in contrast to what has been described in EAE, the role of neutrophils in MS is still a matter of debate. Indeed, the participation of neutrophils in the pathogenesis of MS has not been examined extensively and the scarce data available are contradictory (Aoki et al., 1984; Guarnieri et al., 1985; Naess et al., 1986; Podikoglou et al., 1994; Ziaber et al., 1998; Naegele et al., 2012). It is worth noting that neutrophils exert an important role in the pathogenesis of neuromyelitis optica (NMO), a demyelinating disease of selective areas of the optic nerve and spinal cord in which the humoral response fulfills a crucial role (Lucchinetti et al., 2002). However, differences between NMO and MS have been observed in terms of granulocyte activity in the CSF, and while such cells are present in the CSF of NMO patients they are virtually absent from the CSF of MS patients (Jurynczyk et al., 2015). To our knowledge, only once have occasional $elastase^+$-neutrophils been reported at hyperacute MS lesions, in close association to focal breaches in the BBB, and they are not present in the CNS parenchyma (Aube et al., 2014). However, the extreme specificity of the particular pathological case (a MS patient affected by acute and severe relapse episodes following cessation of natalizumab therapy) means this cannot be accepted as definitive evidence of the histopathological role of neutrophils in the pathogenesis of demyelination in MS.

Concluding remarks

Studying the distribution and activity of myeloid cells is a great challenge for modern scientists, since they not only behave as the main triggers in the pathogenesis of MS but they also control the extent of tissue damage. Indeed, the classic classification of myeloid cells is continuously changing to accommodate their multiple phenotypes and variable states of activation. Indeed, and in parallel to the primitive classification of T cells as Th1 (pro-inflammatory) and Th2 (anti-inflammatory), or activator versus regulatory cells (Th versus Treg), the mature myeloid cell classification has generally followed a similar scheme, discriminating between M1 and M2 macrophages (Shechter and Schwartz, 2013), inflammatory and regulatory DCs (Zozulya et al., 2009; Quintana et al., 2015), and even N1 and N2 neutrophils (Sionov et al., 2015). Whether this classification is derived from incomplete information and whether such activation states exist in pathological/physiological conditions are questions beyond the goal of this review. Probably, both of these issues reflect a much more complicated situation, and these cells can shift between different states in pathological conditions or, at distinct phases during the development of a given disease.

EAE is one of the most used and useful animal models to analyze many pathological aspects of MS and yet, as clearly evident in this review, it far from truly reflects all aspects of the pathological scenario associated with MS. In relation to myeloid cells: i) the large-scale neutrophil invasion of the CNS in EAE has not been observed in MS tissue; ii) it is difficult to translate the observed EAE M1/M2 activation phenotypes to MS tissue, in part due to the absence of suitable histopathological markers; and iii) the role of DCs in EAE and MS is unclear, yet the markers, activity states and histopathological distribution are dissimilar in mice and human tissue (Sriram and Steiner, 2005; Baker

et al., 2011). These differences between the animal model and the true scenario in MS do not convert EAE into a useless animal model. Quite the contrary, all the dispensable drugs on the market have been previously tested in EAE and future treatments for MS must be evaluated in this model as a pre-requirement for entering clinical trials (Croxford et al., 2011; Robinson et al., 2014; Zhang et al., 2015). However, such differences should be an incentive for researchers to look for new animal models, including genetically-designed models, with closer histopathological features to the human disease, thereby ensuring that the drug mechanisms implicated in animals are as similar as possible to those in humans.

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