

Brain Innate Immunity in the Regulation of Neuroinflammation: Therapeutic Strategies by Modulating CD200-CD200R Interaction Involve the Cannabinoid System

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Abstract: The central nervous system (CNS) innate immune response includes an arsenal of molecules and receptors expressed by professional phagocytes, glial cells and neurons that is involved in host defence and clearance of toxic and dangerous cell debris. However, any uncontrolled innate immune responses within the CNS are widely recognized as playing a major role in the development of autoimmune disorders and neurodegeneration, with multiple sclerosis (MS) and Alzheimer's disease (AD) being primary examples. Hence, it is important to identify the key regulatory mechanisms involved in the control of CNS innate immunity and which could be harnessed to explore novel therapeutic avenues. Neuroimmune regulatory proteins (NIReg) such as CD95L, CD200, CD47, sialic acid, complement regulatory proteins (CD55, CD46, fH, C3a), HMGB1, may control the adverse immune responses in health and diseases. In the absence of these regulators, when neurons die by apoptosis, become infected or damaged, microglia and infiltrating immune cells are free to cause injury as well as an adverse inflammatory response in acute and chronic settings. We will herein provide new emphasis on the role of the pair CD200-CD200R in MS and its experimental models: experimental autoimmune encephalomyelitis (EAE) and Theiler's virus induced demyelinating disease (TMEV-IDD). The interest of the cannabinoid system as inhibitor of inflammation prompts us to introduce our findings about the role of endocannabinoids (eCBs) in promoting CD200-CD200 receptor (CD200R) interaction and the benefits caused in TMEV-IDD. Finally, we also review the current data on CD200-CD200R interaction in AD, as well as, in the aging brain.

Keywords: Neuroinflammation, microglia, neurons, neuroimmunoregulatory molecules, CD200, CD200R, multiple sclerosis, aging brain, Alzheimer's disease, cannabinoids.

INTRODUCTION

Immune cells classically involved in innate responses include natural killer cells, neutrophils, dendritic cells and macrophages that participate in the selective recognition and the clearance of pathogens and toxic cell debris during infection or tissue injury [1, 2]. Nevertheless, these peripheral cells show little immunosurveillance of the brain, and it is now evident that resident cells, glial cells, ependymal cells and even neurons are capable of mounting innate immune responses on their own [3]. The local innate immune response is based on the recognition of non-self and altered self-patterns by molecules and receptors expressed by microglia but also present on astrocytes, oligodendrocytes and neurons [4, 5]. The outcome of these interactions is dependent on the microenvironment, as well as, on the nature of the ligands and on the nature and combination of the ligated receptors. From a therapeutic point of view, it is important to manipulate this innate immune response to promote clearance of pathogens or toxic proteins accumulated during brain injury or in central nervous system (CNS) diseases [5, 6]. However, several innate immune molecules can contribute to cytotoxic and cytolytic activities and must be controlled to avoid neuronal loss and excessive inflammation [7]. There is new emphasis on the role of neuroimmune regulatory proteins (NIRegs) involved in silencing and reshaping an adverse innate immune response such as, CD47 and CD200 expressed by neurons as "don't eat me" signals that inhibit microglial activity preventing host cell attack, besides to modulate microglial function and phenotype in situations of harmful and excessive inflammation [8]. Here, we present a review about CNS immunity and the modulation of CD200 and its receptor in multiple sclerosis (MS) and in the aging brain and Alzheimer's

disease (AD). We show original data on the effects of an inhibitor of endocannabinoids (eCBs) uptake in the regulation of CD200-CD200 receptor (CD200R) interaction in an experimental model of MS.

CNS INNATE IMMUNE RESPONSE IN THE RESTORATION OF BRAIN HOMEOSTASIS: ROLE OF MICROGLIAL CELLS

Microglia constitute the highly versatile resident macrophages in the CNS, comprising 5% to 20% of the total glial cell population [9]. As reviewed by Yang *et al.*, [10], in 1919, Ramón y Cajal's disciple, Pío del Río-Hortega recognised microglial cells as a different cell type from the other glial cells. He also characterised microglial transformation from a ramified phenotype into amoeboid phagocytic macrophage-like cells in the stab wounds made in animal brains [11]. From these observations, del Río-Hortega concluded that microglia originated from peripheral mononuclear cells [11].

Interestingly, and after a long debate in which even the existence of microglial cells was questioned [12], microglia has finally been established as a distinct glial cell population from a myelomonocytic origin [13]. Recently, it has been demonstrated that microglial cells are originated from a distinct myeloid precursor that migrates from the haematopoietic islands in the yolk sac to the developing brain parenchyma at embryonic day 8.5 [14].

Microglia are widely distributed in the adult CNS, but differences in their cellular density among different brain areas have been reported in mice [9, 15] and humans [16]. Thus, microglial cells are more abundant in the telencephalon than in the diencephalon or the mesencephalon, with the rhombencephalon containing the lowest amount of these cells [9, 17]. Not only there is an uneven distribution of microglia across the cerebral anatomical regions but between the grey and white matter (the latter containing higher mi-

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croglial cell density than the former). Moreover, cumulative data show that microglial expression of tissue macrophage markers varies within the different brain regions, as well as, their response to different stimuli [18, 19, 20].

In the adult healthy brain, microglia has been described to be in “resting” or “quiescent” state with a ramified morphology. However, this term has limitations and can lead to the misleading idea that microglia are dormant cells awaiting a signal that will wake them up. On the contrary, recent elegant *in vivo* imaging experiments show “resting” microglia as highly dynamic cells, continuously branching thin processes that survey and sample their micro-environment [21, 22] in search for potential threats and danger signals. Thanks to this routine immunosurveillance of the CNS, microglia will remove apoptotic bodies and other potentially toxic cellular debris (myelin debris, amyloid deposits, protein aggregates, etc) [23, 24, 25]. In order to accomplish this task, it is essential that microglial cells are capable of distinguishing between “self” and “nonself” signals (Fig. 1). Clearance of pathogens and toxic cell debris during infection or tissue damage is based on the recognition of “nonself” and “altered-self” patterns by microglia, but also astrocytes, oligodendrocytes and neurons have been shown to be able to recognise those patterns [1, 4]. According to Medzhitov and Janeway [1], there is a plethora of the so-called “eat-me” signals expressed by pathogens and apoptotic or necrotic cells. Some of these signals are a heterogeneous group of molecules known as pathogen-associated molecular patterns (PAMPs) and are characterised by being highly conserved through evolution with little antigenic variability [22]. These PAMPs are constituents of the microbial structure which induce in the host a strong innate immune reaction directed towards the removal of the pathogen by phagocytosis [26].

The classical example of a PAMP is the lipopolysaccharide (LPS), component of the Gram negative external membrane. Analogous to PAMPs, it has been proposed that cells undergoing programmed cell death express *de novo* apoptotic cell-associated molecular patterns (ACAMPs) [22, 27, 28]. These ACAMPs would play a key role in the embryonic process in which whole cell populations need to be cleared out without mounting an inflammatory response and minimising the presence of cellular debris [29]. Some ACAMPs include oxidized low density proteins, alteration of the membrane electrical charges, nucleic acids and phosphatidylinserine [4, 30]. Similarly, damaged or stressed tissues release/express the so-called danger-associated molecular patterns (DAMPs). Some DAMPs including heat shock proteins (HSP), adenosine, ATP, high motility group box chromosomal protein 1 (HMGB-1), galectins and thioredoxin present adjuvant and pro-inflammatory activity [31]. Phagocytic cells recognise these PAMPs, ACAMPs and/or DAMPs, which can be either membrane-bound or soluble, through their pattern recognition receptors (PRRs) [22, 28, 32]. Some of the PRRs include toll-like receptors (TLR), scavenger and manose receptors, CD14, CD36, complement receptors, phosphatidylinserine receptor (PSR) and milk fat globulin (MFG-EGF8) [8]. Therefore, the activation of microglia, rather than an unspecific process, is highly dependent on the stimulus that originated it.

In contrast to this plethora of signals that might elicit an innate immune activation directed to the elimination of the pathogen, the apoptotic cell or the tissue debris; there is a complex set of interactions that silence and reshape microglial response [1]. For instance, electrically active neurons inhibit the interferon- γ (IFN γ)-induced increase in major histocompatibility complex (MHC) class II expression in microglia [33]. Some neurotransmitters have modula-

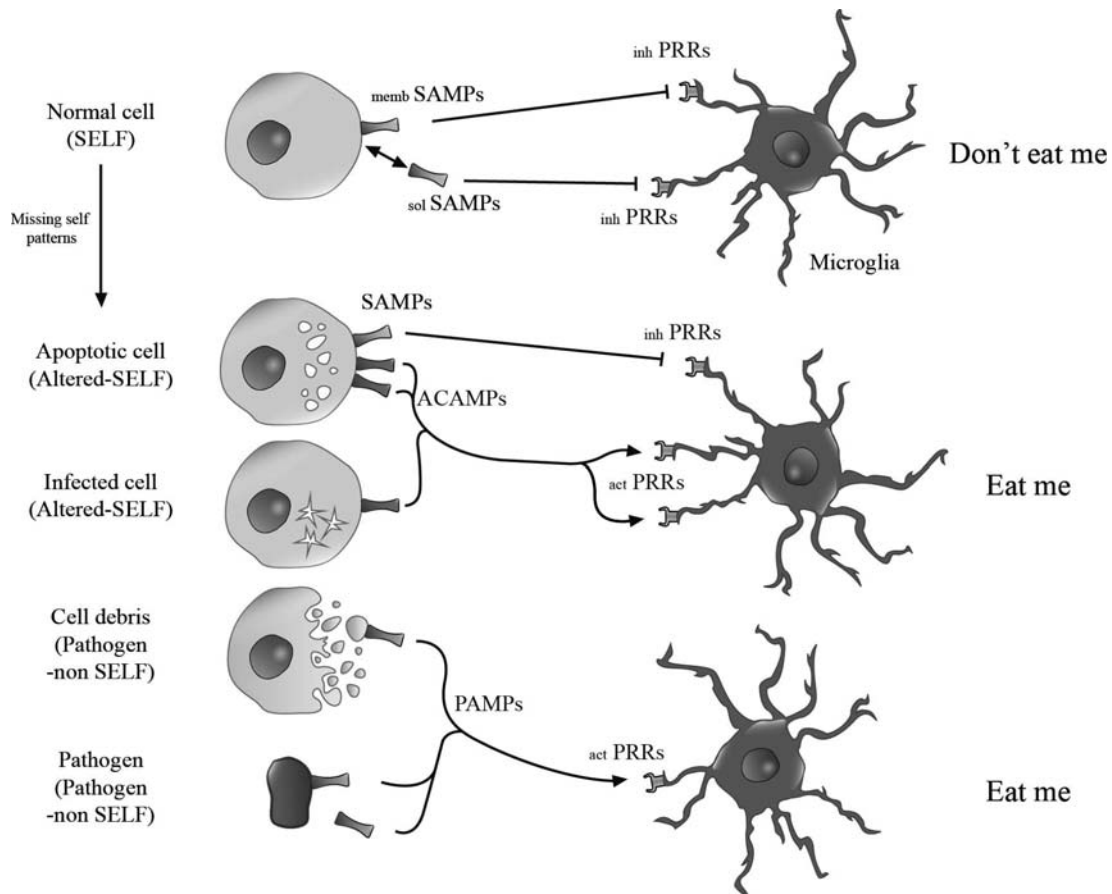


Fig. (1). Roles of “eat me” and “don’t eat me” signals in the CNS innate immune responses. Scheme showing how microglial cells differentiate “self” from “non-self” cues.

tory effects on microglial response whereas others, like substance P enhance the activation of microglia [34]. Neurotrophins secreted by healthy neurons such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and to a lower extent neurotrophin-3 (NT-3) were able to reverse the induction of MHC class II molecules in microglia [35]. Interestingly, neurons express membrane-bound molecules and/or secrete soluble mediators that function as “don’t eat me” signals, reshaping microglial response and inhibiting their phagocytic activity on the site of injury [4, 36]. These signals were originally grouped in a family of heterogeneous molecules called self-associated molecular patterns (SAMPs). It was suggested that these SAMPs could interact with novel inhibitory PPRs, negatively modulating the innate immune response and promoting tissue repair [4]. These SAMPs signals are present in almost all host “normal-self” cells but not in pathogens and they are downregulated in apoptotic/necrotic cells (altered self). Thus, this “normal-self” identity can be lost. An example of a molecular marker of “normal-self” is the presence of sialic acid in the terminal of glycoproteins and glycolipids, which can interact with different microglial receptors [37]. One of these inhibitory receptors belongs to a family known as siglecs [38]. Siglecs contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tail which downregulate phagocytosis in macrophages and microglia [39, 40]. Most microorganisms lack sialic acid in their glycolipids and/or glycoproteins and in some cases, virally infected or transformed cells can show a deficient pattern of sialization, which is a strong signal of “altered-self” to promote phagocytosis. Also in apoptotic cells, a reduced expression of sialic acid has been shown [22].

Of particular interest is the physical interaction (by means of their membrane-bound proteins) between neurons and microglia. Thus, a group of SAMPs were renamed NIREgs to highlight their role in modulating, reshaping an adverse immune response and skewing microglia towards a protective phenotype. Interestingly, many of these NIREgs contain an ITIM domain in their cytoplasmic tails which negatively regulated microglial response to insults.

As mentioned before, siglecs are members of a subgroup of the immunoglobulins (Ig) superfamily that recognize the sialic acid residues on the periphery of cell surface glycolipids and glycoprotein. Siglecs contain an ITIM domain in their cytoplasmic tails which signal via the recruitment of tyrosine phosphatases such as SHP1 [40]. In this sense, when microglial siglecs interact with the neuronal glycolipid, neurotoxicity is alleviated [41].

Another interesting NIREg protein is the pair CD200- CD200R. CD200 is a 41-47 KDa protein belonging to the Ig supergene family characterised by two immunoglobulins superfamily (IgSF) domains [42, 43]. This surface protein is highly conserved and within the CNS is mainly expressed by neurons and vascular endothelium [42, 44, 45, 46], but CD200 is present in almost peripheral cell types, thymocytes [47], T and B cells [43], dendritic cells [48] and even in brain astrocytes and oligodendrocytes [49, 50]. In contrast, CD200R, which also contains two IgSF domains and a longer cytoplasmic tail with an ITIM domain as showed in (Fig. 2) [42], is chiefly expressed by myeloid cells and microglia [46, 51]. However, CD200R has been described to be present also in thymocytes [52] as well as in T and B cells [43, 53]. Functional studies about the role of CD200-CD200R interaction within the brain indicate that this pair of molecules plays a critical role in neuronal protection in the setting of inflammatory-mediated neurodegeneration (Fig. 3). In particular, this immune-regulatory system, when deficient, may contribute to chronic inflammation in MS, AD or in the aging brain as we will show in this review. Deletion of CD200 results in myeloid cells dysregulation and enhanced susceptibility to autoimmune response [51, 54].

Other neuron-microglia ligand-receptor interaction that can modulate microglial responsiveness is the neuronal CD47 (also

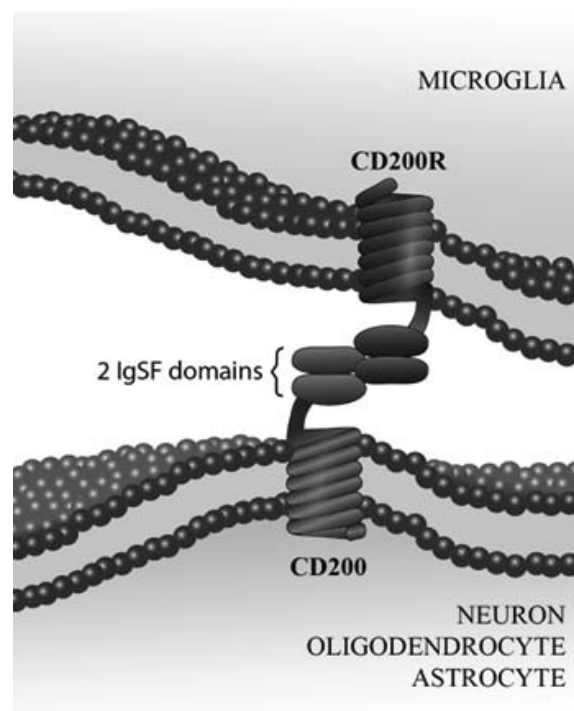


Fig. (2). Binding of CD200-CD200R in the CNS. Scheme showing the binding of CD200 expressed in neurons, oligodendrocytes astrocytes and brain endothelial cells with its receptor CD200R expressed mainly in microglial cells. Both molecules are type-1 membrane glycoprotein that belong to the IgSF and contain two IgSF domains. CD200R has a short intracytoplasmic domain allowing the inhibitory signal to microglial cells. CD200 is expressed in a variety of lymphoid and non-lymphoid cells while CD200R is primarily expressed by myeloid cells (macrophages, monocytes and microglia).

expressed by endothelium, astrocytes and dendritic cells) [55] and the microglial signal regulatory protein SIRP α (CD172) which contains three extracellular Ig domains [56]. CD47-CD172 interaction recruits the tyrosine phosphatases SHP-1 and SHP-2 with inhibition of phagocytosis and synthesis of the anti-inflammatory cytokine TGF- β [22]. Another NIREg with a potential critical role in neuroprotection include the pair neuronal CX3CL1 and microglial CX3CR1 [57].

Interestingly, some membrane receptors contain immunoreceptor tyrosine-based activation motifs (ITAM). ITAM-containing signalling adaptor proteins are associated with receptor subunits. After ligand-receptor interaction, tyrosine residues of the ITAMs become phosphorylated by members of the Src kinase family and subsequently serve as docking sites for Src homology 2 (SH2) domains of Syk protein kinases which then mediate cellular activation via a number of downstream cascades [40]. Triggering receptor expressed on myeloid cells 2 (TREM-2) is an innate immune glycoprotein heterogeneously expressed in brain microglia [58]. It belongs to the immunoglobulin and lectin-like superfamily with a short cytoplasmic tail [59]. Interaction of TREM-2 with its uncharacterized ligand induces its association with the ITAM-containing adaptor molecule DAP12 followed by the recruitment of ZAP70, SYK, PI3K and phospholipase C γ [60]. Activation of TREM-2/DAP12 leads to augmented phagocytosis with reduced expression of tumor necrosis factor- α (TNF α), interleukin (IL)-6 and inducible nitric oxide synthase (iNOS) [61]. Mutation of either TREM2 or DAP12 proteins lead to the rare Nasu-Hakola disease (NHD), also known as polycystic lipomembranous osteodysplasia with sclerosis-

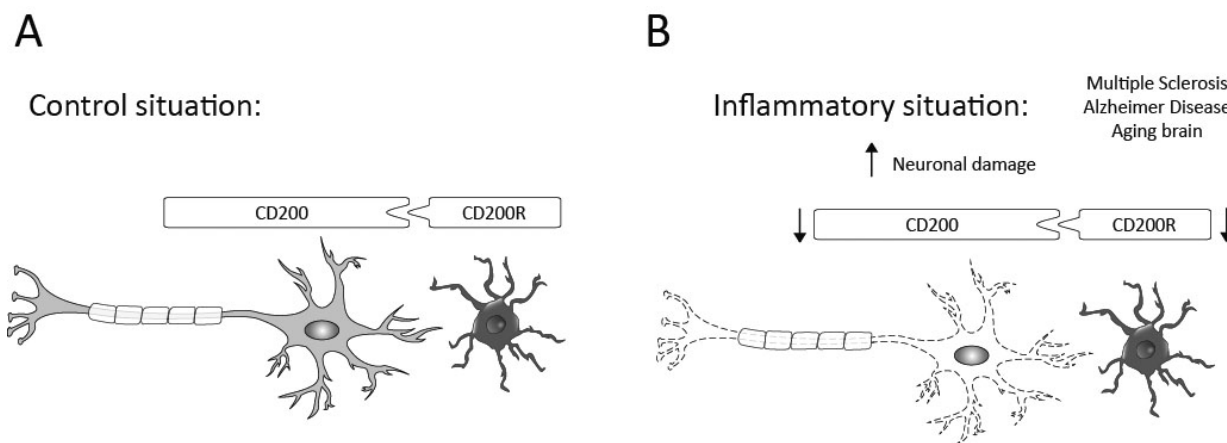


Fig. (3). Neuron-microglia crosstalk. A) In control conditions the crosstalk between neuron-microglia via the CD200-CD200R interaction is working correctly. The inhibitory signal is contributing to the maintenance of brain homeostasis and immune privilege. B) In inflammatory situations the crosstalk between neuron-microglia through the CD200-CD200R interaction is altered. The reduced inhibitory input from CD200 causes a disturbed equilibrium which results in activation of microglia and neuronal damage in MS, in the aging brain and AD.

ing leukoencephalopathy (PLOSL), an autosomal recessive inherited disease [62]. While NHD patients carrying TREM-2 mutation present an early onset presenile dementia followed by delayed bone symptoms [63], patients with mutations in DAP12 display an early onset combination of presenile dementia and systemic bone cysts [64]. Interestingly, Piccio *et al.*, [65, 66] showed that a soluble form of TREM-2 was present in the cerebrospinal fluid (CSF) of patients with MS and that blockade of TREM-2 with a blocking antibody exacerbated experimental autoimmune encephalomyelitis (EAE) in rodents. These observations suggest a crucial role of TREM-2 in preventing neurodegenerative processes. So far the nature of the TREM-2 ligand remains unknown. It has been suggested that TREM-2 bind anionic ligands of the surface of Gram positive and Gram negative bacteria, including *Neisseria gonorrhoeae*, as well as an unidentified ligand in the astrocytoma cell line HTB12 [61]. Importantly, Hsieh *et al.* [67], using a TREM2/Fc chimera, showed that neurons undergoing apoptosis increased the expression of a TREM-2 ligand (TREM-2L), inducing their phagocytosis by BV2 cells (a murine microglia cell line). This effect could be reversed with the use of a blocking antibody against microglial TREM-2 [67].

This review will examine how the CNS innate immune response maintains a critical balance between protective and potentially harmful effects by the interaction of CD200 and its receptor CD200R in neurological diseases which involve neuroinflammation with special emphasis to MS, AD and aging brain. The balance between the destructive and protective effects of the innate immune response must be precisely regulated in order to promote conditions that support brain repair and re-established tissue homeostasis.

MULTIPLE SCLEROSIS

MS belongs to a larger group of inflammatory demyelinating diseases of the CNS which include besides the different manifestations of MS, acute disseminated leukoencephalitis, Devic's neuro-myelitis optica and Balo's concentric sclerosis. Although these diseases differ in clinical course, imaging pathology and immunopathogenesis, they share some essential structural features of their lesions, as they all occur on a background of inflammatory reaction composed of lymphocytes, activated macrophages and microglia and show demyelination. MS is a chronic inflammatory and neurodegenerative disease affecting over 2.5 million individuals worldwide. The pathological hallmark of MS is white matter demyelination but there are other features such as axonal and neuronal damage, grey matter demyelination, composition of vascular cuffs (monocytes, T cells, B cells, plasma cells), loss of oligoden-

drocytes and anatomical lesions that vary between patients [68]. The pathogenesis of MS is still not fully understood, although significant progress has been made in the last decades [69]. Several immunological abnormalities have been identified in patients with MS, such as the presence of oligoclonal bands in the CSF, higher levels of T cell activation and decrease of regulatory T cell function. The presence of inflammation driven by the adaptive immune system in absence of infectious pathogens, the response to immunomodulatory therapy, in addition to the association with human leukocyte antigen (HLA) and the predominance for young women strongly suggest an autoimmune origin. Regarding the susceptibility for suffering MS, familial and twin studies have revealed a concordance in monozygous twin of 30% compared with dizygous twin and sibling of 2-5%. The identification of genomic factors predisposing for MS have revealed the association with HLA class II and specifically with the HLA-DRB1*1501 allele. In respect to environmental factors, several factors have been validated by different studies and meta-analysis including the association with common infections (Epstein-Barr virus, Herpesvirus type 6 and MS retrovirus), levels of vitamin D and sun exposition [70,71]. Regarding association with infections, the picture is complex because no single pathogen has been linked with MS in a clear way. Therapies that have been developed so far are aimed at multiple mechanisms such as broad immune suppression or inhibition of immune cell migration. Some of these therapies reduce the clinical disease activity and the progression of lesion load as determined by magnetic resonance imaging (MRI), but so far no therapy is available that can cure MS or even halt its progression. The search for more effective approaches is therefore necessary.

Macrophages and Microglia as Cell Players in MS

The activation of macrophages and microglia is critical in the development and expansion of MS lesions [72, 73] as clusters of activated microglia are observed before demyelination [74]. Moreover, infiltrated macrophages and activated microglia are the predominant cell types present in expanding MS lesions [75], and are involved in myelin phagocytosis [76]. In that way in order to halt the progression of MS one should particularly target the cells of myeloid origin to dampen neuroinflammation and demyelination.

Other types of macrophages located at the interface of the CNS and the peripheral immune system are the perivascular, meningeal and choroid plexus macrophages. The perivascular macrophages found in the Virchow-Robin space between the endothelial vessel lining and the glia limitans have been involved in scavenging of pathogens and other substances derived from the CNS or the circu-

lation as well as antigen presentation [77]. Moreover, these cells also act as gate keepers, as they facilitate the migration of leukocytes from the perivascular space to the neuropile [78]. Perivascular macrophages increased numbers in the CNS of MS patients, and in experimental models of MS, like EAE and Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD). Since perivascular macrophages increased expression of adhesion molecules and chemokines, these cells are most likely involved in attracting circulating leukocytes in MS. Whether perivascular macrophages themselves remain at their location or also invade the brain parenchyma or how these cells contribute to MS is still unclear. Depending on the activation signals macrophages can turn on a classical or alternative activation program [79, 80]. IFN γ and LPS produce a classical activation of macrophages termed M1 and involve mitogen activated protein kinase (MAPKs) signaling pathways [81]. M1 cells are pro-inflammatory and express high levels of nitric oxide (NO), cytokines like TNF α , IL-1, IL-6, IL-12, IL-23 and opsonin (Fc γ) and the complement type 3 (CR3) receptors. These last receptors are required for antibody or complement mediated phagocytosis. In addition, M1 macrophages can promote T helper 1 (Th1) responses [82].

By contrast, IL-4, IL-13, TGF- β or glucocorticoids induce alternatively activated macrophages (M2) [79]. It has been suggested that the phenotype of M2 cells involves the downregulation of nuclear factor NF- κ B and signal transducer and activator of transcription (STAT-1) [82]. Importantly, M2 cells express high amounts of IL-10 and the mannose receptor possibly to mediate scavenging of debris, tissue remodeling and repair. The M1/M2 distinction should not necessarily invoke a concept of a cellular differentiation state, but as a spectrum of pro- versus anti-inflammatory responses, representing as a continuum between these two extreme activation states.

In the case of microglia, depending on the type of activation, microglial cells can proliferate, lose their ramifications and can induce the expression of neurotrophic factors or pro- or anti-inflammatory cytokines [79, 83]. During neuroinflammation, activation of microglia is initiated through the MAPKs pathways similar to M1 macrophages [84, 85] and upregulate MHC class II molecules and secrete inflammatory mediators like NO, IL-1, IL-6 and TNF α . The upregulation of surface receptors like FC γ and C3 allow microglia to participate in antibody and complement-mediated phagocytosis [86]. In active MS lesions macrophages act mainly in a pro-inflammatory way corresponding with phenotype M1. However, it has been described that foamy macrophages switch their phenotype and start to express markers of M2 phenotype [87]. This change probably contributes to gliosis formation by astrocytes. Microglia also is able to display reactions associated with both protective and deleterious effects in the CNS [18]. It is therefore likely that microglia activation may be polarized depending on activation signal. This has been shown in mice [6] but evidence in humans is still lacking. The cause of classical activation of macrophages and microglia in MS is still subject of debate in terms of questions about if this activation is followed by, or a consequence of T cell activation, oligodendrocyte apoptosis or other pathological events. Once activated, the role of macrophages and microglia in MS has been shown to mediate both beneficial and detrimental effects. By efficiently removing myelin and cellular debris macrophages facilitate remyelination [88] being the cells positive for the receptor of the chemokines CCL3 and CCL5, CCR5⁺, the subpopulation of macrophages involved in this process [89]. Although beneficial effects of activated macrophages/microglia in MS are evident, these effects are only to occur after initial damage which is attributed to the activated macrophages as well. In fact, macrophages and microglia are key players in lesion initiation and development, and therefore in disease progression. Clusters of microglia were present in the MS brain even before cellular infiltrates or demyelination was visible [90]. This first observation was confirmed in various

studies and has been linked to the earliest stages of lesion development [74, 91]. Macrophages and microglia predominate in expanding MS lesions and greatly outnumber lymphocytes [75] localizing in close proximity to damaged axons [92]. Phagocytosis of myelin seems to occur by a common mechanism mediated by complement and immunoglobulin deposition [93]. Macrophages and microglia furthermore secrete many inflammatory cytokines, reactive oxygen species (ROS) and chemokines [84] enhancing inflammation. A disproportional activation of macrophages/ microglia can be a key event in triggering a cascade of events that eventually leads to multiple demyelinated areas in the CNS. In that way, treatments that interfere with the activation of these cells would be a good approach to halt lesion development and further disease progression.

IMMUNOREGULATORY MOLECULES IN NEUROINFLAMMATION

As commented before, in the CNS exists several intrinsic mechanisms that tightly regulate the activities of microglia [94]. This is critical, as an uncontrolled inflammatory reaction could be detrimental to a tissue that is known for its poor regenerating capacity. Quiescent microglia inspect and guard their environment [95], but when a certain threshold is exceeded they become activated and show retraction of ramifications acquiring round phenotype and upregulate a number markers like CD45 and MHC class II. These cells will become motile, phagocytic and might display local proliferation [96]. A proper balance in immune activating and immune inhibitory signals thus regulates their activation. It has been postulated that excessive microglia/macrophage activation in a pathological setting is due to imbalanced control, reflected by impaired immune activation, immune inhibition or both, and could lead to disease progression as seen in MS [97]. This view subsequently implies that correcting the equilibrium by supplying extra inhibitory signals specific for these cells may dampen the pathological inflammatory response and restore the immune suppressed environment of the CNS [8]. A specific therapeutic candidate target to diminish inflammation in MS via the immune inhibitory molecules is CD200 and its receptor CD200R.

CD200-CD200R in MS

CD200 is a membrane glycoprotein belonging to the IgSF, with CNS expression extremely high. Among other cells, neurons express the highest level of CD200 [46]. CD200 has a short cytoplasmic tail with no known signaling motifs, then CD200 itself exerts functions in the cell on which it is expressed its receptor. Precisely, CD200R, homologous to CD200, is expressed mainly on myeloid cells such as macrophages, microglia and dendritic cells [98, 99] and on a subset of B and T cells [53, 100]. CD200R has a cytoplasmic tail which contains three tyrosine residues, one of which forms part of an NPXY motif. Upon binding to its ligand, the tyrosine residues become phosphorylated, and adaptor proteins downstream of tyrosine kinase (DOK) 1 and 2 are recruited. This ultimately leads to inhibition of the MAPK p38, extracellular kinase (ERK) and c-Jun terminal kinase (JNK), the common pathways involved in classical activation of macrophages and microglia. This in turn results in inhibition of release of pro-inflammatory cytokines like IL-1 β , IL-6 and TNF α [101, 102], but promote the secretion of IL-10 [103]. This is an important point as CD200R engagement not only decreases the production of pro-inflammatory cytokines, but enhances the production of an anti-inflammatory cytokine. Therefore, through CD200-CD200R interaction, the activity of macrophages and microglia can be down-regulated. Interestingly, using neuron-microglial cell co-cultures model, as described by Hernández *et al.* [103], IL-10 protected neurons from microglia-induced cytotoxicity. In this context, it has been suggested that CD200-CD200R interaction may be one of the mechanisms by which IL-10 protects neurons from inflammatory damage by increasing the expression of CD200 in neurons [103]. In fact, in time-lapse experiments using co-cultures of neurons-microglial BV2 cells activated

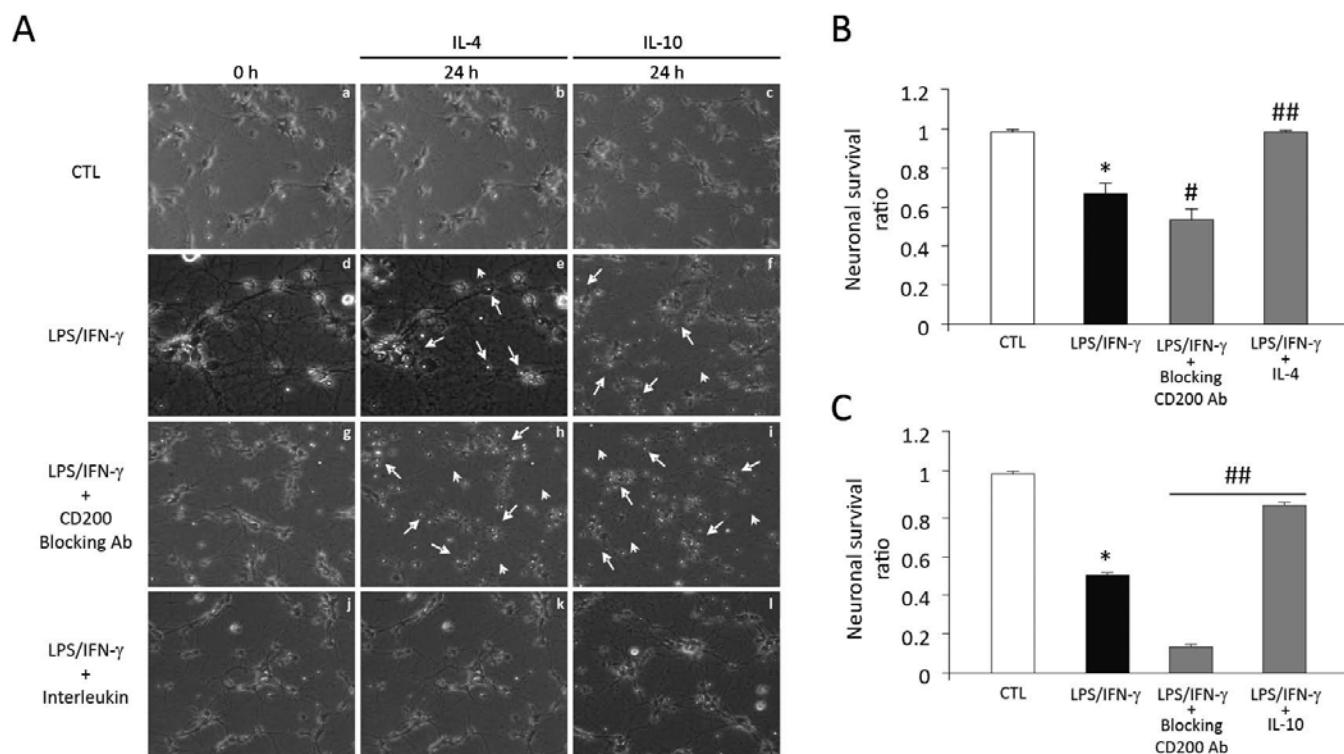


Fig. (4). Microglia-neurons co-cultures: The anti-inflammatory cytokines, IL-4 and IL-10, reduce neuronal death- induced by inflammatory stimuli while the impairment of CD200-CD200R interaction enhanced neuronal death. A) Representative time-lapse experiment of BV2 microglial cells/neurons subjected to LPS (10 ng/ml)/IFN γ (100U) stimulation for 24 h. The addition of IL-4 (10 ng/ml) or IL-10 (10 ng/ml) reduced neuronal death while the impairment of CD200-CD200R interaction using a blocker of CD200, CD200 blocking antibody (5 μ g/mL), augmented neuronal death as reflected in quantification experiments B, and C respectively. Quantification data show the mean \pm SEM from four independent experiments. Statistics: B, * p <0.05 vs control; # p <0.05 vs LPS/IFN γ ; ## p <0.01 vs LPS/IFN γ ; C, * p <0.05 vs LPS/IFN γ ; ## p <0.01 vs LPS/IFN γ .

with LPS/IFN γ , the addition of the anti-inflammatory cytokines IL-4 and IL-10 rescue neurons from inflammation induce death while the blockade of the interaction of CD200 with its receptor enhanced neuronal death induced by the inflammatory stimuli (Fig. 4).

The high expression of CD200 in the CNS is thought to be a mechanism of constitutive immune suppression and is developmentally regulated in the mouse brain [104]. In fact, the blockade of CD200-CD200R interaction, for instance, in CD200^{-/-} deficient mice, leads to spontaneous activation and increased proliferation of microglia and macrophages, demonstrated by upregulation of CD45 which is a hallmark of microglial activation and expression of iNOS [51]. In CD200 deficient mice induction of EAE and experimental uveoretinitis showed enhanced macrophage infiltration and a more rapid and severe disease course compared to wild type mice [51, 105].

A blocking antibody against CD200R aggravated EAE in rats by enhancing infiltration of T cells and activated macrophages [106]. In addition, the interruption of CD200-CD200R interaction increased neuronal death in macrophage/microglia co-cultures [103, 106]. These findings implicate that CD200 is a potent immune inhibitory molecule and that reduced inhibitory input from CD200 causes a disturbed equilibrium which subsequently results in cellular activation and tissue damage. This has been confirmed in MS, since the decreased expression of CD200 and other immune inhibitory molecules like CD47 in chronic active lesions was accompanied of macrophage/microglia activation expanding the lesions [97]. A reduction of CD200 and CD200R gene expression in the spinal cord was also observed at later chronic phases in the viral model of MS, TMEV-IDD [103]. Immune inhibition might be hampered in MS and this could facilitate the activated state of

macrophages/microglia with demyelinating activity as a consequence. Importantly, findings in animal studies show that increased CD200R signaling can be beneficial. For example, mice that have inherently elevated levels of CD200 show ameliorated disease signs in the EAE model accompanied by enhanced neuroprotection and elevated levels of IL-10 [49]. As expected, these animals displayed fewer activated macrophages/microglia and less demyelination and axonal damage. CD200R is likely to mediate several actions of alternatively activated macrophages (M2) such as the reduced secretion of pro-inflammatory cytokines. Moreover, CD200R expression on T cells seemed to be restricted to Th2 cells [107]. In summary, impaired immune inhibition by reduced CD200/CD200R interaction might result in a pro-inflammatory environment that may contribute to demyelination and axonal injury in MS.

THE CANNABINOID SYSTEM: CANNABINOID RECEPTORS AND ENDOCANNABINOIDS PRODUCTION AND INACTIVATION

Cannabinoids (CBs) activate mainly the cloned CB1 and CB2 receptors, but also can interact with the TRPV1 vanilloid receptor, GPR55 and other yet uncloned receptors such as abn-CBD receptors [108, 109]. In addition, CBs can interact with peroxisome proliferator-activated receptors, PPAR γ and PPAR α [110, 111]. CB1 receptors are expressed abundantly throughout the brain by many neurons, as well as outside the CNS, but at much lower levels [112, 113, 114]. CB1 receptors are G_{i/o} protein-coupled receptors that modulate the activity of several plasma membrane proteins and intracellular signaling pathways. Several studies suggest that short-term *versus* long-term activation of CB1 receptors affects different cellular functions. For example, activation of neuronal CB1 recep-

tors for seconds inhibits presynaptic N-type calcium channels and activates inwardly rectifying potassium channels, reducing neurotransmission [115, 116]. In contrast, CB1 receptor activation for minutes to hours changes gene expression, for example, inducing the expression of neuroprotective proteins, such as BDNF, known to counteract cell damage [117]. Under neuropathological conditions whereby the blood-brain barrier (BBB) is disrupted, large quantities of leukocytes enter the CNS and participate in neuroinflammatory responses. Leukocytes express CB1 receptors, although at lower levels compared with neurons, and their activation modulates related immune responses [118]. Thus, CBs may affect neuroinflammation through their action at CB1 receptors expressed by invading leukocytes. CB2 receptors are also $G_{i/o}$ protein-coupled receptors [119]. They share 44% protein identity with CB1 receptors and display a distinct pharmacological profile and expression pattern. Many laboratories reported that CB2 receptors are not expressed in healthy brain [118, 119, 120] suggesting that they are not expressed by healthy neurons, astrocytes, resting microglial cells, and oligodendrocytes. However, more recent studies show that microglia, astrocytes, oligodendrocytes, progenitor neural cells and even neurons can express CB2 receptors [121, 122, 123]. However, CB2 receptors are expressed primarily by immune cells [118]. Δ^9 -Tetrahydrocannabinol (THC) binds to CB2 receptors as a partial agonist [119, 124], an effect that leads to reduced inflammation by inducing apoptosis of immune cells [125] and inhibiting the ability of macrophages to process antigens and prime helper T cells [126, 127]. The expression and pharmacological dichotomy between CB2 and CB1 receptors have tremendous pharmaceutical potential, as compounds that interact selectively with CB2 receptors may pro-

vide anti-inflammatory drugs devoid of CB1-induced psychoactive adverse effects.

In 1992, Mechoulam and coworkers identified the first endogenous compound that binds CB₁ receptors with high affinity [128]. This compound, arachidonylethanolamide (AEA), which was named anandamide, is produced in an activity-dependent manner, activates CB receptors, and is inactivated enzymatically. The activity-dependent increase in AEA production was initially demonstrated with neurons in primary culture and then, with microdialysates from freely moving rats [129, 130, 131]. This response requires a rise in intracellular calcium, which increases the activity of an acyltransferase specific for the precursor of AEA, N-arachidonoylphosphatidylethanolamide (NAPE), and a NAPE-specific phospholipase D, generating AEA (Fig. 5). In 1995, two laboratories identified a second endogenous ligand, 2-arachidonoylglycerol (2-AG) which is approximately 200 times more abundant than AEA in brain and activates CB1 and CB2 receptors [120, 132]. Neurons and glial cells produce and inactivate 2-AG [129, 131, 133, 134]. Its production also requires sustained increase in intracellular calcium levels and the activation of the phospholipase C-diacylglycerol lipase pathway [135, 136]. Several lipids structurally related to AEA and 2-AG can also interact with CB receptors, among them, palmitoylethanolamide (PEA) which contains a 16:0 fatty acid moiety may be a subtype of eCB. PEA is produced and inactivated by cells and induced biological effects that are blocked by a CB2 antagonist although it does not bind to any known CB receptors [131, 137, 138, 139]. The bioactivity of eCBs can be terminated by desensitization and internalization of CB receptors, clearance of eCBs from the extracellular milieu and

Endocannabinoids biosynthesis and degradation

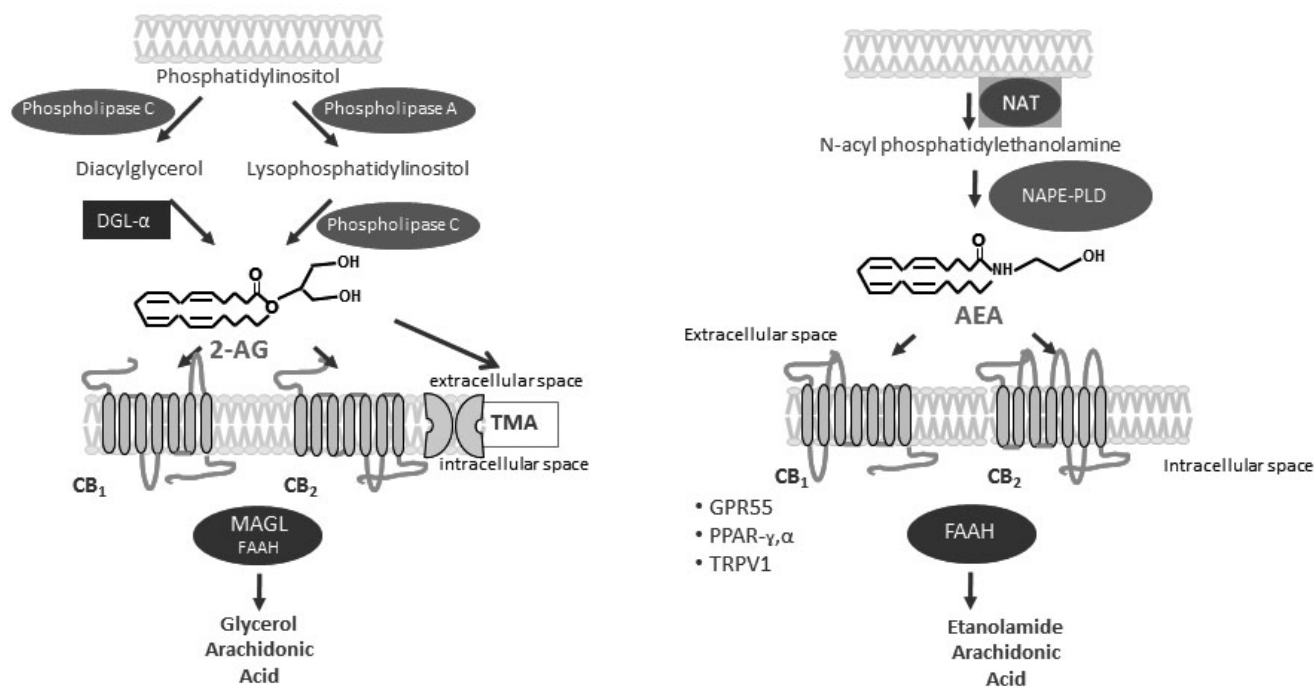


Fig. (5). Models showing AEA and 2-AG production and inactivation. AEA is produced by phospholipase D hydrolysing NAPE which is produced by N-acyltransferase. AEA is released toward the extracellular space and activates CB₁, CB₂ and TRPV1 receptors; AEA is hydrolysed by fatty acid amide hydrolase (FAAH) into arachidonic acid plus ethanolamine. 2-AG is produced by diacylglycerol lipase (DGL) hydrolysing diacylglycerol (DAG) which is produced by phosphatidylinositol-specific phospholipase C. 2-AG is released toward the extracellular space and activates CB₁ and CB₂ receptors, hydrolysed by monoacylglycerol lipase (MGL) into arachidonic acid plus glycerol.

by enzymatic hydrolysis of eCBs, namely AEA by fatty acid amide hydrolase (FAAH) and 2-AG by monoacylglycerol lipase (MGL), ABH serine hydrolase and cyclooxygenase 2 [140, 141, 142, 143]. The characteristics of AEA and 2-AG uptake into cells are similar and suggest a common mechanism [135]. However, whether a protein mediates this uptake is still controversial. In summary, the eCB family consists of several structurally related lipids that interact with CB receptors. Several studies have identified eCBs as retrograde signaling molecules, being generated in a calcium-dependent manner from postsynaptic terminals, diffusing toward presynaptic terminals and inhibiting neurotransmitter release [144, 145, 146]. Figure 6 shows the current signaling relationship between glia and neurons and the role played by eCBs.

Macrophages/Microglia as Targets of Anti-inflammatory Actions of CBs in MS Models

None of the currently available or experimental therapies are specifically aimed at halt the function of macrophages/microglia. Most therapeutic targets originally developed in EAE were aimed at limiting T cell activities and migration. Although T cells are required in the induction phase of EAE their critical role in MS is still elusive [147]. Using a CD200R agonistic compound like the fusion protein CD200 Fc in MS could suppress macrophage/microglia activation, restore the intrinsic immune suppressed environment of the CNS and may thereby restrain disease activity. We here propose to focus on the role of eCBs and exogenous CBs in the regulation of macrophages/microglia function and their effects in MS and its experimental models.

Extracts from the cannabis plant have been used medicinally for thousands of years, but it is only within the last two decades that our understanding of cannabinoid physiology and the evidence for

therapeutic benefit of cannabinoids has begun to accumulate. Here, we will provide the advances in our understanding of eCB system (eCS), and how cannabinoids may help in the management of MS and its experimental models. Recent observations are beginning to suggest positive effects of cannabinoids in neuroinflammatory diseases like MS, with evidence of anti-inflammation, encouragement of remyelination and neuroprotection. Clinical trial studies have demonstrated the benefit of CBs in alleviating MS-related symptoms [148, 149]. Exogenous administration of CBs has also been shown to be beneficial in several animal models of MS [150, 151, 152, 153, 154]. CBs have anti-inflammatory and neuroprotective activities as well as promote oligodendrocyte survival [121, 155].

Cumulative evidence has established that there is a dynamic interplay between the eCS, the immune system and the CNS [156, 157]. Studies on the role played by the eCS during neuroinflammation support the interest of this system as a novel target for pharmacological therapy [153, 158, 159, 160]. In the TMEV-IDD model, exogenous CB agonists attenuated the pathological features of the disease [151, 152]. This effect was also observed when the endogenous levels of the eCB AEA are elevated by pharmacological intervention [161-163]. Microglial cells are possible targets for immunomodulatory activities of eCBs, a hypothesis that is supported by the presence of cannabinoid receptors and the enzymes responsible for their synthesis and degradation [122, 164-166]. The endogenous ligands AEA and 2-AG are also synthesized by microglial cells which produce approximately 20-fold more eCBs than neurons and astrocytes [166]. The eCS is highly activated during brain inflammation and AEA has been shown to be increased in active lesions of MS patients, to protect neurons from inflammatory damage [167, 168].

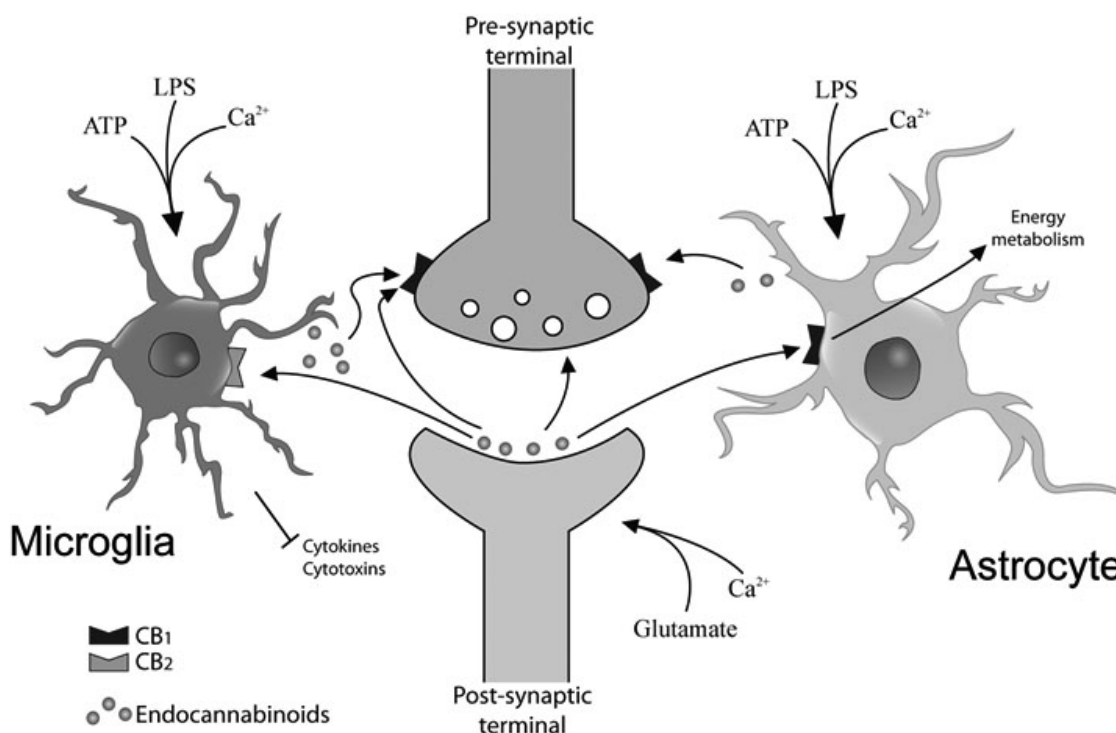


Fig. (6). Current signalling relationship between glia and neurons related to cannabinoids. A sustained elevation in intracellular calcium leads to increased eCBs production. At postsynaptic neurons eCBs production increases when metabotropic glutamate receptors and voltage-sensitive calcium channels are activated. In microglia and astrocytes, eCB production increases when purinergic P2X7 receptors are activated or when LPS or proinflammatory cytokines are acting in these glial cells. CB1 receptors are abundantly expressed on pre-synaptic terminals and inhibit neurotransmitter release. Astrocytes also express CB1 receptors at lower levels than neurons and their activation regulate energy metabolism and cell survival. Activated microglial cells express mainly CB2 receptors that inhibit cytokines and inflammatory mediators.

eCBs Promote CD-200-CD200R Interaction

eCS has arisen as a promising new therapeutic target for the treatment of MS [153, 158, 169]. In animal models of MS, exogenous CB agonists alleviate disease symptomatology [150-152], a benefit also produced by pharmacological interventions aimed at increasing the levels of eCBs [159, 161, 162, 170]. Uncontrolled innate immune responses within the CNS are widely recognized as playing a major role in the development of autoimmune disorders and neurodegeneration, with MS and AD being primary examples. However, the role of the eCS in the regulation of inhibitory immunoregulatory proteins and their impact in microglial function has not been completely elucidated. During the past years, several *in vitro* and *in vivo* studies have suggested that eCS participates in the control of brain immune responses as well as in the protection of the CNS against injury [171]. During immune-mediated attack of the brain, it has been hypothesized that the activation of eCBs represents a protective mechanism, aimed at reducing both neurodegenerative and inflammatory damage through various and partially converging mechanisms that involve neuronal and immune cells. The major eCBs, AEA and 2-AG, have been shown to exert neuroprotective activity largely thought to be mediated through two specific CB receptors, the CB1 and CB2 receptors. Regarding innate immunity and in particular, CD200-CD200R, recently, our lab showed that the treatment with AEA induced the recovery of CD200 and CD200R gene expression that was reduced in the TMEV model of MS [103]. This was accompanied by decreased inflammatory mediators and reduced microglial reactivity. Here, in an attempt to further support the ability of AEA to modulate the interaction of CD200 with its receptor, we show that the administration of an inhibitor of AEA uptake (UCM-707) to TMEV-infected mice also induced a significant recovery in the expression levels of CD200 (Fig. 7A; $p < 0.05$) that was decreased in TMEV-diseased mice. Treatment with UCM-707 did not significantly modify the expression of CD200R mRNA (Fig. 7B), although there was a trend toward increase. The effects of UCM-707 on CD200 mRNA were accompanied by a diminished expression of IL-1 β mRNA (Fig. 7D; $p < 0.05$) and an increased of IL-10 mRNA expression (Fig. 7C; $p < 0.05$). Therefore, increased AEA tone [162] prevented gene expression of a pro-inflammatory cytokine such as IL-1 β and increased the expression of the anti-inflammatory cytokine IL-10, accordingly with a reduction in microglial reactivity, likely by increasing CD200 expression in the spinal cord. Importantly, the axonal damage observed in TMEV-infected mice in comparison to Sham animals ($p < 0.01$) was reduced after treatment with UCM-707 ($p < 0.05$) as evaluated by SMI32 staining (Fig. 7E). Analyses of motor function in TMEV-infected mice treated with UCM-707 confirmed previous data from our group [162], as vertical ($p < 0.05$) and horizontal activity ($p < 0.001$) deficits were ameliorated (Fig. 7F). These data indicate that CD200 negatively correlates with the degree of spinal cord axonal damage induced by TMEV and suggest that increased CD200 expression may be one of the mechanisms contributing to the anti-inflammatory effect observed in the TMEV-IDD model of MS by enhancing eCB tone [161, 162].

The mechanisms that lead to reduction of damage and to promote repair involve switching macrophage functional activation to include decreased pro-inflammatory cytokines [79]. In studies from our lab we have described that macrophages infected with TMEV increased their production of AEA and that both, AEA and inhibitors of AEA cellular re-uptake inhibited IL-12p40 release [161]. The identification of microglia as one of the major sources of IL-23 in brain sections from MS patients points out the importance of microglial cells as cellular targets to down-regulate this family of heterodimeric cytokines. In the TMEV-IDD model of MS, it is unknown whether TMEV-infected microglia exhibit the autocrine regulatory mechanism between IL-10 and the cytokines of IL-12 family [172] but data from our lab have established the molecular mechanisms by which AEA might regulate the IL-12p70/IL-23 axis

and IL-10 in TMEV-infected microglial cells and in the TMEV-IDD model of MS [173, 174].

Taken together, our findings support the hypothesis that enhancing endogenous anti-inflammatory regulatory systems like the eCS, basic mechanisms of innate immunity like CD200-CD200R interaction that are disrupted in chronic inflammatory diseases such as, MS can be restored representing a new therapeutic strategy that prevents harmful inflammation and axonal damage [175, 176].

REGULATION OF MICROGLIAL ACTIVATION BY CD200-CD200R INTERACTION IN OTHER NEUROINFLAMMATORY CONTEXT: ALZHEIMER'S DISEASE AND THE AGING BRAIN

The role of inflammation in AD has been intensively studied [177, 178]. Pathological studies of AD indicated the presence of activated microglia, reactive astrocytes and complement activation on association with amyloid β (A β) containing plaques, suggesting that a kind of chronic inflammation was ongoing [179, 180]. A range of *in vitro* studies using cultured microglia from humans and rodents indicated that aggregated A β peptide activates microglia to a pro-inflammatory state capable to produce a wide range of neurotoxic mediators [181, 182, 183]. In this line, anti-inflammatory therapies have been considered for treating this disease [184]. Because clinical trials with anti-inflammatory compounds did not generally show effectiveness at slowing the progression of AD, new inflammatory therapeutic targets are needed. These would include the enhancing of the function of endogenous immune regulatory molecules such as CD200 and its CD200R.

CD200 and CD200R in Human Elderly Brain and in AD

Recent studies have focused in the characterization of CD200 and CD200R expression in elderly human brains, as well as in AD. One of these studies showed a decrease in CD200 mRNA expression in the hippocampus of rats with increasing age [50], in agreement with those observed in MS lesions [97]. The main findings obtained from brain tissues affected by AD showed that there is a deficit of CD200 but also of CD200R [181]. As confirmed in other studies CD200 is abundant in the human brain while CD200R is expressed at lower levels [46]. In fact, low expression of CD200R by microglia was confirmed at the mRNA and protein levels using cultured human microglia compared to blood-derived macrophages [181]. Neuronal expression of CD200 was decreased in brain regions affected by AD pathology evaluated by quantitative measurements of protein and mRNA in brain extracts as well as in antibody stained tissue sections. Interestingly, in the normal human brain differences in CD200 immunoreactivity can be observed depending on the brain area studied, while in AD brain the main decreases of CD200 labeling correspond to the hippocampus and inferior temporal gyrus. In agreement with previous data obtained from C57/BL6 mice [49] not only neurons but also astrocytes were positive for CD200 labeling in the human brains [181]. CD200R mRNA expression was also reduced in AD brains, specifically in regions affected by the pathology in comparison with matched no disease brains. In LPS models of neuroinflammation a decreased expression of CD200R has been described within the brain [185]. In the case of MS, no changes in CD200R were observed in brain lesions from MS patients [97], but in the experimental model of MS, TMEV-IDD a decreased expression of CD200 mRNA was observed at chronic phases of the disease [103]. It is suggested that a deficiency of CD200 in AD brain could contribute to maintaining chronic inflammation as has been hypothesized in MS, but also a deficiency in CD200R provokes a reduced efficiency of the CD200-CD200R complex to limit or control inflammation and therefore, the CD200-CD200R interaction in elderly brain might not be functioning in a correct way. Previous studies showed that the inhibitory response to CD200 is dependent on the level of expression of CD200R in responding cells [101]. Increasing the expression of CD200 and

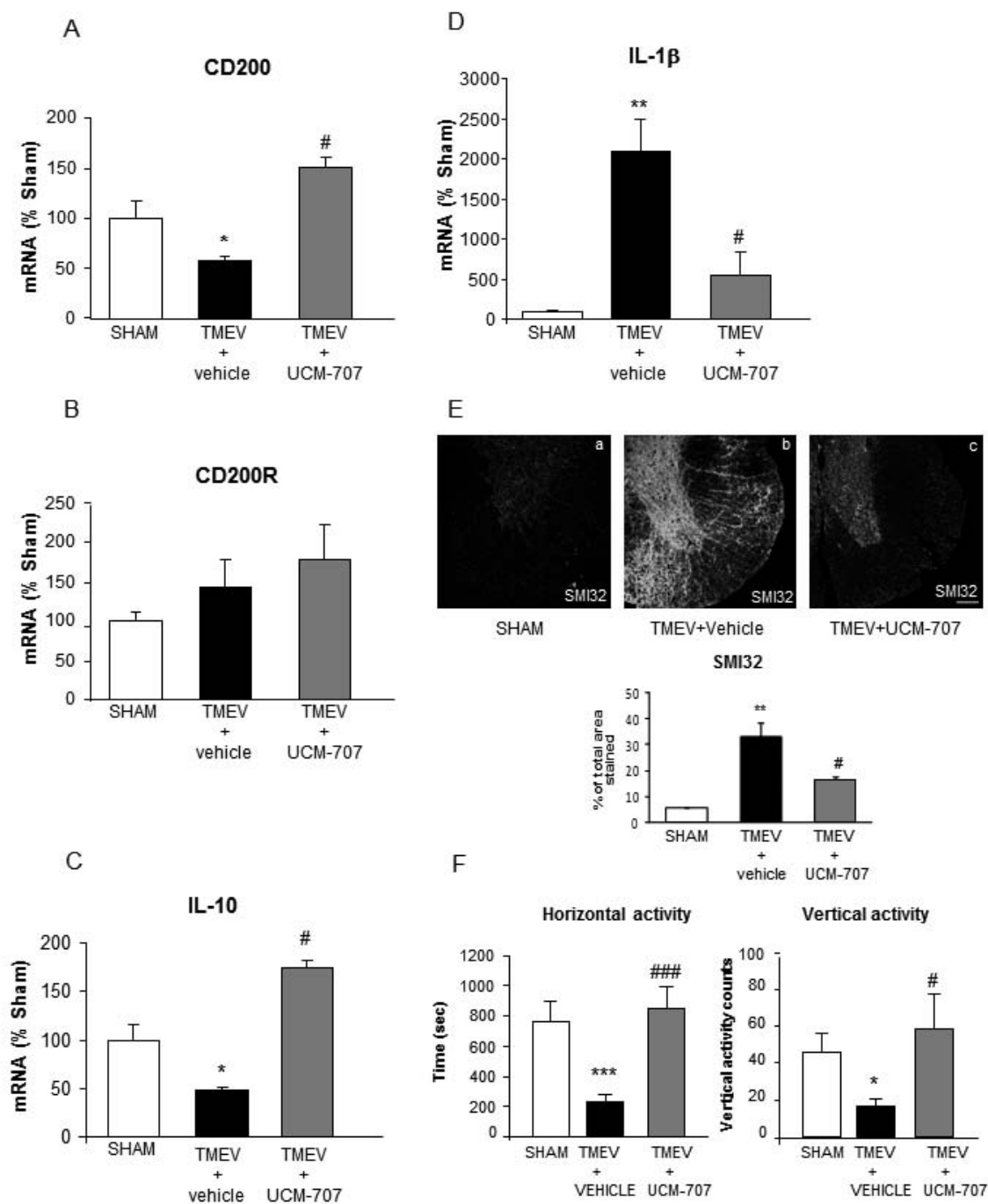


Fig. (7). The inhibitor of AEA uptake, UCM-707, induces a recovery in the expression of CD200 mRNA in the spinal cord of TMEV-IDD mice. TMEV-infected mice were subjected at 60 days pi (during established disease) to the administration of the selective inhibitor of AEA uptake, UCM-707 (5 mg/kg) for 12 consecutive days. **A)** Level of expression of CD200 mRNA, evaluated by real time-PCR, in Sham mice and TMEV-infected mice subjected to UCM-707 or vehicle administration. TMEV-infected mice show downregulation of CD200 mRNA expression ($p < 0.05$) compared to Sham animals. UCM-707 treatment significantly increased the expression of CD200 in the spinal cord of TMEV-infected mice ($p < 0.05$). **B)** Levels of expression of CD200R mRNA, evaluated by real time-PCR, in Sham mice and TMEV-infected mice subjected to UCM-707 or vehicle administration. No significant changes in the level of CD200R expression were found among the different groups. UCM-707 treatment did not modify the level of CD200R expression. **C)** Expression of IL-1 β mRNA, evaluated by real time-PCR, in Sham mice and in TMEV-infected mice subjected to UCM-707 treatment or vehicle administration. TMEV-infected mice show elevated levels of IL-1 β ($p < 0.01$) vs Sham mice in the spinal cord. UCM-707 treatment significantly down-regulates IL-1 β expression ($p < 0.05$). **D)** Expression of IL-10 mRNA, evaluated by real time-PCR, in Sham mice and TMEV-infected mice subjected to UCM-707 or vehicle administration. TMEV-infected mice show decreased level of expression of IL-10 in the spinal cord ($P < 0.05$) vs Sham mice. UCM-707 treatment significantly up-regulates IL-10 expression in the spinal cord of TMEV-infected mice ($P < 0.05$). **E)** Representative images of coronal spinal cord sections derived from Sham or TMEV-infected mice subjected or not to UCM-707 treatment stained with SMI32 for labelling axonal damage. The quantification of the images analysis shows that TMEV-infected mice present axonal degeneration ($p < 0.01$) and UCM-707 treatment reduces axonal damage ($p < 0.05$). Scale bars: 100 μ m. **F)** Activity cage performance: horizontal activity (left panel), vertical activity (right panel). TMEV-infected mice display decreased horizontal ($P < 0.001$) and vertical activity ($P < 0.05$) vs Sham mice. UCM-707 treatment significantly improves motor activity of TMEV-infected mice ($p < 0.001$ for horizontal activity and $p < 0.05$ for vertical activity). Mice were assessed one day after the end of the 12-day treatment protocol. All values in **A**, **B**, **C**, **E** and **F** represent mean \pm SEM from 7-8 mice per group.

CD200R might provide a way of enhancing the efficiency of the system relevant from a therapeutic point of view [186]. Thus, the treatment with the CD200 fusion protein (CD200 Fc) was able to decrease microglial activation in the hippocampus of aged rats [187] and CD200 immunoadhesin diminished in a murine model of rheumatoid arthritis [188]. In this way, anti-inflammatory cytokines play a relevant role in regulating CD200-CD200R axis as IL-4 has been described to increase the expression of CD200 protein in neuronal cultures from rodents and IL-4^{-/-} mice had lower levels of CD200 in the brain [189]. Similarly, IL-10 has been recently shown that augmented the expression of CD200 in neurons [103]. Additional studies revealed that the expression of CD200R was also strongly increased by IL-4 in microglia and macrophages and IL-13, that share common receptors with IL-4, exert a positive effect in increasing the expression of CD200R [181, 190]. In agreement with the above findings a significant age-related decline of IL-4 has been observed in the hippocampus of rats between 4 months and 22 months which correlated with increased levels of IL-1 β [191]. In summary, the above reports suggest that the impairment of CD200-CD200R interaction may initiate a disturbed equilibrium in microglia–neuron interaction, in the way of sensitizing the pro-inflammatory reactivity of microglia and resulting in CNS injuries or in the maintenance of chronic inflammatory states such as observed in AD and in the aging brain.

CONCLUSION AND FUTURE RESEARCH

The CNS innate immune response maintains a critical balance between the protective and potentially harmful effects of activating the innate immune system in CNS pathologies like MS and AD, as well as, in the aging brain that loss its homeostatic capacity with the consequent augmentation of morbidity and mortality. The balance between the protective and the deleterious effects of the innate immune response must be tightly regulated to favor conditions that support brain repair and the return to tissue homeostasis. The CD200/CD200R inhibitory immune ligand-receptors system constitutes one of the most suitable endogenous immunoregulatory molecule candidate to restore the immune suppressive status of the CNS altered in chronic neuroinflammatory situations. The elucidation of the immunoregulatory pathways shared between the CNS innate immune system and microglial cells and their crosstalk with neurons, oligodendrocytes and cells of the immune compartment represents an important challenge, but one that is of great therapeutic potential in preventing damage caused by macrophages/microglia in neuroinflammatory and neurodegenerative disorders.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

2-AG	=	2-Arachidonoyl glycerol
Abn-CBD	=	Abnormal cannabidiol
ACAMPS	=	Apoptotic cell-associated molecular patterns
AD	=	Alzheimer's disease
AEA	=	Anandamide
ATP	=	Adenosin Triphosphate

BBB	=	Blood-brain barrier
BDNF	=	Brain-derived growth factor
C3a	=	marker of complement activation
CBs	=	Cannabinoids
CB1	=	Cannabinoid receptor 1
CB2	=	Cannabinoid receptor 2
CD200	=	ligand of CD200R
CD200R	=	CD200 receptor
CNS	=	Central nervous system
CSF	=	Cerebrospinal fluid
DAMPs	=	Danger-associated molecular patterns
DOK	=	Downstream of tyrosine kinase
EAE	=	Experimental autoimmune encephalomyelitis
eCBs	=	Endocannabinoids
eCS	=	Endocannabinoid system
ERK	=	Extracellular receptor kinase
FAAH	=	Fatty acid amide hydrolase
Fcy	=	Opsonin
fH	=	factor H
GPR	=	G-protein receptors
HLA	=	Human leukocyte antigen
HMGB-1	=	High motility group box chromosomal protein1
HSP	=	Heat shock protein
IFN	=	Interferon
Ig	=	Immunoglobulin
IL	=	Interleukin
iNOS	=	Inducible nitric oxide synthase
ITAMs	=	Immunoreceptor tyrosine-based activation motifs
ITIMs	=	Immunoreceptor tyrosine-based inhibitory motifs
JNK	=	c-Jun N-terminal kinase
LPS	=	Lipopolysaccharide
M1	=	Macrophage type 1
M2	=	Macrophage type 2
MAPK	=	Mitogen activated protein kinase
MFG-EGF 8	=	Milk fat globulin
MGL	=	Monoacylglycerol lipase
MHC class II	=	Major histocompatibility complex class II
MRI	=	Magnetic resonance imaging
MS	=	Multiple Sclerosis
NAPE	=	N-arachidonoylphosphatidylethanolamide
NGF	=	Nerve growth factor
NHD	=	Nasu-Hakola disease
NIRegs	=	Neuroimmune regulatory proteins
NO	=	Nitric oxide
NT-3	=	Neurotrophin-3
PAMPs	=	Pathogen-associated molecular patterns
PEA	=	Palmitoylethanolamide
PI3K	=	Phosphatidylinositol3kinase
PLOSL	=	Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy

PPAR	=	Peroxisome proliferator-activated receptor
PRRs	=	Pattern recognition receptors
PSR	=	Phosphatidylserine receptor
ROS	=	Reactive oxygen species
SAMPs	=	Self-associated molecular patterns
SH2	=	Src homology 2
SHP1	=	Tyrosine phosphatase 1
SHP2	=	Tyrosine phosphatase 2
STAT-1	=	Signal Transducers and Activators of Transcription 1
TGF- β	=	Transforming growth factor β
Th1	=	Lymphocyte T helper 1
Th2	=	Lymphocyte T helper 2
THC	=	Δ 9-Tetrahydrocannabinol
TLR	=	Toll like receptors
TMEV-IDD	=	Theiler's murine encephalomyelitis virus-induced demyelinating disease
TNF α	=	Tumor necrosis factor alpha
TREM-2	=	Triggering receptor expressed on myeloid cells 2
TREM-2L	=	Triggering receptor expressed on myeloid cells 2 ligand
TRPV1	=	Transient receptor potential vanilloid 1
ZAP70	=	ζ associated protein of 70kDa

REFERENCES

- Medzhitov R, Janeway CA, Jr. Decoding the patterns of self and nonself by the innate immune system. *Science* 2002; 296: 298-300.
- Stuart LM, Stuart R, Ezekowitz A. Phagocytosis: elegant complexity. *Immunity* 2005; 22: 539-50.
- Nguyen MD, Julien JP, Rivest S. Innate immunity the missing link in neuroprotection and neurodegeneration. *Nature Rev Neurosci* 2002; 3: 216-27.
- Elward K, Gasque P. "Eat me" and "don't eat me" signals govern the innate immune response and tissue repair in the CNS: emphasis on the critical role of the complement system. *Mol Immunol* 2003; 40: 85-94.
- Hauwel M, Furon E, Canova C, Griffiths M, Neal J, Gasque P. Innate (inherent) control of brain infection, brain inflammation and brain repair: the role of microglia, astrocytes "protective" glial stem cells, and stromal endymal cells. *Brain Res Rev* 2005; 48: 220-33.
- Butovsky O, Koronyo-Hamaoui M, Kunis G, *et al.* Glatiramer acetate fights against Alzheimer's disease by inducing dendritic like microglia expressing insulin-like growth factor 1. *Proc Natl Acad Sci USA* 2006; 103: 11784-9.
- Minghetti L. Role of inflammation in neurodegenerative diseases. *Curr Opin Neurol* 2005; 48: 315-21.
- Griffiths M, Neal JW, Gasque P. Innate immunity and protective neuroinflammation: new emphasis on the role of neuroimmune regulatory proteins. *Int Rev Neurobiol* 2007; 82: 29-55.
- Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 1990; 39: 151-70.
- Yang I, Han SJ, Kaur G, Crane C, Parsa AT. The role of microglia in central nervous system immunity and glioma immunology. *J Clin Neurosci* 2010; 17: 6-10.
- del Río-Hortega P. El tercer elemento de los centros nerviosos. I La microglía en estado normal. II Intervención de la microglía en los procesos patológicos. III Naturaleza probable de la microglía. *Bol de la Soc Española de Biología* 1919; 9: 69-120.
- Graeber MB. Changing face of microglia. *Science* 2010; 330: 783-8.
- Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia* 1993; 7: 9-18.
- Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. *Nat Rev Immunol* 2011; 11: 775-87.
- Barnham KJ, Cappai R, Beyreuther K, Masters CL, Hill AF. Delineating common molecular mechanisms in Alzheimer's and prion diseases. *Trends Biochem Sci* 2006; 31: 465-72.
- Mittelbronn M, Dietz K, Schluesener HJ, Meyermann R. Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathol* 2001; 101: 249-55.
- Olah M, Biber K, Vinet J, Boddeke HW. Microglia phenotype diversity. *CNS Neurol Disord Drug Targets* 2011; 10: 108-18.
- Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* 2007; 10: 1387-94.
- Kraft AD, McPherson CA, Harry GJ. Heterogeneity of microglia and TNF signaling as determinants for neuronal death or survival. *Neurotoxicology* 2009; 30: 785-93.
- Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. *Annu Rev Immunol* 2009; 27: 119-45.
- Davalos D, Grutzendler J, Yang G, *et al.* ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci* 2005; 8: 752-8.
- Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 2005; 308: 1314-8.
- Gasque P, Dean YD, McGreal EP, VanBeek J, Morgan BP. Complement components of the innate immune system in health and disease in the CNS. *Immunopharmacology* 2000; 49: 171-86.
- Barnum SR. Complement in central nervous system inflammation. *Immunol Res* 2002; 26: 7-13.
- Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease--a double-edged sword. *Neuron* 2002; 35: 419-32.
- Matzinger P. Friendly and dangerous signals: is the tissue in control? *Nat Immunol* 2007; 8: 11-3.
- Franc NC, White K, Ezekowitz RA. Phagocytosis and development: back to the future. *Curr Opin Immunol* 1999; 11: 47-52.
- Gregory CD. CD14-dependent clearance of apoptotic cells: relevance to the immune system. *Curr Opin Immunol* 2000; 12: 27-34.
- Imler JL, Hoffmann JA. Toll receptors in Drosophila: a family of molecules regulating development and immunity. *Curr Top Microbiol Immunol* 2002; 270: 63-79.
- Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekowitz RA, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 2000; 405: 85-90.
- Amor S, Puentes F, Baker D, van der Valk P. Inflammation in neurodegenerative diseases. *Immunology* 2010; 129: 154-69.
- Parnaik R, Raff MC, Scholes J. Differences between the clearance of apoptotic cells by professional and non-professional phagocytes. *Curr Biol* 2000; 10: 857-60.
- Neumann H, Boucraut J, Hahnel C, Misgeld T, Wekerle H. Neuronal control of MHC class II inducibility in rat astrocytes and microglia. *Eur J Neurosci* 1996; 8: 2582-90.
- McCluskey LP, Lampson LA. Local neurochemicals and site-specific immune regulation in the CNS. *J Neuropathol Exp Neurol* 2000; 59: 177-87.
- Neumann H, Wekerle H. Neuronal control of the immune response in the central nervous system: linking brain immunity to neurodegeneration. *J Neuropathol Exp Neurol* 1998; 57: 1-9.
- Grimsley C, Ravichandran KS. Cues for apoptotic cell engulfment: eat-me, don't eat-me and come-get-me signals. *Trends Cell Biol* 2003; 13: 648-56.
- Meri S, Pangburn MK. Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. *Proc Natl Acad Sci USA* 1990; 87: 3982-6.
- Angata T, Kerr SC, Greaves DR, Varki NM, Crocker PR, Varki A. Cloning and characterization of human Siglec-11. A recently evolved signaling molecule that can interact with SHP-1 and SHP-2 and is expressed by tissue macrophages, including brain microglia. *J Biol Chem* 2002; 277: 24466-74.
- Crocker PR. Siglecs in innate immunity. *Curr Opin Pharmacol* 2005; 5: 431-7.
- Linnartz B, Wang Y, Neumann H. Microglial immunoreceptor tyrosine-based activation and inhibition motif signaling in neuroinflammation. *Int J Alzheimers Dis* 2010.

- [41] Wang Y, Neumann H. Alleviation of neurotoxicity by microglial human Siglec-11. *J Neurosci* 2010; 30: 3482-8.
- [42] Barclay AN, Wright GJ, Brooke G, Brown MH. CD200 and membrane protein interactions in the control of myeloid cells. *Trends Immunol* 2002; 23: 285-90.
- [43] Wright GJ, Jones M, Puklavec MJ, Brown MH, Barclay AN. The unusual distribution of the neuronal/lymphoid cell surface CD200 (OX2) glycoprotein is conserved in humans. *Immunology* 2001; 102: 173-9.
- [44] Webb M, Barclay AN. Localisation of the MRC OX-2 glycoprotein on the surfaces of neurones. *J Neurochem* 1984; 43: 1061-7.
- [45] Barclay AN, Clark MJ, McCaughan GW. Neuronal/lymphoid membrane glycoprotein MRC OX-2 is a member of the immunoglobulin superfamily with a light-chain-like structure. *Biochem Soc Symp* 1986; 51: 149-57.
- [46] Koning N, Swaab DF, Hoek RM, Huitinga I. Distribution of the immune inhibitory molecules CD200 and CD200R in the normal central nervous system and multiple sclerosis lesions suggests neuron-glia and glia-glia interactions. *J Neuropathol Exp Neurol* 2009; 68: 159-67.
- [47] Barclay AN, Ward HA. Purification and chemical characterization of membrane glycoproteins from rat thymocytes and brain recognised by monoclonal antibody MRCOX2. *Eur J Biochem* 1982; 129: 447-58.
- [48] Gorczynski RM, Khatri I, Lee L, Boudakov I. An interaction between CD200 and monoclonal antibody agonists to CD200R2 in development of dendritic cells that preferentially induce populations of CD4(+)CD25(+) T regulatory cells. *J Immunol* 2008; 180: 5946-55.
- [49] Chitnis T, Imitola J, Wang Y, *et al.* Elevated neuronal expression of CD200 protects Wlds mice from inflammation-mediated neurodegeneration. *Am J Pathol* 2007; 170: 1695-712.
- [50] Frank MG, Barrientos RM, Biedenkapp JC, Rudy JW, Watkins LR, Maier SF. 2006. mRNA up regulation of MHC class II and pivotal proinflammatory genes in normal brain aging. *Neurobiol Aging* 2006; 27: 717-22.
- [51] Hoek RM, Ruuls SR, Murphy CA, *et al.* Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 2000; 290: 1768-71.
- [52] Rygiel TP, Meyaard R. CD200R signalling in tumor tolerance and inflammation: A tricky balance. *Curr Opin Immunol* 2012; 24: 233-8.
- [53] Rijkers ES, Baridi RT, Veninga A, Hoek RM, Meyaard L. The inhibitory CD200R is differentially expressed on human and mouse T and B lymphocytes. *Mol Immunol* 2008; 45: 1126-35.
- [54] Simelyte E, Criado G, Essex D, Uger RA, Feldmann M, Williams RO. CD200-Fc, a novel antiarthritic biologic agent that targets proinflammatory cytokine expression in the joints of mice with collagen-induced arthritis. *Arthritis Rheumatism* 2008; 58: 1038-43.
- [55] de Vries HE, Hendriks JJ, Honing H, *et al.* Signal-regulatory protein alpha-CD47 interactions are required for the transmigration of monocytes across cerebral endothelium. *J Immunol* 2002; 168: 5832-9.
- [56] Brown EJ, Frazier WA. Integrin-associated protein (CD47) and its ligands. *Trends Cell Biol* 2001; 11: 130-5.
- [57] Cardona AE, Pioro EP, Sasse ME, *et al.* Control of microglial neurotoxicity by the fractalkine receptor. *Nat Neurosci* 2006; 9: 917-24.
- [58] Schmid CD, Sautkulis LN, Danielson PE, *et al.* Heterogeneous expression of the triggering receptor expressed on myeloid cells-2 on adult microglia. *J Neurochem* 2002; 83: 1309-20.
- [59] Bouchon A, Hernandez-Munain C, Cella M, Colonna M. A DAP12-mediated pathway regulates expression of CC chemokine receptor 7 and maturation of human dendritic cells. *J Exp Med* 2001; 194: 1111-22.
- [60] Lanier LL, Corliss BC, Wu J, Leong C, Phillips JH. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. *Nature* 1998; 391: 703-7.
- [61] Ford JW, McVicar DW. TREM and TREM-like receptors in inflammation and disease. *Curr Opin Immunol* 2009; 21: 38-46.
- [62] Colonna M. DAP12 signaling: from immune cells to bone modeling and brain myelination. *J Clin Invest* 2003; 111: 313-4.
- [63] Chouery E, Delague V, Bergougnoux A, Koussa S, Serre JL, Megarbane A. Mutations in TREM2 lead to pure early-onset dementia without bone cysts. *Hum Mutat* 2008; 29: E194-204.
- [64] Paloneva J, Kestila M, Wu J, *et al.* Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. *Nat Genet* 2000; 25: 357-61.
- [65] Piccio L, Buonsanti C, Mariani M, *et al.* Blockade of TREM-2 exacerbates experimental autoimmune encephalomyelitis. *Eur J Immunol* 2007; 37: 1290-301.
- [66] Piccio L, Buonsanti C, Cella M, *et al.* Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. *Brain* 2008; 131: 3081-91.
- [67] Hsieh CL, Koike M, Spusta SC, *et al.* A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. *J Neurochem* 2009; 109: 1144-56.
- [68] Sospedra M, Martin R. Immunology of multiple sclerosis. *Ann Rev Immunol* 2005; 23: 683-47.
- [69] Hauser SL, Oksenberg J. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. *Neuron* 2006; 52: 61-7.
- [70] Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Ann Neurol* 2007; 61: 288-99.
- [71] Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. *Ann Neurol* 2007; 61: 504-13.
- [72] Benveniste EN. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. *J Mol Med* 1997; 75: 165-73.
- [73] Platten M, Steinman L. Multiple sclerosis:trapped in deadly glue. *Nat Med* 2005; 11: 252-3.
- [74] Marik C, Felts PA, Bauer J, Lassman H, Smith KJ. Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? *Brain* 2007; 130: 2800-15.
- [75] Prineas JW, Wright RG. Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. *Lab Invest* 1978; 38: 409-21.
- [76] Bogie JF, Stinissen P, Hellings N, Hendriks JJ. Myelin-phagocytosing macrophages modulate autoreactive T cell proliferation. *J Neuroinflammation* 2011; 8: 85.
- [77] Fabrick BO, Van Haastert ES, Galea I, *et al.* CD163-positive perivascular macrophages in the human CNS express molecules for antigen recognition and presentation. *Glia* 2005; 51: 297-305.
- [78] Owens T, Bechmann I, Engelhardt B. Perivascular spaces and the two steps to neuroinflammation. *J Neuropathol Exp Neurol* 2008; 67: 1113-21.
- [79] Colton CA. Heterogeneity of microglial activation in the innate immune response in the brain. *J Neuroimmune Pharmacol* 2009; 4(4): 399-418.
- [80] Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity* 2010; 32: 593-604.
- [81] Rao KM. MAP kinase activation in macrophages. *J Leukoc Biol* 2001; 69: 3-10.
- [82] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004; 25: 677-86.
- [83] Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 2005; 308: 1314-8.
- [84] Garden GA, Moller T. Microglial biology in health and disease. *J Neuroimmune Pharmacol* 2006; 1: 127-37.
- [85] Correa F, Docagne F, Mestre L, *et al.* A role for CB2 receptors in anandamide signalling pathways involved in the regulation of IL-12 and IL-23 in microglial cells. *Biochem Pharmacol* 2009; 77: 86-100.
- [86] Smith ME. Phagocytic properties of microglia *in vitro*: implications for a role in multiple sclerosis and EAE. *Microsc Res Tech* 2001; 54: 81-94.
- [87] Boven LA, Van Meurs M, Van Zwam M, *et al.* Myelin-laden macrophages are anti-inflammatory, consistent with foam cells in multiple sclerosis. *Brain* 2006; 129: 517-26.
- [88] Kotter MR, Zhao C, Van RN, Franklin RJ. Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression. *Neurobiol Dis* 2005; 18: 166-75.
- [89] Trebst C, Koning F, Ransohoff R, Bruck W, Stangel M. CCR5 expression on macrophages/microglia is associated with early remyelination in multiple sclerosis lesions. *Mult Scler* 2008; 14: 728-33.

- [90] Adams RD, Kubik CS. The morbid anatomy of the demyelinating disease. *Am J Med* 1952; 12: 510-46.
- [91] Van der Valk P, De Groot CJ. Staging of multiple sclerosis (MS) lesions: pathology of the time frame of MS. *Neuropathol Appl Neurobiol* 2000; 26: 2-10.
- [92] Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; 338: 278-85.
- [93] Breyer EC, Brink BP, Veerhuis R, *et al.* Homogeneity of active demyelinating lesions in established multiple sclerosis. *Ann Neurol* 2008; 63: 16-25.
- [94] Galea I, Bechmann I, Perry VH. What is immune privilege (not)? *TRENDS Immunol* 2006; 28: 12-8.
- [95] Wake H, Moorhouse AJ, Jinno S, K ohsaka S, Nabekura J. Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J Neurosci* 2009; 29: 3974-80.
- [96] Muzio L, Martino G, Furlan R. Multifaceted aspects of inflammation in multiple sclerosis: the role of microglia. *J Neuroimmunol* 2007; 191: 39-44.
- [97] Koning N, Bo L, Hoek RM, Huitinga I. Downregulation of macrophage inhibitory molecules in multiple sclerosis lesions. *Ann Neurol* 2007; 62: 504-14.
- [98] Preston S, Wright RG, Starr K, Barclay AN, Brown MH. The leukocyte/neuron cell surface antigen OX2 binds to a ligand on macrophages. *Eur J Immunol* 1997; 27: 1911-8.
- [99] Wright GJ, Cherwinski H, Foster-Cuevas M, *et al.* Characterization of the CD200 receptor family in mice and humans and their interaction with CD200. *J Immunol* 2003; 171: 3034-46.
- [100] Wright GJ, Puklavec MJ, Willis AC, *et al.* Lymphoid/neuronal cell surface OX2 glycoprotein recognizes a novel receptor on macrophages implicated in the control of their function. *Immunity* 2000; 13: 233-42.
- [101] Jenmalm MC, Cherwinski H, Bowman EP, Phillips JH, Sedgwick JD. Regulation of myeloid cell function through the CD200 receptor. *J Immunol* 2006; 176: 191-9.
- [102] Zhang S, Chervinski H, Sedgwick JD, Phillips JH. Molecular mechanisms of CD200 inhibition of mast cell activation. *J Immunol* 2004; 173: 6786-93.
- [103] Hernangómez M, Mestre L, Correa FG, *et al.* CD200-CD200R1 interaction contributes to neuroprotective effects of anandamide on experimentally induced inflammation. *Glia* 2012; 60: 1437-50.
- [104] Shivastava K, Gonzalez P, Acarín L. The immune inhibitory complex CD200-CD200R is developmentally regulated in the mouse brain. *J Comp Neurol* 2012; 520: 2657-75.
- [105] Banerjee D, Dick AD. Blocking CD200-CD200 receptor axis augments NOS-2 expression and aggravates experimental uveoretinitis in Lewis rats. *Ocul Immunol Inflamm* 2004; 12: 115-25.
- [106] Meuth SG, Simon OJ, Grimm A, *et al.* CNS inflammation and neuronal degeneration is aggravated by impaired CD200-CD200R-mediated macrophage silencing. *J Neuroimmunol* 2008; 194: 62-9.
- [107] McWhirter JR, Kretz-Rommel A, Saven A, *et al.* Antibodies selected from combinatorial libraries block a tumor antigen that plays a key role in immunomodulation. *Proc Natl Acad Sci USA* 2006; 103: 1041-6.
- [108] Pertwee RG, Howlett AC, Abood ME, *et al.* Cannabinoid receptors and their ligands: beyond CB1 and CB2. LXXIX International Union of basic and clinical pharmacology. *Pharmacol Rev* 2010; 62: 588-631.
- [109] Stella N. Cannabinoid and cannabinoids like receptors in microglia, astrocytes and astrocytomas. *Glia* 2010; 58: 1017-30.
- [110] O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol* 2007; 152: 576-82.
- [111] O'Sullivan SE, Kendall DA. Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory diseases. *Immunobiology* 2010; 215: 611-26.
- [112] Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990; 346: 561-4.
- [113] Tsou K, Brown S, Sañudo-Peña MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 1998; 83: 393-411.
- [114] Howlett AC, Barth F, Bonner TI, *et al.* Classification of cannabinoid receptors. XXVII International Union of Pharmacology. *Pharmacol Rev* 2002; 54: 161-202.
- [115] Mackie K, Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* 1992; 89: 3825-9.
- [116] Mackie K, Lai Y, Westenbroek R, Mitchell R. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptors. *J Neurosci* 1995; 15: 6552-61.
- [117] Marsicano G, Goodenough S, Monory K, *et al.* CB1 cannabinoid receptors and on demand defense against excitotoxicity. *Science* 2003; 302: 84-8.
- [118] Galiègue S, Mary S, Marchand J, *et al.* Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* 1995; 232: 54-61.
- [119] Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993; 365: 61-5.
- [120] Sugiura T, Kondo S, Sukagawa A, *et al.* 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 1995; 215: 89-97.
- [121] Molina-Holgado E, Vela JM, Arévalo-Martín A, *et al.* Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3-kinase/Akt signaling. *J Neurosci* 2002; 22: 9742-53.
- [122] Benito C, Romero JP, Tolon RM, *et al.* Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis. *J Neurosci* 2007; 27: 2396-402.
- [123] Brusco A, Tagliaferro P, Saez T, Onaivi ES. Postsynaptic localization of CB2 cannabinoid receptors in the rat hippocampus. *Synapse* 2008; 62: 944-9.
- [124] Bayewitch M, Avidor-Reiss T, Levy R, Barg J, Mechoulam R, Vogel Z. The peripheral cannabinoid receptor: adenylate cyclase inhibition and G protein coupling. *FEBS Lett* 1995; 375: 143-7.
- [125] McKallip RJ, Lombard C, Martin BR, Nagarkatti M, Nagarkatti PS. Delta9 tetrahydrocannabinol-induced apoptosis in the thymus and spleen as a mechanism of immunosuppression *in vitro* and *in vivo*. *J Pharmacol Exp Therap* 2002; 302: 451-65.
- [126] McCoy KL, Matveyeva M, Carlisle SJK, Cabral GA. Cannabinoid inhibition of the processing of intact lysozyme by macrophages: evidence for CB2 receptor participation. *J Pharmacol Exp Therap* 1999; 289: 1620-5.
- [127] Buckley NE, McCoy KL, Mezey E, *et al.* Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB2 receptor. *Eur J Pharmacol* 2000; 396: 141-9.
- [128] Devane WA, Hanus L, Breuer A, *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992; 258: 1946-9.
- [129] Di Marzo V, Fontana A, Cadas H, *et al.* Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 1994; 372: 686-91.
- [130] Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D. Dopamine activation of endogenous cannabinoid signalling in dorsal striatum. *Nat Neurosci* 1999; 2: 358-63.
- [131] Stella N, Piomelli D. Receptor dependent formation of endogenous cannabinoids in cortical neurons. *Eur J Pharmacol* 2001; 425: 189-96.
- [132] Mechoulam R, Ben-Shabat S, Hanus L, *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 1995; 50: 83-90.
- [133] Walter L, Stella N. Endothelin-1 increases 2-arachidonoyl glycerol (2-AG) production in astrocytes. *Glia* 2003; 44: 85-90.
- [134] Carrier EJ, Kearn CS, Barkmeier AJ, *et al.* Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonoyl glycerol, which increases proliferation via a CB2 receptor-dependent mechanism. *Mol Pharmacol* 2004; 65: 999-1007.
- [135] Beltramo M, Piomelli D. Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonoyl glycerol. *Neuropharmacology* 2000; 11: 1231-5.
- [136] Witting A, Walter L, Wacker J, Moller T, Stella N. P2X7 receptors control 2-arachidonoylglycerol production by microglial cells. *Proc Natl Acad Sci USA* 2004; 101: 3214-9.
- [137] Facci L, dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc Natl Acad Sci USA* 1995; 92: 3376-80.

- [138] Di Marzo V, de Petrocellis L, Sepe N, Buono A. Biosynthesis of anandamide and related acylethanolamides in mouse J774 macrophages and N18 neuroblastoma cells. *Biochem J* 1996; 316: 977-84.
- [139] Bisogno T, Maurelli S, Melck D, de Petrocellis L, di Marzo V. Biosynthesis, uptake and degradation of anandamide and palmitoylethanolamide in leukocytes. *J Biol Chem* 1997; 272: 3315-23.
- [140] Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuro-modulatory fatty-acid.amides. *Nature* 1996; 384: 83-7.
- [141] Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonoyl glycerol, to glyceril prostaglandins by cyclooxygenase-2. *J Biol Chem* 2000; 275: 33744-9.
- [142] Yu M, Ives D, Ramesha CS. Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. *J Biol Chem* 1997; 272: 21181-6.
- [143] Marrs WR, Blankman JL, Horne EA, *et al.* The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-arachidonoyl glycerol at cannabinoid receptors. *Nat Neurosci* 2010; 95: 1-7.
- [144] Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. *Science* 2002; 296: 678-82.
- [145] Brenowitz SD, Regehr WG. Associative short-term synaptic plasticity mediated by endocannabinoids. *Neuron* 2005; 45: 419-31.
- [146] Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M. Subcellular arrangement of molecules for 2-arachidonoyl glycerol mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J Neurosci* 2007; 27: 3663-76.
- [147] Fletcher JM, Lalor SJ, Sweeney CM, Tubridy N, Mills KH. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin Exp Immunol* 2010; 162: 1-11.
- [148] Zajicek JP, Sanders HP, Wright DE, *et al.* Cannabinoids and multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up. *J Neurol Neurosurg Psychiatry* 2005; 76: 1664-9.
- [149] Zajicek JP, Apostu VI. Role of cannabinoids in multiple sclerosis. *CNS Drugs* 2011; 25: 187-201.
- [150] Baker D, Pryce G, Croxford JL, *et al.* Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature* 2000; 404: 84-7.
- [151] Arévalo-Martín A, Vela JM, Molina-Holgado E, Borrell J, Guaza C. Therapeutic action of cannabinoids in a murine model of multiple sclerosis. *J Neurosci* 2003; 23: 2511-6.
- [152] Croxford J L, Miller SD. Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R+WIN55,212. *J Clin Invest* 2003; 111: 1231-40.
- [153] Arévalo-Martín A, García-Ovejero D, Gómez O, *et al.* CB2 cannabinoid receptors as an emerging target for demyelinating diseases: from neuroimmune interactions to cell replacement strategies. *Br J Pharmacol* 2008; 153: 216-25.
- [154] de Lago E, Moreno-Martel M, Cabranes A, Ramos JA, Fernández-Ruiz J. Cannabinoids ameliorate disease progression in a model of multiple sclerosis in mice acting preferentially through CB₁ receptors mediated anti-inflammatory effects. *Neuropharmacology* 2012; 62: 2299-308.
- [155] Docagne F, Mestre L, Loria F, Hernangómez M, Correa F, Guaza C. Therapeutic potential of CB₂ targeting in multiple sclerosis. *Expert Opin Ther Targets* 2008; 12: 185-95.
- [156] Wolf SA, Ullrich O. Endocannabinoids and the brain immune system: new neurons at the horizon? *J Neuroendocrinol* 2008; 20: 15-9.
- [157] Molina-Holgado E, Molina-Holgado F. Mending the broken brain: Neuroimmune interactions in neurogenesis. *J Neurochem* 2010; 114: 1277-90.
- [158] Lambert DM, Fowler CJ. The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. *J Med Chem* 2005; 48: 5059-87.
- [159] Cabranes A, Venderova K, de Lago E, *et al.* Decreased endocannabinoid levels in the brain and beneficial effects of agents activating cannabinoid and/or vanilloid receptors in a rat model of multiple sclerosis. *Neurobiol Dis* 2005; 20: 207-17.
- [160] Cabral GA, Griffin-Thomas L. Emerging role of the cannabinoid receptor CB₂ in immune regulation: therapeutic prospects for neuroinflammation. *Expert Rev Mol Med* 2009; 20: 11-24.
- [161] Mestre L, Correa F, Arévalo-Martín A, *et al.* Pharmacological modulation of the endocannabinoid system in a viral model of multiple sclerosis. *J Neurochem* 2005; 92: 1327-39.
- [162] Ortega-Gutiérrez S, Molina-Holgado E, Arévalo-Martín A, *et al.* Activation of the endocannabinoid system as therapeutic approach in a murine model of multiple sclerosis. *FASEB J* 2005; 19: 1338-45.
- [163] Loria F, Petrosino S, Hernangómez M, *et al.* An endocannabinoid tone limits excitotoxicity *in vitro* and in a model of multiple sclerosis. *Neurobiol Dis* 2010; 37(1): 166-76.
- [164] Cabral GA, Marciano-Cabral F. Cannabinoid receptors in microglia of the central nervous system: immune functional relevance. *J Leukoc Biol* 2005; 78: 1192-7.
- [165] Stella N. Cannabinoid signaling in microglial cells. *Neuropharmacology* 2009; 56: 244-53.
- [166] Walter L, Franklin A, Witting A, *et al.* Non-psychotropic cannabinoid receptors regulate microglia cell migration. *J Neurosci* 2003; 23: 1398-405.
- [167] Centonze D, Bari M, Rossi S, *et al.* The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain* 2007; 130: 2543-53.
- [168] Eljaschewitsch E, Witting A, Mawrin C, *et al.* The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron* 2006; 49: 67-79.
- [169] Pryce G, Baker D. Potential control of multiple sclerosis by cannabis and the endocannabinoid system. *CNS Neurol Disord Drug Targets* 2012; 11: 624-41.
- [170] Ligresti A, Cascio MG, Pryce G, *et al.* New potent and selective inhibitors of anandamide reuptake with antispastic activity in a mouse model of multiple sclerosis. *Br J Pharmacol* 2006; 147: 83-91.
- [171] Scotter EL, Abood ME, Glass M. The endocannabinoid system as a target for the treatment of neurodegenerative diseases. *Br J Pharmacol* 2010; 160: 480-98.
- [172] D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* 1993; 178: 1041-8.
- [173] Correa F, Hernangómez M, Mestre L, *et al.* Anandamide enhances IL-10 production in activated microglia by targeting CB(2) receptors: roles of ERK1/2, JNK, and NF-kappaB. *GLIA* 2010; 58: 135-47.
- [174] Correa F, Hernangómez M, Mestre L, Loria F, Docagne F, Guaza C. The endocannabinoid anandamide downregulates IL-23 and IL-12 subunits in a viral model of multiple sclerosis: Evidence for a cross-talk between IL-12p70/IL-23 axis and IL-10 in microglial cells. *Brain Behav Immun* 2011; 25: 736-49.
- [175] Correa FG, Mestre L, Docagne F, Borrell J, Guaza C. The endocannabinoid anandamide from immunomodulation to neuroprotection. Implications for multiple sclerosis. *Vitam Horm* 2009; 81: 207-30.
- [176] Sánchez AJ, García-Merino A. Neuroprotective agents: cannabinoids. *Clin Immunol* 2012; 142: 57-67.
- [177] Akiyama H, Barger S, Barnum S, *et al.* Inflammation and Alzheimer's disease. *Neurobiol Aging* 2000; 21: 383-421.
- [178] Wilkinson BL, Landreth GE. The microglial NADPH oxidase complex as a source of oxidative stress in Alzheimer's disease. *J Neuroinflammation* 2006; 3: 30-41.
- [179] McGeer EG, McGeer PL. Inflammatory processes in Alzheimer disease. *Prog Neuro-Psychopharmacol* 2003; 27: 741-9.
- [180] Sastre M, Klockgether T, Heneka MT. Contribution of inflammatory processes to Alzheimer's disease: molecular mechanisms. *Int J Dev Neurosci* 2006; 24: 167-76.
- [181] Walker DG, Dalsing-Hernández JE, Campbell NA, Lue LF. Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation. *Exp Neurol* 2009; 215: 5-19.
- [182] Ramirez BC, Blazquez C, Gomez del Pulgar T, Guzman M, de Ceballos M. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci* 2005; 25: 1904-13.
- [183] Combs CK, Karlo JC, Kao SC, Landreth GE. Beta-Amyloid stimulation of microglia and monocytes results in TNF-alpha-dependent expression of inducible nitric oxide synthase and neuronal apoptosis. *J Neurosci* 2001; 21: 1179-88.
- [184] Mackenzie IR, Munoz DG. Non-steroidal antiinflammatory drug use and Alzheimer type pathology in aging. *Neurology* 1998; 50: 986-90.

- [185] Masocha W. Systemic lipopolysaccharide (LPS)-induced microglial activation results in different temporal reduction of CD200 and CD200 receptor gene expression in the brain. *J Neuroimmunol* 2009; 214: 78-82.
- [186] Gorczynski RM. CD200 and its receptor as targets for immunoregulation. *Curr Opin Investig Drugs* 2005; 6: 483-8.
- [187] Cox FF, Carney D, Miller AM, Lynch MA. CD200 fusion protein decreases microglial activation in the hippocampus of aged rats. *Brain Behav Immun* 2012; 26: 789-96.
- [188] Gorczynski RM, Chen Z, Yu K, Hu J. CD200 immunoadhesin suppresses collagen-induced arthritis in mice. *Clin Immunol* 2001; 101: 328-34.
- [189] Lyons A, Downer EJ, Crotty S, Nolan YM, Mills KHG, Lynch M. CD200 ligand receptor interaction modulates microglial activation *in vitro* and *in vivo*: A role for IL-4. *J Neurosci* 2007; 27: 8309-13.
- [190] Lue LF, Kuo YM, Beach T, Walker DG. Microglia activation and anti-inflammatory regulation in Alzheimer's disease. *Mol Neurobiol* 2010; 41: 115-28.
- [191] Nolan Y, Maher FO, Martin DS, *et al.* Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus. *J Biol Chem* 2005; 280: 9354-62.

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