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REVIEW

CB_2 cannabinoid receptors as an emerging target for demyelinating diseases: from neuroimmune interactions to cell replacement strategies

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Amongst the various demyelinating diseases that affect the central nervous system, those induced by an inflammatory response stand out because of their epidemiological relevance. The best known inflammatory-induced demyelinating disease is multiple sclerosis, but the immune response is a common pathogenic mechanism in many other less common pathologies (e.g., acute disseminated encephalomyelitis and acute necrotizing haemorrhagic encephalomyelitis). In all such cases, modulation of the immune response seems to be a logical therapeutic approach. Cannabinoids are well known immunomodulatory molecules that act through CB_1 and CB_2 receptors. While activation of CB_1 receptors has a psychotropic effect, activation of CB_2 receptors alone does not. Therefore, to bypass the ethical problems that could result from the treatment of inflammation with psychotropic molecules, considerable effort is being made to study the potential therapeutic value of activating CB_2 receptors. In this review we examine the current knowledge and understanding of the utility of cannabinoids as therapeutic molecules for inflammatory-mediated demyelinating pathologies. Moreover, we discuss how CB_2 receptor activation is related to the modulation of immunopathogenic states.
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Abbreviations: APCs, antigen-presenting cells; CNS, central nervous system; CREAE, chronic relapsing experimental allergic encephalomyelitis; CXCL12, stromal cell-derived factor; DCs, dendritic cells; DHT, delayed-type hypersensitivity; EAE, experimental allergic encephalomyelitis; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; KO, knockout; LPS, lipopolysaccharide; MBP, myelin basic protein; MHC, major histocompatibility complex; MOG, myelin oligodendroglial glycoprotein; MS, multiple sclerosis; NSCs, neural stem cells; PI3K/Akt, phosphoinositide 3-kinase/protein kinase B; PNS, peripheral nervous system; PSA-NCAM, polysialylated neural cell adhesion molecule; SGZ, subgranular zone; SVZ, subventricular zone; Ths, T-helper cells; TMEV-IDD, Theiler's murine encephalomyelitis virus-induced demyelinating disease; TNF-α, tumour necrosis factor alpha; VCAM-1, vascular cellular adhesion molecule-1; WT, wild type; Δ^9-THC, delta-9-tetrahydrocannabinol

Introduction

Demyelination is the loss of the myelin sheath that surrounds axons and it may affect both the central nervous system (CNS) and peripheral nervous system (PNS). Demyelinating pathologies may have a primary genetic aetiology (leukodystrophies) or may be the secondary effect of infections, vascular alterations, toxic insults or inflammatory reactions. Regardless of the cause, the result will be the total or partial loss of function in the demyelinated area, depending on the region of the nervous system affected. In this review we shall focus on the CNS demyelinating diseases in which inflammation plays a role in their pathogenesis, like multiple sclerosis (MS). However, we will also discuss other effects mediated by CB_2 that are fundamental in demyelinating diseases, including neuronal and glial protection, the regulation of stem/precursor cells and cell replacement. MS is the most frequent neurological disease in young adults. In addition to myelin loss, there is also neuronal damage in MS that further contributes to the symptomatology

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(Ferguson *et al*., 1997; Mews *et al*., 1998; Trapp *et al*., 1998, 1999). Even though several hypotheses have been proposed to explain its aetiopathology, this issue remains unresolved. Epidemiological studies suggest that MS occurs in a genetically susceptible population that has been in contact with an environmental factor, most probably a viral infection. This triggers an immune response that, by diverse mechanisms is finally directed towards myelin self-peptides (Kurtzke, 1993; Johnson, 1994; Coraddu *et al*., 1998; Haines *et al*., 1998). Accordingly, the CSF of MS patients contains antibodies against myelin basic protein (MBP) and myelin oligodendroglial glycoprotein (MOG; Olsson and Nilsson, 1979; Miller *et al*., 1983). This autoimmune response against myelin peptides has been reproduced in animal models of MS, such as experimental allergic encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD; Rauch *et al*., 1987; Rodriguez *et al*., 1988; Fujinami, 1989; Yamada *et al*., 1990). In both MS- and EAE-, activated lymphocytes are detected that recognize several epitopes of myelin proteins and these self-reactive clones have been reported to be involved in the pathogenesis of EAE and TMEV-IDD (Gerety *et al*., 1994; Steinman, 1996; Schmidt *et al*., 1997; Pope *et al*., 1998; Hellings *et al*., 2002). The main reason for inflammatory-mediated damage is thought to be the release of reactive oxygen and nitrogen species by immune cells, as well as that of proteases, which directly mediate cell damage (Correa *et al*., 2005a, b). In addition, immune cells release cytotoxic/cytostatic cytokines, that not only cause damage but that may also enhance the release of more reactive species and glutamate by the cells in the surrounding tissue (the pathogenic mechanisms of demyelinating diseases are summarized in Figure 1). Modulation of the immune response could therefore be an important therapeutic approach to treat demyelinating conditions.

Since the late 1970s, it was clear that the psychoactive compounds of *Cannabis sativa*, such as delta-9-tetrahydrocannabinol (Δ^9-THC), modulate the inflammatory response (Zimmerman *et al*., 1977; Smith *et al*., 1978). In the following years, numerous studies showed that cannabinoids increased the susceptibility of animals to experimentally induced infections, as these molecules acted as anti-inflammatory drugs. The discovery of this undesirable effect of cannabinoids within the context of infection led to the idea that they could be used against inflammation in diseases like MS. Indeed, in the 1980s, a cannabinoid agonist was first shown to be effective in a CNS demyelinating pathology (Lyman *et al*., 1989). In this study, animals pretreated with Δ^9-THC displayed a delay in the onset of the symptoms and a reduction in severity when EAE was subsequently induced. In corresponding histological studies, a decrease in cell infiltration into the spinal cord was observed, which correlated with the symptomatology of the inflammatory process. The identification of the so-called peripheral cannabinoid receptor (Munro *et al*., 1993) and further studies on the function of this receptor identified the new CB_2 receptor as the major participant in cannabinoid-mediated immune modulation. Indeed, the CB_2 receptor was found to be expressed primarily in cells of the immune and haematopoietic systems (Munro *et al*., 1993) although more recently it was found in the brain (Van Sickle *et al*., 2005; Gong *et al*., 2006). CB_2 receptors are G-protein-coupled receptors that primarily associate with the Gi/o subtypes of G proteins, and they activate signalling pathways that may involve adenylate cyclase inhibition or activation of mitogen-activated protein kinases (reviewed in Howlett and Mukhopadhyay, 2000; Pacher *et al*., 2006; Fernandez-Ruiz *et al*., 2007).

The effects of CB_2 activation on demyelination through modulation of the inflammatory response

The first studies on the potential therapeutic role of cannabinoids in demyelinating diseases were aimed at evaluating its usefulness as a symptomatic treatment for spasticity and tremor in patients with MS (Clifford, 1983; Ungerleider *et al*., 1987). However, the involvement of CB_1 or CB_2 was not assessed since they were yet to be cloned at that time. While the administration of a CB_2 agonist to mice with chronic relapsing experimental allergic encephalomyelitis (CREAE) ameliorated spasticity, the use of a CB_2 antagonist worsened it (Baker *et al*., 2001). As well as using CB_2 agonists for symptomatic treatment of demyelinating pathologies, cannabinoids may serve as therapeutic agents. In this scenario, CB_2 is expressed in MS plaques by microglia, lymphocytes and astrocytes (Benito *et al*., 2007). Indeed, intraperitoneal injection of a selective CB_2 agonist over 10 days to mice with established TMEV-IDD improves their motor function by modulating microglia and lymphocyte infiltration into the spinal cord (Arévalo-Martin *et al*., 2003). As with MS, there is an autoimmune response against myelin peptides in TMEV-IDD that contribute to the pathology (Gerety *et al*., 1994; Merrill and Benveniste, 1996; Steinman, 1996). In essence, myelin-specific $CD4^+$ peripheral T cells are activated and enter the spinal cord where, after recognizing their specific epitope, they differentiate into a T-helper cell (Th1) phenotype and elaborate a delayed-type hypersensitivity (DTH) response (Figure 1). In DTH responses, not only the immune cells are involved but also, the surrounding tissue cells respond to the signals released by the immune system. CB_2 receptors are expressed by all cells in the immune system both in humans and rodents (Galiegue *et al*., 1995; Lee *et al*., 2001; Matias *et al*., 2002), as well as by resident CNS cells. We shall analyse, here, how CB_2 receptor activation can improve an immune-mediated demyelinating disorder through its effects on the following cell types: $CD4^+$ T cells, B lymphocytes, dendritic cells (DCs), microglia/macrophages and astrocytes. However, it should be noted here that CB_2 is highly inducible and its presence or absence in culture models may or may not reflect the native situation *in vivo*.

$CD4^+$ T cells

Treatment of TMEV-IDD mice with a CB_2 agonist reduces the infiltration of $CD4^+$ T cells to the spinal cord (Arévalo-Martín *et al*., 2003). Among other effects, this could be the result of inhibiting the migration of these cells from the periphery to the CNS since the CB_2 selective ligands JWH-133 and JWH-015 inhibit stromal cell-derived factor

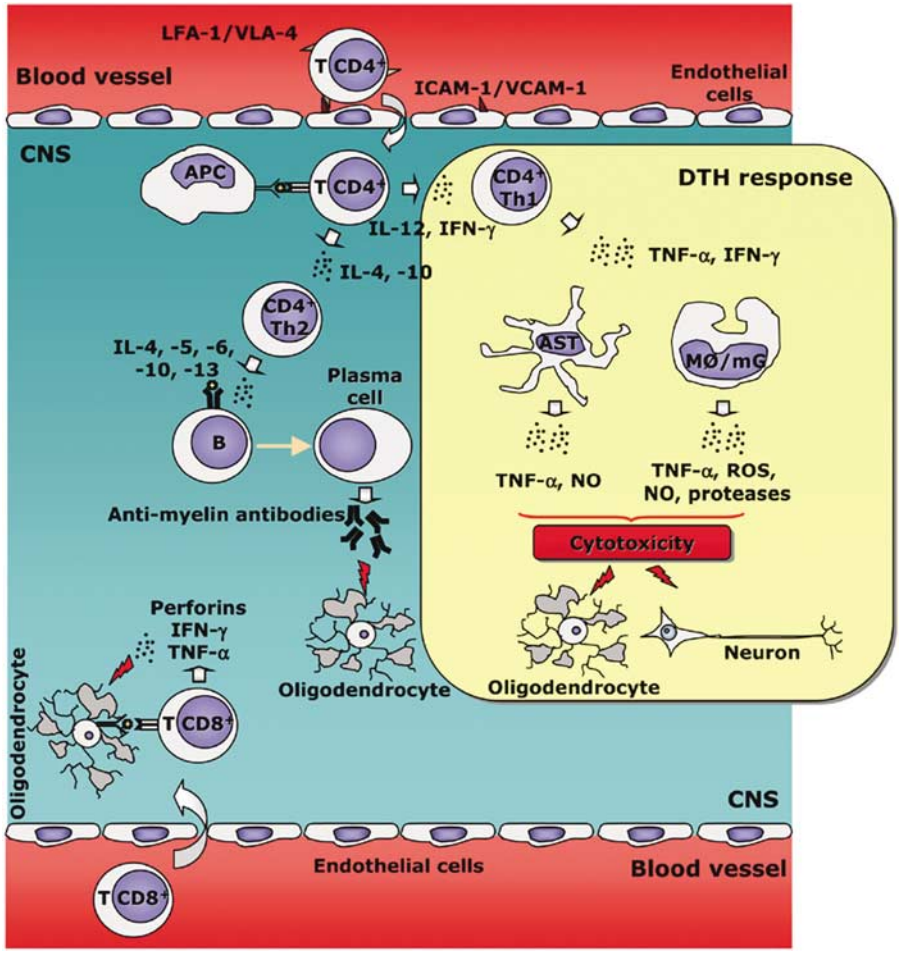

Figure 1 Hypothetic immune-mediated demyelinating disease pathogenesis. Primed $CD4^+$ T lymphocytes migrate to the CNS through blood vessels enriched in adhesion molecules. Once in the CNS, DCs or macrophages/microglia acting as APC present the antigen to antigen-specific $CD4^+$ lymphocytes. Depending on the environmental signals that these cells receive, the $CD4^+$ lymphocytes acquire different T helper (Th) phenotypes. Th1 cells will establish an antigen-specific DTH response that will lead to the liberation of several cytotoxic molecules by the Th1 themselves, astrocytes and microglia/macrophages. In addition, the generation of the Th2 phenotype will result in the activation of infiltrated B cells into plasma cells that will secrete antibodies against myelin antigens. This response has been related both with oligodendroglial damage and recovery. Even though their pathogenic contribution to MS and EAE is not clear, $CD8^+$ T cells recognize specific antigens and they may elaborate a cytotoxic response against the cells that presented the antigen. CNS, central nervous system; DCs, dendritic cells; DTH, delayed-type hypersensitivity; EAE, experimental allergic encephalomyelitis; LFA-1, lymphocyte function-associated antigen-1; MS, multiple sclerosis; VLA-4, very late activation antigen-4.

(CXCL12)-induced chemotaxis and transendothelial migration (Ghosh *et al*., 2006; Coopman *et al*., 2007). The reduced infiltration of $CD4^+$ T cells into the spinal cord may also be explained through CB_2 activation to induce apoptosis of these cells (Sanchez *et al*., 2006; Lombard *et al*., 2007). Remarkably, some of the effects induced by CB_2 agonists are not completely blocked by CB_2 antagonists and they seem to be independent of CB_1 receptors (Sanchez *et al*., 2006). Thus, it should be borne in mind that there is increasing evidence of non-CB_1- and non-CB_2-mediated effects of many cannabinoids. Nevertheless, here we will focus on the studies where the effects are mediated partially or entirely by CB_2 receptors.

Whether by inhibiting the migration of leukocytes or promoting the apoptosis of these cells, the effect of the reduced infiltration of these cells into the CNS is beneficial as there is a decrease in the release of Th1 cytokines (interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) or interleukin-12 (IL-12)) into the surrounding tissue, which is involved in tissue damage. It is known that cannabinoids alter the profile of cytokine expression from a Th1 to a Th2 phenotype in a CB_2-dependent manner (Yuan *et al*., 2002). In addition, the treatment of TMEV-IDD mice with the non-selective CB_1 and CB_2 agonist WIN 55212-2 inhibits the myelin-specific DTH response and the release of IFN-γ from $CD4^+$ T cells (Croxford and Miller, 2003). IFN-γ has been shown to exacerbate MS, reducing the survival of oligodendrocytes and affecting normal myelination (Panitch *et al*., 1987; Corbin *et al*., 1996; Horwitz *et al*., 1997; Molina-Holgado *et al*., 2001). In addition IFN-γ induces the expression of the vascular cellular adhesion molecule-1 (VCAM-1) by endothelial cells, which favours the access of activated lymphocytes to the CNS (Groves *et al*., 1993; Weiser *et al*., 2007). Therefore, reducing the release of IFN-γ by the Th1 cells could itself block the 'calling' signal and therefore, decrease the migration of more primed T cells to the CNS that would amplify the damage.

B lymphocytes

As commented above, antibodies against myelin proteins are detected in both MS patients and in EAE and TMEV-IDD mice. Although their pathological contribution is not clear, since autoantibodies have been related both with damage and recovery (Rodriguez and Lennon, 1990; Brosnan and Raine, 1996), cannabinoids modulate the function of the cells that produce them through a mechanism that involves CB_2. For instance, cannabinoids are involved in the maturation of B lymphocytes to antibody-secreting plasma cells, and they are known to induce the apoptosis of these cells (Carayon *et al*., 1998; Lombard *et al*., 2007).

Microglia/macrophages

In both MS patients and animal models of the disease, activated microglia/macrophages have been related to tissue damage. The release of reactive oxygen and nitrogen species, seem to be two critical events involved in the pathogenesis of these cells, producing severe damage to myelin and toxicity in oligodendroglia and neurons (Chia *et al*., 1983; Konat and Wiggins, 1985; Rodriguez and Quddus, 1986; Liuzzi *et al*., 1995; Molina-Hogado *et al*., 2001). Treatment of activated microglia cultures with anandamide, UCM707 (an endocannabinoid uptake blocker) or WIN 55212-2, decreases the expression of the inducible NOS-2 and the production of nitrites in a CB_1- and CB_2-dependent manner (Ortega-Gutierrez *et al*., 2005; Eljaschewitsch *et al*., 2006). In addition, microglia and macrophages produce several cytokines considered to be pro-inflammatory, such as TNF-α, IL-1β or IL-12. Through CB_2 receptors, cannabinoids inhibit the expression of TNF-α, IL-1β and the p40 subunit of IL-12 and IL-23 by microglia/macrophages (Klegeris *et al*., 2003; Correa *et al*., 2005a, b). TNF-α causes neuronal death through an excitotoxic mechanism and recruits more lymphocytes to the CNS by upregulating the expression of VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) on endothelial cells (Dobbie *et al*., 1999; Weiser *et al*., 2007). The benefits derived from inhibiting IL-1β release may be related to the increase in NMDA receptor-mediated intracellular calcium provoked by this cytokine, which in turn augments glutamate-mediated neurodegeneration (Viviani *et al*., 2003). On the other hand, cannabinoids may abrogate the beneficial effects of IL-1β, which promotes the survival of mature oligodendrocytes and therefore, impedes demyelination (Vela *et al*., 2002). IL-12 is necessary for the differentiation of the lymphocytes that enter into the CNS in the Th1 phenotype, explaining the therapeutic effect of CB_2-mediated inhibition of the p40 subunit of IL-12 in monocytic cells (Shevach *et al*., 1999; Correa *et al*., 2005a, b). In addition, the p40 subunit is a constituent of IL-23, which is directly involved in the generation of the Th17 lymphocyte phenotype. This has recently been uncovered as a major participant in tissue damage (Langrish *et al*., 2005; Kroenke and Segal, 2007).

Microglia/macrophages are also involved in antigen presentation, which is necessary for the activation of primed lymphocytes that enter the CNS. Treatment of TMEV-IDD mice with a CB_2 agonist decreases microglial activation and major histocompatibility complex-II (MHC-II) expression in the spinal cord (Arévalo-Martin *et al*., 2003; Ortega-Gutierrez *et al*., 2005). Finally, specific stimulation of CB_2 receptors by JWH-015 suppresses IFN-γ-induced CD40 expression, which is involved in phagocytosis, antigen presentation and the production of cytokines. CD40 is becoming considered as a new therapeutic target for experimental autoimmune encephalomyelitis (EAE), since blocking CD40–CD154 interactions with a neutralizing antibody diminishes murine EAE disease activity (Ehrhart *et al*., 2005 and references therein).

It should be noted that CB_2 expression is highly inducible in macrophages or microglia, and its levels *in vitro* depend on the local environment and the combination of inflammatory molecules (Carlisle *et al*., 2002; Maresz *et al*., 2005). In addition, *in vivo* CB_2 is not expressed equally in all microglial populations, but rather it is predominantly present in perivascular or activated microglia (Benito *et al*., 2003; Nunez *et al*., 2004). This variability in the expression of CB_2 *in vitro* and *in vivo* must be taken into account when extrapolating the effects mediated by CB_2 in culture models to the *in vivo* situation, not only for microglia but also for other cell types.

Dendritic cells

DCs, the major antigen-presenting cells (APCs), drive the cellular fate of lymphocytes and therefore, they are critical for the development of immune responses. These cells have been involved in the pathogenesis of MS and related animal models (Greter *et al*., 2005) even though there is no direct evidence that activation of CB_2 receptors modulates their activity in MS or in animal models. However, CB_2 receptor activation does induce apoptosis of these cells, as well as promoting their migration to lymph nodes (Maestroni, 2004).

Astrocytes

Astrocytes express CB_1 and CB_2 receptors and they respond to cytokines secreted by the immune cells by regulating the production of molecules involved both in the bystander injury and in the protection of CNS tissue (Molina-Holgado F *et al*., 2002; Sheng *et al*., 2005). Among these molecules, astrocytes express NOS-2 and they produce NO in response to several inflammatory signals. Cannabinoids inhibit the release of nitrites by astrocytes through a mechanism involving the CB_2 receptor (Molina-Holgado *et al*., 1997; Molina-Holgado F *et al*., 2002; Sheng *et al*., 2005). In these cells, cannabinoids also inhibit the inflammation-induced expression of TNF-α, IL-1β and IL-6 through CB_1 and CB_2 receptors (Molina-Holgado *et al*., 1997, 1998; Ortega-Gutierrez *et al*., 2005). The implications of modulating TNF-α and IL-1β expression in inflammatory-demyelinating diseases have already been discussed. Regarding IL-6, this controversial cytokine is thought to be involved in disease development, in the protection of both neurons and oligodendroglial cells from excitotoxicity, and in remyelination (Rodriguez *et al*., 1994; Okuda *et al*., 1998; Pizzi *et al*., 2004). Regarding excitotoxic damage, it was recently shown that cannabinoids can prevent axonal damage in a viral model of MS, interfering with the excitotoxic component in

the progression of this disease in a way that requires activation of CB_2 receptors (Docagne *et al*., 2007).

CB_2 activation affects remyelination through modulation of the inflammatory response

In experimental models of demyelination there is, both neurological and histopathological, evidence of the therapeutic benefit of cannabinoids. Most data indicate that the activation of CB_1 or CB_2 receptors reduces deficits such as spasticity, tremor or neuropathic pain (Baker *et al*., 2000), whereas CB_2 receptors also regulate inflammatory aspects related to disease progression (Maresz *et al*., 2007). Recent studies show that demyelination and axonal defects that initially occur after humoral and cellular immune-mediated attacks to elements of the CNS are affected by cannabinoid treatment. We previously reported extensive remyelination of the spinal cord in TMEV-infected mice, which paralleled the functional recovery of mice treated with WIN 55212-2, ACEA and JWH-015, CB_1/CB_2, CB_1 and CB_2 receptor agonists, respectively (Arèvalo-Martín *et al*., 2003). In this model, cannabinoid-induced attenuation of the inflammatory response was linked to axon remyelination. Oligodendrocyte death and the resulting destruction of myelin plays an important role in axonal degeneration, as demyelinated axons are highly vulnerable to oxidative stress and to cytokine and glutamate toxicity (Werner *et al*., 2001). Similarly, oligodendrocytes are sensitive to microglial-derived free-radicals and mediators of inflammation (Molina-Holgado *et al*., 2001; Back *et al*., 2002; Li *et al*., 2005). Therefore, strategies aimed at reducing or slowing down the demyelination and neurodegeneration process will certainly be beneficial in MS.

It is now well accepted that once oligodendrocytes have lost their myelin membranes, they are unable to remyelinate axons even if they survive the insult (Keirstead and Blakemore, 1997). Consequently, oligodendrocyte progenitors located in the brain parenchyma and as stem cells in the subventricular zone (SVZ) are recruited to demyelinated areas following experimental demyelination and in MS to remyelinate naked axons (Keirstead and Blakemore, 1999; Chang *et al*., 2000; Menn *et al*., 2006). The specific action of cannabinoids in neural stem cells is described in the next section. However, the failure in remyelination could either be due to the constant presence of lymphocytes invading the CNS, which release IFN-γ or to the activated microglia/astrocytes that produce IL-1β, TNF-α and IL-6, among other mediators. Cannabinoids may directly enhance myelin repair by acting on oligodendrocyte progenitors, or they may act indirectly by inhibiting the immune response that might be contributing to demyelination or hampering remyelination.

The synthetic cannabinoid agonists HU210 or WIN 55212-2 act on both CB_1 and CB_2 receptors to protect oligodendrocytes from apoptosis produced by deprivation of trophic support, a mechanism dependent on phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signalling (Molina-Holgado E *et al*., 2002). Moreover, cannabinoids suppress the production of inflammatory molecules by astrocytes and microglial cells including IL-1β, TNF-α and NO (Molina-Holgado *et al*., 1997; Molina-Holgado E *et al*., 2002; Puffenbarger *et al*., 2000; Cabral *et al*., 2001), as well as enhancing the release of the anti-inflammatory cytokines IL-4, IL-10, IL-6 and interleukin-1 receptor antagonist (IL-1ra) (Molina-Holgado *et al*., 1998, 2003; Klein *et al*., 2000).

Apoptotic death of oligodendrocytes occurs following treatment with IFN-γ or TNF-α (Vartanian *et al*., 1995; Baerwald and Popko, 1998; Ye and D'Ercole, 1999). Moreover, NO released by microglia or generated from exogenous NO donors is known to induce oligodendrocyte death (Merrill *et al*., 1993). In contrast, IL-1β is not toxic for oligodendrocytes in pure culture, but it does produce apoptosis of oligodendrocytes in mixed glial cultures containing astrocytes and microglia. However, this apoptosis can be blocked by antagonists of AMPA/kainate glutamate receptors (Takahashi *et al*., 2003). Interestingly, the cannabinoid HU-210 reduced neural cell death in response to S-AMPA or NMDA receptor activation, although neurons from IL-1ra knockout (KO) mice were not protected. These results suggest that the neuroprotective and anti-inflammatory effects of cannabinoids are mediated by the release of endogenous IL-1ra in primary neurons or glial cells *in vitro*. The specific CB_1 and CB_2 receptor antagonists (SR-141716A and SR-144528, respectively) abrogated lipopolysaccharide (LPS)-induced IL-1ra release, implicating both CB receptors in this effect (Molina-Holgado *et al*., 2003). However, in chronic relapsing EAE only cannabinoid agonists acting through CB_1 receptors provide significant neuroprotection (Pryce *et al*., 2003).

The anti-inflammatory cytokines, IL-4 and IL-10 and their receptors are present in oligodendroglial cells (Molina-Holgado *et al*., 2001), and some of the effects attributed to these cytokines in models of MS (Kennedy *et al*., 1992) may have a direct influence on oligodendrocytes. Remarkably, IL-10 offered protection against oligodendroglial death evoked by LPS/IFN-γ. These data raise the question of whether IL-10 may play a protective role in demyelinating diseases, not only downregulating the function of inflammatory cells but also promoting the survival of progenitors and differentiated oligodendrocytes (Molina-Holgado *et al*., 2001).

Finally, the activation of cannabinoid receptors exerts protective effects on neurons and oligodendrocytes and suppresses chronic inflammatory responses through the attenuation of pro-inflammatory mediators. Therefore, cannabinoids might well modulate the progression of demyelinating diseases.

The effect of CB_2 receptor activation on neural stem/precursor cells

CB_2 receptors in the CNS

The past decade has seen a dramatic increase in our understanding of CB_2 cannabinoid receptor expression in the CNS, where CB_2 receptors display a more limited pattern of expression than CB_1 receptors (Pazos *et al*., 2005; Van Sickle *et al*., 2005; Gong *et al*., 2006). CB_2 receptors are thought to be mainly expressed in the immune system

(Munro *et al*., 1993); however, recent findings have clearly demonstrated that CB_2 receptors are also located in discrete neural cell populations (Van Sickle *et al*., 2005), oligodendrocyte progenitors and mature cells (Molina-Holgado E *et al*., 2002), cerebellar granular cells (Skaper *et al*., 1996), microglial cells (Benito *et al*., 2003; Walter and Stella, 2004) and endothelial cells (Golech *et al*., 2004). Moreover, cannabinoid CB_2 receptors have been identified in neural stem cells (NSCs) both *in vivo* and *in vitro*, and CB_2 selective cannabinoid agonists and antagonists modulate NSC formation and precursor cell proliferation through phosphoinositide-3 kinaseAkt and extracellular regulated kinase signalling (Palazuelos *et al*., 2006; Arevalo-Martin *et al*., 2007; Molina-Holgado *et al*., 2007). Indeed, this pathway was previously shown to mediate cannabinoid-induced survival of oligodendrocyte progenitor cells deprived of trophic support *in vitro* (Molina-Holgado E *et al*., 2002).

Neural stem cells express CB_2 receptors in vivo

Some discrete regions of active neurogenesis are maintained in the adult mammalian brain, with the capacity to generate functional neurons. These neurogenic areas include the SVZ and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, and adult NSCs appear to be capable of proliferating and differentiating within these zones (for a review see Alvarez-Buylla and Lim, 2004). In both these areas, the SVZ and SGZ, functional CB_2 receptors have been identified in neural progenitor cells and their activation has been associated with NSC proliferation (Palazuelos *et al*., 2006; Molina-Holgado *et al*., 2007). Mounting interest has focused on the dual function of the endocannabinoid system on neurogenesis in health and disease (Galve-Roperh *et al*., 2007). Hence, further studies will be necessary to determine how CB_2 signalling pathways participate in the regulation of neurogenesis during CNS development, and whether CB_2-associated signalling cascades are activated in neurodegenerative disorders.

The SGZ

Neural progenitor cells located in the SGZ of the hippocampus proliferate and give rise to immature neurons (Kempermann *et al*., 2003), which become functional neurons within 4–6 weeks (Van Praag *et al*., 2002). There is evidence that CB_2 receptors play an active role in the modulation of hippocampal neurogenesis. Indeed, hippocampal progenitors produce endocannabinoids in a developmentally regulated pattern (Fernandez-Ruiz *et al*., 2000; Aguado *et al*., 2005). Moreover, the expression of CB_2 receptors in the SGZ is restricted to neural progenitor cells (Palazuelos *et al*., 2006). The presence of functional CB_2 receptors in SGZ progenitor cells was confirmed by assessing BrdU incorporation in response to HU-308 treatment in CB_2 KO animals and their wild-type (WT) littermates. Pharmacological blockage with the CB_2 antagonist SR-144528 blocked basal progenitor proliferation while HU-308 induced proliferation, suggesting that CB_2 signalling modulates progenitor cell proliferation in the SGZ (Palazuelos *et al*., 2006).

The SVZ

In the SVZ, NSC proliferate and are a source of neurons and oligodendrocytes (Doetsch *et al*., 1999; Menn *et al*., 2006). CB_2 cannabinoid receptors are present in the postnatal rat SVZ and exogenous administration of selective CB2 receptor agonists such as JWH-056 to newborn animals affects the development of this zone (Arevalo-Martin *et al*., 2007). Additional studies confirmed that CB_2 receptors are expressed in the rat SVZ using PCR, western blotting and immunohistochemistry. This expression is somewhat complementary to that of the CB_1 receptor since CB_2 is predominantly expressed in polysialylated neural cell adhesion molecule (PSA-NCAM)-positive precursors, although there are a small number of stem cells that are immunoreactive for CB_2 receptors. Furthermore, CB_2 receptors affect SVZ function, since treatment with a selective agonist (JWH-056) increases the expression of PSA-NCAM. PSA-NCAM-expressing cells in the SVZ have been identified as migratory neuroblasts that enter the rostral migratory stream, as well as oligodendrocyte precursors that migrate towards the adjacent areas of the white matter (Doetsch *et al*., 1999; Menn *et al*., 2006). The signals controlling the physiology of postnatal SVZ development remain unknown, despite the fact that some candidates control these processes have been already identified (Lie *et al*., 2004). These findings indicate that cannabinoids may provide such signals, and they suggest that both CB_1 and CB_2 receptors may be implicated in postnatal oligodendrogenesis.

Neural stem cells express CB_2 receptors in vitro

In vitro studies, using neurospheres (clonal cellular aggregates of neural stem cells/precursors cells), stem cell lines or primary cultures of NSC derived from embryonic and adult brain, have confirmed that cells proliferating *in vitro* express functional CB_2 receptors (Palazuelos *et al*., 2006; Molina-Holgado *et al*., 2007). Indeed, CB_2 receptors are expressed in PSA-NCAM-positive cells of the rat SVZ (Arevalo-Martin *et al*., 2007). Accordingly, NSCs express fully functional CB_2 receptors and their activation promotes their proliferation leading to the generation of more neurospheres. Hence, CB_2 receptor activation could be involved in maintaining the self-renewal capacity of stem cells. Moreover, the expression of the stem cell marker Sox-2 is maintained through several passages in cultures stimulated with CB_2 agonists. Since the activation of CB_2 receptors does not produce any psychoactive effects, these results open the possibility to study whether neural stem cell behaviour could be manipulated *in vivo* through the administration of exogenous CB_2-selective agonists.

Collectively, these findings demonstrate that NSCs are targets for CB_2 agonists, and in conjunction with recent synthesis of non-psychotropic CB_2 agonists (Galve-Roperh *et al*., 2006; Zhang *et al*., 2007), this raises the possibility that CB_2 agonists could have the potential to promote brain repair. In fact, brain or spinal cord lesions activate stem/precursor cells and recruit new cells to the injured areas (Hallbergson *et al*., 2003; Romanko *et al*., 2004). Thus, promoting remyelination by endogenous progenitors or transplantation of new exogenous progenitors are promising

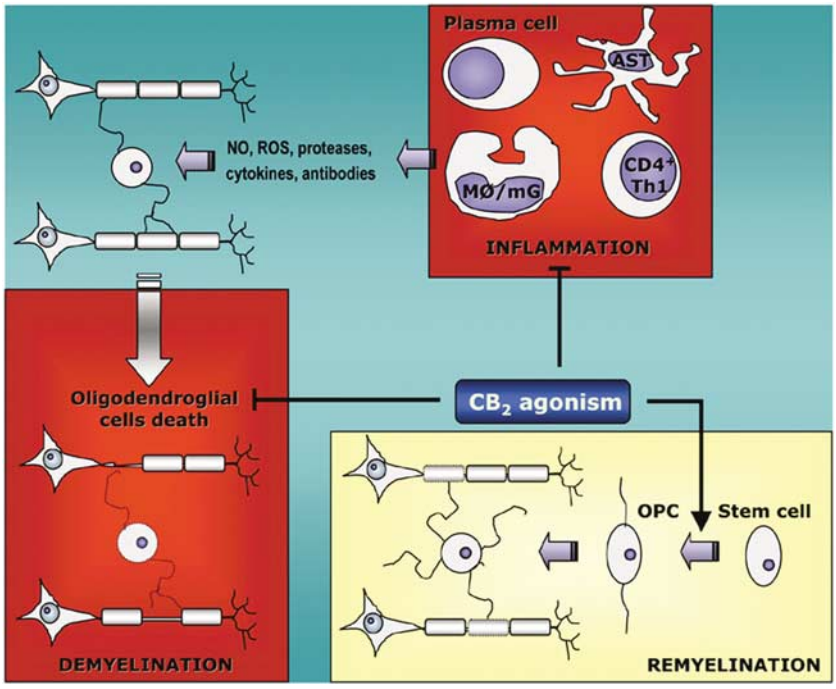

Figure 2 Points of interaction of CB_2 receptors with the pathogenesis of demyelinating diseases. The activation of CB_2 receptors decreases the deleterious inflammatory response that results in the death of both oligodendrocyte progenitors and mature oligodendrocytes in immune-mediated demyelinating pathologies. Also, CB_2 stimulation in oligodendroglial cells promotes their survival and therefore, CB_2 agonists may be useful in other non-immune-mediated demyelinating pathologies. In addition, the activation of CB_2 receptors may not only have an effect in protecting from demyelination but also, in promoting repair. In this sense, CB_2 agonists promote the proliferation of neural stem/precursor cells and increase the expression of migration-related molecules such as PSA-NCAM in the SVZ. Therefore, CB_2 receptor agonists could be therapeutic molecules that prevent the loss of myelin and promote the activation of the neural stem/precursor cells involved in the recovery of the myelin sheath. PSA-NCAM, polysialylated neural cell adhesion molecule; SVZ, subventricular zone.

therapeutic strategies for demyelinating diseases (Imitola *et al*., 2003). Either way, CB_2 agonists could be a powerful tool to aid future therapies.

Conclusions

Resident immune and CNS cells express functional CB_2 receptors. The activation of CB_2 receptors results in the modulation of the inflammatory response, restraining one of the agents responsible for the progress of demyelination and neuronal death, the ultimate causes of the symptoms in pathologies such as MS and EAE. The modulation of inflammatory molecules through CB_2 receptors could also enhance remyelination, stimulating the survival of oligodendrocyte precursors and neural stem/precursor cells, and their development into mature oligodendrocytes.

However, the role of CB_2 in controlling demyelination and enhancing remyelination is not limited to autoimmune diseases and it is not restricted to the control of the immune system (Figure 2). Both in MS, EAE and other non-immune-mediated demyelinating diseases, the protective effect of CB_2 agonists on neural cells is a remarkable advantage. Moreover, CB_2 receptor activation may be a relevant strategy in cellular replacement. However, before proposing the usefulness of CB_2 agonists for myelin disorders, it is necessary to obtain a deeper understanding of what effects may be attributable to the activation of CB_2 receptors alone, and which are also due to the participation of the CB_1 receptor. Furthermore, we must be aware of what effects may be mediated by other receptors, since there is increasing evidence that cannabinoids can induce certain effects independently of CB_1 or CB_2. Nevertheless, given our current understanding of CB_2 receptors and the pathogenesis of immune-mediated or other demyelinating disorders, these receptors seem to be of potential therapeutic interest.

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Conflict of interest

The authors state no conflict of interest.

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