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Antigen presentation in EAE: role of microglia, macrophages and dendritic cells

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1.ABSTRACT

Experimental autoimmune encephalomyelitis (EAE), a well-established model of multiple sclerosis, is characterised by microglial activation and lymphocytic infiltration. Lymphocytic activation through the antigen presentation process involves three main signals, the first provided by the engagement of major histocompatibility complex molecules (MHC) with the receptor of T-cells (TCR), the second by the binding of co-stimulatory molecules and the third by the secretion or expression of T-cell polarising molecules in specific populations of antigen presenting cells (APC). Microglial cells are considered to be the main APC population in the central nervous system (CNS). Specifically in EAE an increase in MHCs, co-stimulatory molecules and different T-cell polarising factors have been reported in microglia. However, a growing number of evidences suggest that dendritic cells (DCs), the main APC in the peripheral immune system, may also participate in the regulation of T-cell responses within the CNS. In this review we summarize the principal knowledge regarding microglial/macrophage function in EAE and their role in T-cell modulation, as well as the participation of DCs in the immune response associated to this disease.

2. INTRODUCTION

Microglial cells are considered to be the innate immune cell population in the central nervous system (CNS) (1-3). These cells originate from highly proliferative blood-borne myeloid cells infiltrating the brain parenchyma during foetal and early post-natal development to become amoeboid cells that, later, transform into the ramified microglial cells observed in adult animals (4-10). At least two subsets of microglial cells are nowadays recognised in adult CNS: 1) the so-called "resting" microglia, which are ramified cells distributed in all grey and white matter areas of the parenchyma, and 2) the so-called perivascular microglia (also called perivascular macrophages), which represent a minority population specifically located in the perivascular space of blood vessels (11). Ramified microglia are a permanent population of cells with a low turnover (12), with a very low CD45 expression and no expression of MHC-class II (13). In contrast, perivascular microglia/macrophages are periodically replaced (14), do not present the characteristic prolongations of microglia and express high levels of CD45, MHC-class II (15) and ED2 (16, 17). Due to their strategic location, these perivascular cells seem to play a key role in the initiation of immune responses in the CNS (11).

Over the years, numerous studies have demonstrated the fundamental role played by microglial cells in the CNS, not only in normal conditions where they control tissue homeostasis (18, 19), but also in all of those situations in which the integrity of the tissue is disturbed, as a result of a wide variety of situations including lesions, neurotoxicity or infections (20-23). In these circumstances, microglial cells are activated, showing specific reactivity patterns that fully depend on changes that take place in the specific micro-environment where they are located as well as the magnitude and type of injury. The process of microglial reactivity involves changes in their gene activation and phenotype, manifested in morphological modifications, increase/decrease or *de novo* expression of surface molecules and secretion of a wide range of substances such as cytokines, chemokines and trophic factors (21, 22, 24-26). Nowadays, this great variety of changes, coupled with increasing evidence suggesting that different microglial subpopulations can co-exist within the CNS (27), indicates that microglial reactivity cannot be considered as homogeneous, but rather as a heterogeneous process that may have different outcomes according to where, how and what population of cells are activated.

Microglial cells not only interact with resident CNS cells as neurons or other glial cells, but are also able to establish a cross-talk with cells of the immune system that can be recruited to the CNS parenchyma under inflammatory conditions, through the production and secretion of the different molecules mentioned above. In particular, accumulating evidence *in vitro* and *in vivo* suggests that microglial cells may act as antigen presenting cells playing a role in the modulation of lymphocyte activation.

3. ANTIGEN PRESENTATION MECHANISM

Antigen processing and presentation is a crucial mechanism in the modulation of the immune response, by which foreign molecules or intracellular antigens are processed and presented for recognition by T cells, thereby inducing their activation. In general, intracellular antigens, such as those produced by viruses, defective self-molecules or tumour-associated antigens, are presented by the major histocompatibility complex class I (MHC-class I). In contrast, antigens from extracellular pathogens, such as bacteria, parasites and toxins, are presented in the context of MHC-class II molecules. Whereas all nucleated cells express MHC-class I, only specialised cells, the antigen-presenting cells (APCs), such as dendritic cells (DCs), macrophages and B cells, in addition to expressing MHC-class I have the appropriate machinery for the processing and presentation of extracellular antigens through the MHC-class II molecules.

It has been shown that two main signals are involved in the mechanism of antigen presentation. The first signal is provided by the binding between MHC molecules bearing the antigen, with the receptor of T-cells (TCR) present on the surface of T-lymphocytes. This signal confers the specificity of the mechanism, as MHC-class I is specifically recognised by CD8+ T-lymphocytes, whereas MHC-class II exclusively binds CD4+ T-cells. The second signal, the so-called co-stimulatory signal, is antigen-independent and is produced by the engagement of different receptors and their respective co-receptors expressed on the surface of both APCs and lymphocytes (28). The presence of these co-stimulatory signals has been demonstrated to be essential for the full activation of T-cells, as binding of the TCR with the MHCs in the absence of co-stimulation can lead to apoptosis or anergy of T-cells (29). Different combinations of co-stimulatory molecules providing stimulatory or inhibitory signals have been described (30). For example, the binding of ICOS, CD154 or OX40 with their corresponding ligands B7h, CD40 and OX40L delivers a stimulatory signal in lymphocytes, inducing their activation and proliferation, whereas the engagement of other co-stimulatory molecules like PD-1, with its receptor PD-L1, triggers an inhibitory signal causing the apoptosis of lymphocytes. Among the different co-stimulatory molecules currently described, the pair that plays a more relevant role in T-cell activation is the one formed by B7 molecules (B7.1 and B7.2, also known as CD80 and CD86), present in the APC surface and their receptors CD28 and CTLA-4 expressed in lymphocytes (31-34). Binding of B7.1 or B7.2 with CD28 generates a stimulatory signal in T-cells inducing their proliferation, expression of anti-apoptotic molecules and secretion of different cytokines. In contrast, the binding of B7.1 and B7.2 with CTLA-4 results in an inhibitory signal that mediates the stoppage in proliferation and cytokine secretion, leading to the end of the immune response (32, 35). Therefore, the delicate balance between positive and negative signals conducted by different co-stimulatory molecules may provide different outcomes of the immune response (See Figure 1 for a summary).

In addition to these two well-known signals, a growing body of evidence indicates the existence of a third type of signal by which APCs, particularly dendritic cells (DCs), may also regulate the immune response. This third signal consists of the expression of specific sets of T-cell polarising molecules either soluble or membrane-bound (see Figure 2). In general, Type 2 IFNs, IL12 and ICAM-1 are considered to be Th1 polarising molecules; IL4, MCP-1 and OX40L are Th2 polarising molecules; IL10, PD-L1 and ILT3/4 are viewed as regulatory T-cell polarising molecules; and TGF-beta and/or IL6 as polarising molecules for Th17 and inducible T-regulatory cells (36-38).

The selective expression profile of all of these polarising molecules is fully dependent on the signals that the DCs receive during their maturation process (Figure 2). The binding of pathogens to selective "pattern recognition receptors" or factors produced by tissue cells in response to these pathogens induce different signalling in immature DCs that promotes the specific expression and production of these polarising factors. Basically, DCs exposed to viruses or intracellular bacteria promote Th1 responses, some helminths induce Th2, and the presence of regulatory factors or specific pathogens such as *Schistosoma mansoni* support the development of T-regulatory cells (39).

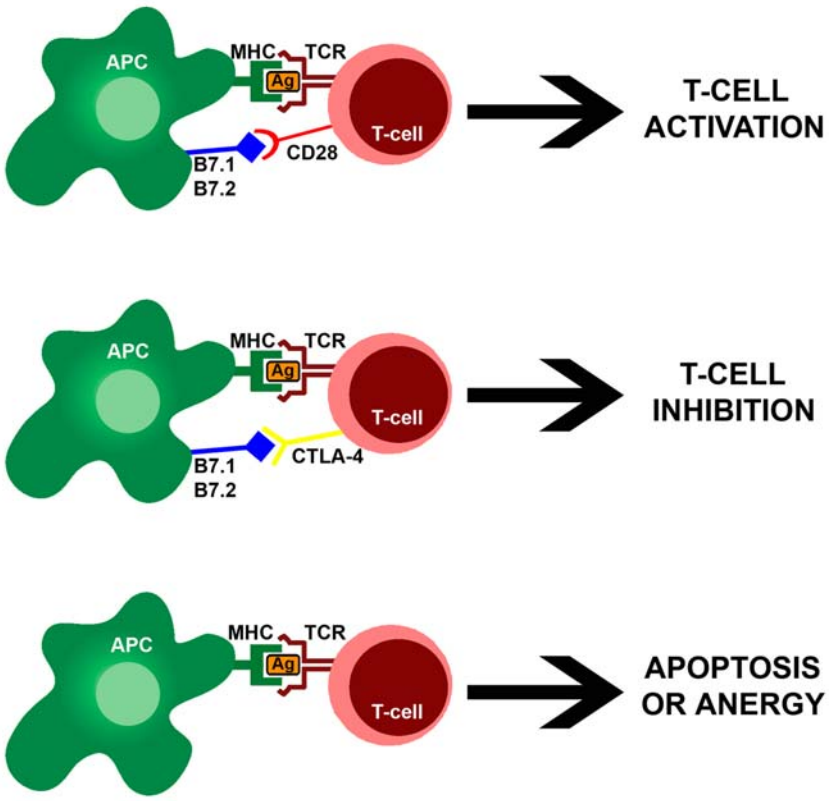

Figure 1. Putative effects derived from the interaction between antigen presenting cells (APC) and T-cells.

4. ANTIGEN PRESENTING CELLS

In general terms, it is assumed that those cell populations that have the capacity to process and present antigens and display them to T-cells are APCs. DCs are considered to be the most specialised APC type in the periphery. They are generated in the bone marrow and migrate as precursor cells to sites considered as potential points of entry for pathogens. Thus, there are subsets of resident DCs in the skin, in the gastrointestinal and respiratory tracts and in lymphoid organs which can be distinguished by the expression of different cell-surface markers that are not only dependent on the specific organ but also on the animal species (40-42). In steady-state situations, DCs display an immature phenotype (43, 44) characterised by: 1) expression of specific receptors and molecules such as Fc- and mannose-receptors whose presence confers to them a high capacity to phagocytose or endocytose antigens, and 2) high expression of MHC molecules, but low or absent expression of co-stimulatory or other molecules involved in T-cell activation. This phenotype renders these immature DCs able to process antigens but not to activate T-cells. Immature DCs are, however, not inactive cells but they rather continuously circulate through tissues and into lymph nodes capturing self-antigens as well as innocuous environmental proteins, playing an active role in the maintenance of tolerance (45). Under inflammatory conditions, immature DCs become activated and differentiate into mature DCs. This maturation process involves the down-regulation of molecules related to the antigen capture and processing mechanism, and the up-regulation of the antigen presenting machinery, including an increase or *de novo* expression of co-stimulatory molecules (43). Moreover, mature DCs undergo changes in their expression pattern of chemokine receptors that allow them to migrate to the lymph nodes where they activate T-cells. For instance, DCs decrease molecules related to their tissue homing such as CCR5 and on the other hand, increase CCR7 expression, a molecule involved in their migration to the nearest lymph node.

In addition to DCs, macrophages and B-lymphocytes can also act as APCs (46). They are considered non-professional APCs because, in contrast to the professional DCs, they cannot activate naïve T-cells and they do not express MHCs constitutively. Nevertheless, they have the necessary machinery to capture and process antigens, and under inflammatory conditions they increase MHC expression, becoming able to activate T-cells.

For many years it has been assumed that there were not DCs in the CNS. This, together with the presence of the blood brain barrier, the lack of lymphatic vessels and the fact that skin grafts, viruses, bacterias or antigens directly inoculated in the parenchyma do not induce an immune response (47-50), contributes to the initial views of the CNS as an immune-isolated site. In this context, microglial cells were considered to be incompetent immune

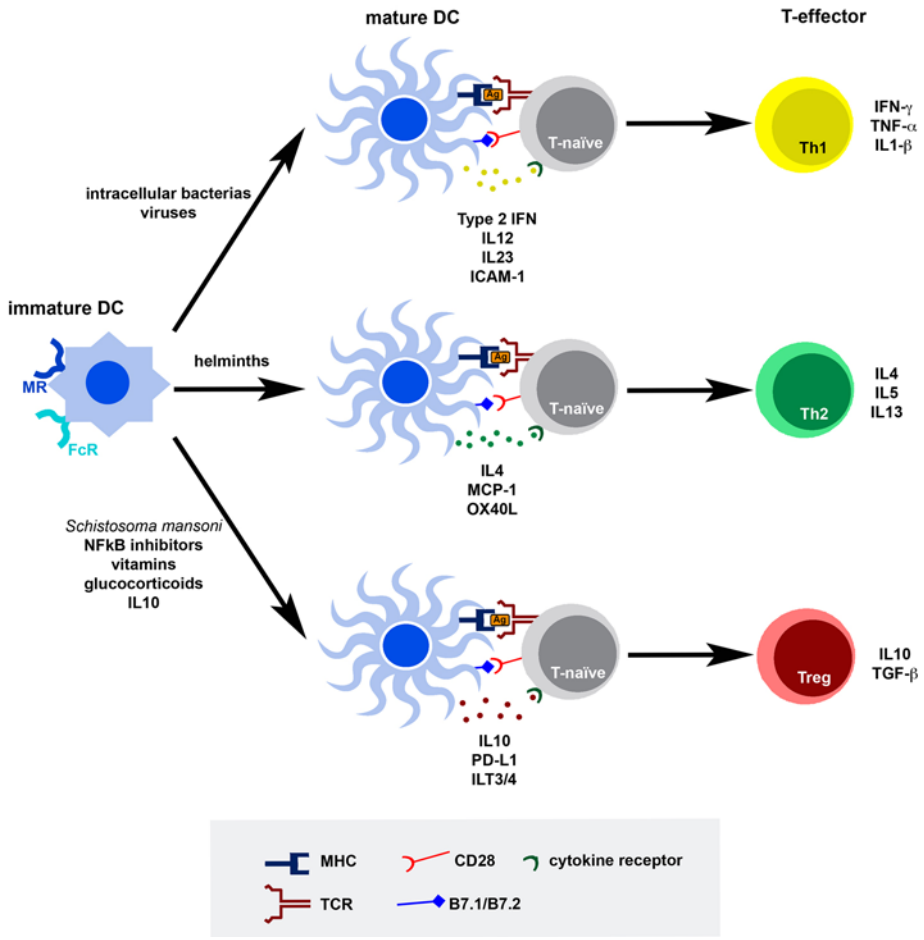

Figure 2.Summary of signals leading to the maturation of immature dendritic cells (DCs) into different subtypes of mature DCs. These mature DCs secrete different T-cell polarising molecules that regulate the transformation of T-naïve cells into T-effector cells.

cells. However, during the last decade, the capacity of microglial cells to interact with immune cells recruited into the CNS parenchyma under several situations has been widely reported (3, 23, 51, 52), suggesting that microglia may be considered as being APCs in the CNS and under certain circumstances they differentiate into DC-like cells. Furthermore, as shall be discussed in detail later, in addition to DCs of a microglial origin, an increasing number of evidence suggests that specific subpopulations of bone marrow-derived DCs may reach the CNS parenchyma under certain inflammatory conditions, such as focal cortical ischemia (53), stroke (54), excitotoxic lesion (55), delayed type-hypersensitivity lesions (56) or experimental autoimmune encephalomyelitis (15, 56).

5. EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

One of the most useful animal models for the study of the interactions established between CNS resident and peripheral immune cells is experimental autoimmune encephalomyelitis (EAE), which is a commonly used model of human multiple sclerosis (MS) although it may not represent all pathophysiological aspects of the human disease. EAE is characterised by progressive muscle weakness and paralysis starting in the tail and hindlimbs and may eventually lead to paraplegia or even tetraplegia. Associated with this clinical symptomatology, animals induced with EAE experience a progressive and significant loss of weight, mimicking what also happens in MS. There are several models of EAE with particular symptomatological and histopathological features that reproduce different forms of human MS (57, 58). Thus there are models, such as that induced by myelin oligodendrocyte protein (MOG) immunisation in C57BL/6 mice, which resemble a form of chronic MS; others, such as that induced in SJL mice by proteolipid protein (PLP) injection, which reproduce relapsing-remitting MS, which alternate stages of paralysis with stages of recovery, and also some models like that caused in Lewis rats by myelin basic protein (MBP) immunisation, which mimic an acute phase of MS characterised by a single peak of disability followed by a full and spontaneous recovery. Multiple factors including the species, strain, sex and age of animals used, as well as the peptide and the protocol utilised in the immunization process, seem to be crucial to yield the

specific type of EAE. Susceptible animals, mainly rodents, can be induced by: 1) active immunisation, which consists of the subcutaneous injection of an emulsion containing nervous tissue or any peptide of myelin proteins together with a coadjuvant, or 2) passive immunisation, based on the intravenous injection of T-lymphocytes activated against myelin proteins.

EAE primarily affects the white matter of the spinal cord although today it has also been reported that the spinal cord grey matter and other CNS areas such as the cerebellum and the brainstem are affected (59, 60). As occurs in human MS, the main histopathological features of EAE are glial reactivity, mainly microglial activation, and a large infiltration of peripheral immune cells, principally T-cells (3, 24, 61). Due to the autoimmune nature of EAE and MS, it was widely accepted that CD4+T cells with a Th1 pro-inflammatory phenotype were the principal lymphocytes involved in the disease (62). Over the last few years, however, the identification of different subsets of lymphocytes with varied functions and cytokine profiles (63-65), have led to the re-evaluation of this initial assumption. It has been demonstrated that Th17 lymphocytes, a subtype of CD4+ T-cells (66, 67), are able to induce EAE when injected into mice (68). It has also been shown that Th17 cells are present in the spinal cord at the onset of EAE in mice (69), suggesting a pathogenic role of these lymphocytes. On the other hand, subtypes of regulatory cells, such as the extensively studied Foxp3 T-regulatory cells (Treg), are also reported in EAE. Treg cells have been detected during recovery in mice (70-72), and their beneficial role in EAE has been clearly demonstrated (71, 73, 74). Furthermore, in addition to CD4+ T-cells, some authors have also suggested the participation of other types of lymphocytes, such as CD8+ T-cells and gamma-delta T-cells (75-78).

6. MICROGLIA, MACROPHAGES AND MONOCYTES IN EAE

Microglial reactivity has been reported in different models of EAE (79-85). The major part of these studies has focused on the peak of the disease, where maximal levels of microglial activation were found. However, only few reports have analysed the temporal pattern of microglial reactivity along the course of EAE. Specifically in acute EAE, microglial reactivity has been detected in close correspondence with the increase in clinical symptomatology along the inductive and peak phases of the disease (85). Despite the large amount of literature describing microglial reactivity in EAE and human MS, the exact function played by these cells in these diseases still remains to be established. On the one hand, the observations of maximal microglial reactivity at the peak of the disease, together with the fact that the decrease in microglial reactivity, induced by treatments such as macrophage inhibitor factor (MIF) (86) and MW01-5-188WH (87) or observed in microglia-depleted animals (88), has been associated with beneficial effects in EAE, led to the perception of microglia as detrimental cells in EAE pathogenesis. Moreover, it has been reported that activated microglia can release a wide range of molecules such as cytokines, chemokines and nitric oxide which may contribute to the recruitment of immune cells and the spread of the inflammatory response in the CNS (2, 24).

On the other hand, it has also been reported that during EAE remissions, despite the gradual recovery of animals, microglial cells remained very reactive in both mice (84) and rat EAE models (85), suggesting a putative beneficial role of these microglial cells. Microglial reactivity has also been described in EAE-resistant rats (89) and in normal-appearing white matter in human MS (90). Furthermore, earlier recovery from EAE symptoms has been reported in mice administered with tufsin, a microglial/macrophage activator (86). In this sense, the capacity of microglial cells to also produce protective factors such as anti-inflammatory cytokines, prostanoids and trophic factors that may contribute to the termination of the inflammatory response in the CNS, has been extensively demonstrated (2, 24).

In addition to morphological changes, an increase in the number of reactive microglia/macrophage cells in both chronic (81) and acute EAE (85) has been reported. This increase could be due to resident microglial cell proliferation or it could be the result of the recruitment of blood-borne monocytes, a population whose infiltration along the course of EAE has been described (91-93). The lack of good markers to distinguish between macrophages coming from the monocytic lineage and reactive macrophage-like microglial cells makes the analysis of the contribution of infiltrated monocytes to the final number of microglial/macrophage cells difficult.

Some evidence indicates that besides microglia, the perivascular macrophage population may also play a role in the pathogenesis of EAE. They are commonly distinguished from microglial cells by their specific expression of ED2 in rats (16) and its counterpart CD163 in humans (17). Data in the acute EAE model induced in Lewis rat has reported an increase in the number of ED2+ cells before the appearance of clinical signs (94), which is in close relationship with the progressive increase in EAE symptomatology (85). Moreover, these ED2+ cells infiltrate the CNS parenchyma specifically at the peak of the disease (85). Due to their strategic location and their constitutive expression of MHC-class II (15), perivascular macrophages seem to be involved in the initiation of the immune response associated with EAE, perhaps contributing to T-cell activation, a step required for the subsequent infiltration of T-cells into the parenchyma (95, 96). Indeed, experimental evidence based on the selective elimination of perivascular macrophages by the use of chlorodate lyposomes (97) revealed that their depletion produced a decrease in the symptomatology (94), reinforcing the idea of an active participation of these cells in EAE evolution.

7. MICROGLIA REGULATE THE LYMPHOCYTE RESPONSE

Infiltration and retention of T-cells in the CNS requires their interaction with local APCs (95, 96). As

microglial cells are currently considered to be the principal APC within the CNS parenchyma (2, 24), it is assumed that, in the CNS, they may play a key role in modulating the lymphocyte response including their proliferation, differentiation and apoptosis through the antigen presenting mechanism. Although in basal conditions, microglia do not express MHC-class I and MHC-class II, it has been shown that once activated they can rapidly express these molecules in a variety of situations (1, 98).

Specifically in EAE, it has been widely reported that when microglial cells become activated, they increase the expression of MHC molecules in both mouse (81, 99-101) and rat models (102-106). The role played by these MHC molecules in EAE showed controversial results. On the one hand, some studies pointed towards a detrimental role, as it has been reported that some treatments with a positive effect in EAE were associated with a decrease in MHC-class II expression (107, 108). However, on the other hand, a growing number of evidence indicated a putative beneficial effect of these molecules. It has been shown that MHC-class I KO mice present aggravation of EAE symptoms (109, 110) and MHC-class II KO showed impairment of remyelination after Theiler's murine encephalomyelitis (111). Interestingly, in the acute EAE model induced in Lewis rats, the expression of both MHC-class I and class II molecules increased progressively during the inductive and peak phases correlating with the increase in clinical symptomatology, but levels of MHC expression remained high during the spontaneous recovery phase, even in animals that completely recovered and do not show any symptomatology (15).

At this point, it is important to highlight that MHC expression *per se* is not sufficient for antigen presentation, and co-stimulatory signals are necessary for the complete activation of T-cells (28, 31). The involvement of co-stimulatory molecules in EAE evolution comes principally from studies showing the effects of the absence of these co-stimulatory molecules (112). Thus, a reduction of EAE severity has been correlated with the blockage of CD28 (113, 114) or B7 (115, 116), whereas the blockage of CTLA-4, the co-stimulatory signal that drives the ending of the immune responses, has been shown to produce a worsening of the disease (117).

The question of whether a specific pattern of MHC and co-stimulatory molecule expression governs the evolution of EAE remains unresolved, and so far it is not clearly known which are the cells that express these molecules. Differences in the expression of B7.1 and B7.2 have been detected among different models of EAE. Whereas in mouse models B7.1 has been commonly described during the relapses, being nominated as one of those responsible for the epitope-spreading phenomenon and the induction of clinical relapses (115, 118-120), no B7.1 expression has been detected at any phase of acute EAE in rats (15). In fact, in this acute EAE model, despite the high expression of MCH molecules in microglia and the infiltration of CD28+ T-cells, neither B7.1 nor B7.2 expression was found during the inductive and peak phases (15), leading the authors to suggest that this expression of MHCs without a concomitant co-stimulatory signal can mediate either the anergy or the apoptosis of infiltrating T-cells, as described in the peripheral immune system (29). Induction of T-cell anergy or apoptosis may, therefore, be the cause of the immune response resolution leading to the spontaneous clinical recovery (15). In fact, apoptosis of encephalitogenic T-lymphocytes has been widely described in EAE models (121-124).

In contrast, during the recovery phase the aforementioned study (15) revealed the expression of B7.2 in a subpopulation of microglial cells located in the vicinity of blood vessels. This pattern of expression of MHCs with B7.2, in the absence of B7.1, has been reported in reactive microglia in acute CNS injuries, such as facial-nerve axotomy (125), enthorinal-cortex lesion (126, 127) and cuprizone-induced demyelination (128), situations in which myelin destruction does not lead to autoimmunity. Furthermore, B7.2 expression has been described accompanied by an accumulation of cells expressing CTLA-4 (15), which is the B7.2 co-receptor that inhibits T-cell activation (129). CTLA-4 was found constitutively expressed in T-regulatory cells (130), a specialised subpopulation of T-lymphocytes that suppressed the activation of the immune system, whose principal function is the maintenance of immunological homeostasis and tolerance to autoantigens (For detailed reviews, see (131-133). The role played by CTLA-4 in T-reg activation is still not fully determined. However, it has been shown that signaling through this co-stimulatory molecule is necessary for the induction of Foxp3, the master transcription factor of Treg (134, 135), and for the activation of this population of lymphocytes (135-137).

Finally, it is also important to mention that an increasing number of reports suggests the involvement of other co-stimulatory molecules in EAE progression (138). CD40/CD40L molecules have been described in MS plaques in humans (139) and during relapses in mice with EAE (118). It has been shown that CD40/CD40L recognition is instrumental in the development of EAE, since EAE induction is prevented in CD40KO mice or by treatment of wildtype mice with antibodies to CD40L (139, 140). Other molecules whose expression has been involved in EAE are PD1/PDL1,2 and ICOS/ICOS-L. The involvement of PD1 and its receptors PDL1 and PDL2, in EAE, came from studies showing the upregulation of these molecules at the peak of the disease (141, 142). Some years later, the use of KO mice demonstrated that a deficiency in either PD1 or PDL1, but not PDL2, induces an increase in EAE severity (143), suggesting a putative beneficial role of this signalling in EAE progression. Moreover, recently it has been shown that EAE can be induced in PD1-deficient mice without the use of pertussis toxin (PTX), whereas in wild-type animals, PTX administration is essential for EAE development (144). Only few studies have addressed the role of signalling through ICOS and ICOS-L. These molecules may exert a beneficial role, as induction of EAE in ICOS-deficient mice results in severe disease (145). Nonetheless, treatment with anti-ICOS antibody suggested a dual role of ICOS in EAE: treatment during the first days post-induction (1-10 days post-induction) exacerbated EAE

severity, whereas administration during the effector phase (9-20 days post-induction) promotes a delay in onset and severity (146). However, no reports about ICOS expression in microglia or any other CNS resident cells are currently available.

8. DENDRITIC CELLS IN EAE: RECRUITED FROM THE PERIPHERY OR DERIVED FROM MICROGLIA?

Different subtypes of DCs showing specific expression of surface molecules are currently identified in both human and mouse (41, 147). They are continuously produced from stem cells located in the bone marrow and they migrate as DC-precursor cells to peripheral organs where they differentiate into specific populations of resident DCs, by the action of different factors such as GM-CSF (granulocyte-macrophage colony stimulating factor), Flt-3 and c-Kit, in an antigen-independent manner (148).

The presence of DCs within the CNS has been an issue of debate for many years. Nowadays the presence of these cells in selective areas of the healthy CNS, such as the meninges and the choroids plexus, is accepted (149, 150). Studies in recent years have reported the existence of specific populations of DCs within the brain parenchyma under inflammatory conditions (151, 152).

The presence of DCs within the CNS parenchyma in different models of EAE in rat (15, 56) and mouse (153-155) has been reported. The role played by DCs in the evolution of EAE is still controversial. Some authors point towards a detrimental role showing that intracerebral injection of DCs, pulsed *in vitro* with MOG, exacerbates EAE pathology (156). In addition, it has been reported that DCs are responsible for the immune infiltration in EAE (157) and promote the polarisation of lymphocytes towards Th17-pathogenic cells (155). However, some studies have reported that if DCs were previously cultured with TNF-alpha, a tolerogenic phenotype would be induced in these cells which, when injected, produced a decrease in EAE symptomatology (156, 158). These results suggest a beneficial role of DCs and indicate that further research is needed to clarify the exact role of DCs in CNS (159). In addition, the emergence of new subsets of DCs with regulatory cues, such as the tolerogenic DCs, opens new perspectives in the study of CNS DCs. Tolerogenic DCs are considered as an incomplete or modulated form of DC maturation, involved in peripheral tolerance via induction of anergy, apoptosis or Treg activation (160, 161). *In vitro* studies have demonstrated that certain cytokines such as IL10 and TFG-beta, some vitamins (1,25-dihydroxy vitamin D3, vitamin C), immunosuppressive treatments using glucocorticoids or NFkB inhibitors can generate a tolerogenic phenotype in DCs (162). In contrast to immature DCs, also considered as inductors of tolerance, tolerogenic DCs display a stable phenotype, which is resistant to further maturation. Characteristic features of these tolerogenic DCs have been described including the secretion of the anti-inflammatory enzyme indoleamine 2,3-dioxygenase (IDO), expression of MHC molecules but low levels of co-stimulatory molecules and expression of surface molecules involved in T-cell inhibition such as PDL1 or CD95L (163). All of these molecules contribute to the inhibition of T-cell proliferation and induction of apoptosis. Despite the large amount of information in the peripheral immune system on the phenotype, role and modulation of these tolerogenic DCs, little is known regarding the presence and participation of these cells in the CNS during EAE. Some evidence, nevertheless, raises the possibility that tolerogenic DCs can be involved in EAE evolution. The expression of IDO in EAE has been described in mouse, coinciding with the remission phase (164). IL10 and TGF-beta expression have also been detected during the remission stage (165, 166). Moreover, low levels of co-stimulatory molecules have been detected in acute EAE (15).

Another issue that remains to be elucidated is what the origin and the differentiation cues of CNS DCs are. Two different theories can be considered to explain the possible sources of these cells: differentiation from resident microglial cells or recruitment from the periphery. Regarding the putative microglial origin, *in vitro* studies have demonstrated the capacity of microglial cells to differentiate into cells with a DC phenotype after exposure to GM-CSF (13, 154). In addition, recent studies have shown the existence of a subpopulation of microglial cells that constitutively displayed CD11c in the healthy brain (167). In accordance with these observations, the expression of CD1, a marker commonly used for the identification of immature DCs (168), has been demonstrated in a subpopulation of microglial cells located in the vicinity of blood vessels during the course of EAE (15). In agreement with this idea, an increase in CD1 expression in CD11b+CD45low microglial cells and CD11b+CD45high macrophages has been reported during the peak of EAE in mouse by flow cytometry (169). If this theory of the microglial origin of DCs were true, microglial cells should be considered as a population of immature DCs residing in the CNS parenchyma, as already suggested by some authors (170).

Alternatively, it has also been proposed that CNS DCs can be recruited from the periphery. Although monocytes were initially described as the precursors of all of the subpopulations of tissue macrophages, it is now recognised that they can also differentiate into DCs. In steady-state conditions, monocytes are the precursor of tissue-resident DCs such as the Langerhans cells in the skin, but under inflammatory situations the differentiation of monocytes into monocyte-derived DCs has recently been described (171). The choice of monocytes to differentiate into DCs or macrophages is likely determined by the cytokine milieu and the signals that these cells received from the environment (172). The presence of Ly6C+ monocytes, the putative source of monocyte-DCs, has been recently reported in EAE in mouse (92, 173). Reinforcing the idea of a putative peripheral origin of the CNS DC population, recent findings have revealed the presence of a specific population of mature DCs, which does not correspond to microglia, within the CNS parenchyma (15). These mature DCs were characterised by the expression of fascin, an actin-bundling protein whose expression has been linked to DC maturation (174-176).

Taking into consideration all of these observations, the origin and function of DCs in CNS remain to be clarified and further research is warranted.

9. ACKNOWLEDGMENTS

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