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REVIEW

Cannabinoid CB_2 receptors in human brain inflammation

C Benito, RM Tolón, MR Pazos, E Núñez, AI Castillo, and J Romero

Laboratorio de Apoyo a la Investigación, Fundación Hospital Alcorcón, C/Budapest 1, Alcorcón, Madrid, Spain

The presence of functional cannabinoid CB_2 receptors in the CNS has provoked considerable controversy over the past few years. Formerly considered as an exclusively peripheral receptor, it is now accepted that it is also present in limited amounts and distinct locations in the brain of several animal species, including humans. Furthermore, the inducible nature of these receptors under neuroinflammatory conditions, in contrast to CB_1, makes them attractive targets for the development of novel therapeutic approaches. In fact, the undesired psychoactive effects caused by CB_1 activation have largely limited the clinical use of cannabinoid-related compounds that act on these receptors. In this review some recent findings on the antiinflammatory properties of CB_2 receptors are presented, as well as new perspectives that have been obtained based on studies of human postmortem brain samples. In addition, various working hypotheses are also proposed and discussed.
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Keywords: human brain; chronic neuroinflammation; glia; CB_2 receptor; neuropathology; endocannabinoid system

Abbreviations: Aβ, β-amyloid peptide; AD, Alzheimer's disease; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; IL-1, interleukin-1; MS, multiple sclerosis; THC, Δ-9-tetrahydrocannabinol

CB_2 receptors in neuroinflammation

Data obtained during the past few years have shown that natural and synthetic cannabinoids are neuroprotective after various types of insults (reviewed by Fernández-Ruiz *et al*., 2005). These beneficial effects were thought to be mediated mainly by cannabinoid receptors of the CB_1 type, as this receptor is expressed at a high level in the CNS and its activation triggers several mechanisms that protect neurons from death (Howlett *et al*., 2002). Included in these are inhibition of cell excitability and a decrease in the release of glutamate and other neurotransmitters. Cannabinoids act on glia and neurons to inhibit the release of proinflammatory molecules, including interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), IL-6 and nitric oxide (Molina-Holgado *et al*., 1997, 1998, 2002b; Shohami *et al*., 1997; Puffenbarger *et al*., 2000; Cabral *et al*., 2001), and enhance the release of the anti-inflammatory cytokines IL-4, IL-10 (Klein *et al*., 2000) and an IL-1 receptor antagonist (Molina-Holgado *et al*., 2003). Due to the neuronal and, specifically, presynaptic location of CB_1 receptors, these actions were thought to be exerted directly on local neural circuits. More recently, however, non-CB_1-mediated protective effects of cannabinoids have also been reported (such as antioxidative actions and *N*-methyl-D-aspartic acid-antagonism; reviewed by Fernández-Ruiz *et al*., 2005) thus prompting the search for additional mechanisms. In addition, other elements of the endocannabinoid system (ECS) such as the enzymes involved in endocannabinoid degradation or the yet to be characterized uptake carrier have been considered as putative pharmacological targets.

Interestingly, growing attention is being paid to the second cannabinoid receptor, CB_2. Initial studies revealed that this receptor was expressed exclusively in peripheral tissues. Specifically, CB_2 receptors have been demonstrated in cells and tissues of the immune system, such as the marginal zone of the spleen (Lynn and Herkenham, 1994). In contrast to the constitutive presence of CB_1, recent studies have confirmed the inducible nature of this receptor in other tissues and organs including the CNS, although CB_1 receptors may also be upregulated under pathological conditions, such as, for instance, ischaemia (Jin *et al*., 2000; Fernández-López *et al*., 2006). This latter finding is supported by results obtained by Cabral and co-workers, from observing the pattern of expression of both receptors during microglia differentiation using an *in vitro* model of multistep activation (Carlisle *et al*., 2002; Cabral and Marciano-Cabral, 2005). Additional studies have confirmed a key role for CB_2 in macrophage/microglia functions (Table 1). At present, it is known that the anti-inflammatory properties of cannabinoid agonists also involve CB_2

Correspondence: Dr J Romero, Laboratorio de Apoyo a la Investigación, Fundación Hospital Alcorcón, C/Budapest 1, Alcorcón, Madrid 28922, Spain.
E-mail: Jromerop@fhalcorcon.es
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Table 1 CB_2 receptor and microglial function (*in vitro* data)

Reference	*Insult/ challenge*	*Ligand(s)*	*Receptor mediation*	*Effects of cannabinoid agonists*	*Signalling pathways involved*	*Cell type*
Carlisle *et al.* (2002)	Thioglycolate LPS IFN-γ	None	CB_2 mediated	↑ CB_2 expression with cell activation	Not studied	Murine and rat peritoneal macrophages Murine RAW264.7 Murine P388D1 Rat microglia
Walter *et al.* (2003)	ATP	AEA 2-AG PEA SR141716A SR144528 Cannabinol Cannabidiol O-1918	CB_2 mediated abn-CBD mediated	↑ Cell migration	ERK1/2	BV-2 Mouse microglia
Klegeris *et al.* (2003)	LPS + IFN-γ	JWH-015 SR141716A SR144528	CB_2 mediated	↓ IL-1β ↓ TNF-α	Not studied	THP-1 Human microglia
Franklin and Stella (2003)	None	ACPA Cannabinol Cannabidiol O-1918 SR141716A SR144528	CB_2 mediated abn-CBD mediated	↑ Cell migration	Gi/Go	BV-2
Carrier *et al.* (2004)	M-CSF	2-AG AEA JWH133 SR144528	CB_2 mediated	↑ Proliferation	ERK1	RTMGL1
Ramírez *et al.* (2005)	Aβ25-35 and Aβ1-40	HU-210 WIN55212-2 JWH-133	CB_1 mediated CB_2 mediated	↓ Morphological changes ↓ MTT ↓ TNF-α ↑ Neuronal survival	Not studied	Mouse microglia
Ortega-Gutierrez *et al.* (2005a)	LPS	UCM707 OMDM1 AEA Methanandamide SR141716A SR144528	CB_1 mediated CB_2 mediated	↓ NO ↓ iNOS ↓ Cytokines (IL-1β, IL-6, TNF-α)	Not studied	Mouse microglia
Ehrhart *et al.* (2005)	Aβ1-42	JWH-015	CB_2 mediated	↓ IFN-γ-mediated CD40 expression ↓ TNF-α ↓ NO ↑ Phagocytosis	JAK/STATI	Mouse microglia
Maresz *et al.* (2005)	GM-CSF IFN-γ LPS	None	Not studied	↑ CB_2	Not studied	Mouse microglia
Eljaschewitsch *et al.* (2006)	NMDA OGD	AEA WIN55212-2 AM251 AM630	CB_1 mediated CB_2 mediated	↑ MKP-1 ↓ NO ↓ iNOS	ERK-1/2	Rat microglia BV-2 OHSCs
Mukhopadhyay *et al.* (2006)	LPS	None	Not studied	↑ CB_2	NF-κB PKA PKC	RAW264.7
Kreutz *et al.* (2007)	NMDA	THC AEA 2-AG AM630	CB_2 mediated (THC, 2-AG) Non-CB_2 mediated (AEA)	↓ Number of microglial cells ↓ Number of degenerating neurons (2-AG)	Not studied	OHSCs

receptors. CB_2 receptor activation decreases the *in vitro* production of proinflammatory molecules in a number of neural cell types, such as rat microglial cells (Puffenbarger *et al.*, 2000; Facchinetti *et al.*, 2003), primary mouse astrocytes (Molina-Holgado *et al.*, 1997, 2002a), human microglial and THP-1 cells (Klegeris *et al.*, 2003), and human astrocytes (Sheng *et al.*, 2005). The activation of CB_2 receptors also reduces the release of proinflammatory factors in animal models of perinatal hypoxia–ischaemia (Fernández-López *et al.*, 2006) and Huntington's disease (Fernández-Ruiz *et al.*, 2005).

Most of these data have been obtained in primary microglial cultures or with murine cell lines *in vitro*. As pointed out by Maresz *et al.* (2005), the upregulation of

Table 2 CB_2 receptors and microglial function (*in vivo* data)

Reference	*Insult/disease model*	*Ligand(s)*	*Receptor mediation*	*Molecular effects of cannabinoid agonists*	*Symptomatic effects of cannabinoid agonists*	*Animal species*
Arevalo-Martin *et al.* (2003)	Theiler's virus	WIN55212-2 ACEA JWH-015	CB_1 mediated CB_2 mediated	↓ Microglial activation ↓ MHC-II expression ↓ CD4+ infiltration	Motor recovery Remyelination	Mouse
Zhang *et al.* (2003)	Chronic constriction injury Freund's complete adjuvant injection Spinal nerve ligation	None	↑ CB_2 mRNA	Not studied	Not studied	Rat
Maresz *et al.* (2005)	Experimental autoimmune enchepalomyelitis	None	Not studied	↑ CB_2	Not studied	Mouse
Beltramo *et al.* (2006)	Spinal cord ligation	AM1241 L768242 SR144528	CB_2 mediated	↑ CB_2	↓ Hyperalgesia	Mouse
Mukhopadhyay *et al.* (2006)	LPS	None	Not studied	↑ CB_2	Not studied	Rat
Ashton *et al.* (2007)	Middle cerebral artery occlusion Hypoxia–ischemia	None	Not studied	↑ CB_2	Not studied	Rat

Table 3 CB_2 receptors in neurodegenerative diseases

Control	*Alzheimer's disease*	*Down's syndrome*	*SIVE*	*HIVE*	*Multiple sclerosis*
Perivascular microglia	Activated microglia	Activated microglia	Activated microglia Perivascular microglia T-lymphocytes	Activated microglia Perivascular microglia T-lymphocytes	Activated microglia Macrophages T-lymphocytes Astrocytes

Abbreviations: HIVE, type 1-human immunodeficiency virus-induced encephalitis; SIVE, Simian immunodeficiency virus-induced encephalitis.

cannabinoid CB_2 receptors also takes place *in vivo*, and seems to be triggered by chronic inflammatory conditions (Table 2). These authors were the first to show that the increased expression of these receptors was a direct consequence of microglial cell activation occurring during an experimentally induced autoimmune process. More recently, additional studies have further corroborated the *in vivo* link between chronic neuroinflammation and CB_2 upregulation in animal models of pain (Beltramo *et al.*, 2006), inflammation (Mukhopadhyay *et al.*, 2006) and ischaemia-induced hypoxia (Ashton *et al.*, 2007). From these studies, it can be concluded that macrophage/microglia activation, whether by inherent changes to *in vitro* conditions or by experimentally induced neuroinflammatory processes, leads to a dramatic increase in CB_2 expression. It should be noted that this glial expression also affects other elements of the ECS, such as, for example, fatty acid amide hydrolase (FAAH). Albeit the expression of FAAH in microglia is negligible (Stella, 2004), it seems to play a significant role in astrocytic function.

Few data exist on the role that CB_2 receptors may play in humans. Due to its abundant presence in immune-related cells, it seems reasonable to think that they are involved in the well-known effects of cannabinoids on immunological function (Klein, 2005). As discussed in subsequent sections of this review, in the human CNS, CB_2 receptors seem to follow a similar pattern of inducible expression as that described in animal models.

CB_2 receptors in neuroinflammatory conditions of the human brain

Although inflammation serves as a protective function in controlling infections and promoting tissue repair, it can also cause tissue damage and disease. Recently, 'neuroinflammation' became a commonly used term and neuroscientists spoke of 'reactive gliosis' when describing endogenous CNS tissue responses to injury. Neuroinflammation incorporates a wide spectrum of complex cellular responses that include activation of microglia and astrocytes and elaboration of cytokines and chemokines, complement proteins, acute phase proteins and related molecular processes. In addition, invasion of peripheral immune cells is also usually present. These events may have detrimental effects on neuronal function, leading to neuronal injury with further glial activation and, ultimately, neurodegeneration. Neuroinflammation occurs in myelin degenerative disorders, such as multiple sclerosis (MS, reviewed by Martino *et al.*, 2002) and also in neurodegenerative disorders, such as Alzheimer's disease (AD, McGeer and Rogers, 1992) Parkinson's disease (McGeer *et al.*, 2001) and Huntington's disease (Sapp *et al.*, 2001), viral encephalitis (Gendelman *et al.*, 1994), ischaemia (Chopp *et al.*, 1994) and traumatic brain injury (Dusart and Schwab, 1994). The release of proinflammatory and neurotoxic mediators (interferon-γ, tumour necrosis factor-α, IL-1β, IL-6, eicosanoids, nitric oxide and reactive oxygen species) may induce or aggravate

brain damage. These factors are produced by glial cells and invading immune cells (mainly reactive microglia) and can be deleterious to neurons (for review see Boje and Arora, 1992; Chao *et al*., 1992; Mc Guire *et al*., 2001; Liu and Hong, 2003).

On the other hand, the study of the changes in the expression pattern of several elements of the ECS in the healthy versus diseased human brain has provided new perspectives to the field (Pazos *et al*., 2005; Benito *et al*., 2007c). Specifically, recent evidence suggests that the ECS may participate in the pathogenesis and/or the adaptive changes taking place in the human CNS after chronic neuroinflammatory conditions. As previously mentioned, this participation would include, in addition to the known neuroprotection exerted by neuronal CB_1 receptors, glial CB_2 receptors and FAAH. Both proteins seem to be significantly upregulated in microglial and astroglial cells, respectively, in areas of active neuroinflammation. Among these, β-amyloid enriched neuritic plaques in AD, infiltrative areas in viral encephalitis and regions of active demyelination in MS show marked increases in CB_2 and FAAH levels of glial expression (reviewed by Benito *et al*., 2007c).

Alzheimer's disease

AD accounts for the most frequent form of dementia in the elderly and is one of the most important health challenges in western countries. In 2001, more than 5 million people with dementia lived in European countries and more than 4 million people were affected in the United States alone. Furthermore, a 100% increase in the number affected is expected in developed regions by the year 2040 (Ferri *et al*., 2005). The initial symptoms presented in AD patients usually include slight losses of memory and progress to a total inability to control basic functions of the body. Current treatments for AD only provide symptomatic relief and much effort is being directed to the search for curative and/or preventive treatments (Lleó *et al*., 2006).

Although the neuropathological and molecular changes that underlie these symptoms are now well characterized thanks to the analysis of postmortem samples, controversial aspects still await further clarification. In general terms, the 'amyloid hypothesis' is now accepted (Walsh and Selkoe, 2004). According to this hypothesis, the aberrant processing of a peptide located on the cell membrane leads to the synthesis of a small 42 amino-acid peptide (the amyloid peptide, Aβ) that exhibits a remarkable tendency to acquire a tridimensional structure that makes it precipitate in the extracellular space. This is the origin of the 'amyloid or neuritic plaques' that accumulate in the brain parenchyma of AD patients and that are thought to trigger a cascade of events that leads to massive neuronal death (Giulian, 1999). Among these events, amyloid deposition (i) stimulates the hyperphosphorylation of the cytoskeletal tau protein, thus triggering its precipitation and leading to the formation of intraneuronal 'neurofibrillary tangles'; (ii) triggers a potent, local inflammatory reaction, involving microgial and astroglial cells that attempt to encapsulate and degrade the amyloid deposit; (iii) results in a massive local accumulation of inflammatory cytokines and reactive oxygen species ('cytokine cycle'; Mrak and Griffin, 2005) and (iv) leads ultimately to neuronal death and massive loss of functional synapses, thus dramatically altering neurotransmission (Wyss-Coray and Mucke, 2002).

Therapeutic approaches intended to decrease endogenous Aβ levels are considered to be among the most promising strategies for the treatment of AD. Clinical trials with vaccines against this peptide have been carried out, although the appearance of meningoencephalitis in approximately 6% of patients has led to this treatment being prematurely stopped (Goni and Sigurdsson, 2005). The possible application of anti-inflammatory compounds is also under debate; in particular, non-steroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors and peroxisome-proliferator-activated receptor ligands have been tested (reviewed by Aisen, 2002).

As mentioned above, undesired psychoactive effects have limited the clinical use of cannabinoid-related chemicals. Memory impairment is one of the most frequent side effects of these compounds, so their usefulness for the treatment of AD has been seriously questioned. However, a pilot study by Volicer *et al*. (1997) performed in patients with dementia who were refusing to eat indicated that dronabinol (Δ-9-tetrahydrocannabinol, THC) significantly improved this condition and the behaviour of these patients, with few side effects. From these results, the investigators proposed that dronabinol could be a suitable compound for the treatment of anorexia and disturbed behaviour in AD patients. Further, in accordance with this proposal, Walther *et al*. (2006) found that dronabinol also improved nocturnal agitation and motor activity in six patients in the late stages of dementia. However, more research is needed to substantiate the effectiveness of this compound for the treatment of AD.

Several studies performed *in vitro* and in animal models of AD have shown a protective role for cannabinoids. The molecular basis of these effects includes CB_1-, CB_2- and also receptor-independent mechanisms (for a recent review, see Benito *et al*., 2007a).

The analysis of human post-mortem brain samples from AD patients has provided information on the neuropathology of the ECS and has allowed new hypotheses to be formulated on the possible role of this system in the prevention and/or treatment of AD. Data from AD patients may be summarized as follows: (i) in AD brains, CB_1 receptor binding in hippocampus and basal ganglia structures is decreased (Westlake *et al*., 1994), but not in neocortex or frontal cortex and the receptor is less efficiently coupled to signal transduction mechanisms (Ramírez *et al*., 2005); (ii) Aβ deposition induces dramatic changes in the phenotype of glial cells, including upregulation of some elements of the ECS (such as CB_2 receptors and FAAH) (Benito *et al*., 2003); (iii) overexpression of CB_2 and FAAH seems to be a phenomenon directly linked to Aβ deposition, as suggested by the study of human samples of Down's syndrome, a natural model of AD (Núñez *et al*., submitted) and, (iv) the upregulation of these elements of the ECS is a cell-specific event, as CB_2 receptors seem to be restricted to microglia and FAAH to astrocytes. Although the functions of these receptors in the CNS are far from clear, they may be now considered as diagnostic markers for microglial activation

and as relevant candidates for the development of anti-Aβ therapies. Indeed, in the next section of the review, we will cover the *in vitro* data illustrating the anti-inflammatory effects of CB_2 agonists.

More recently, we have explored a possible functional role for microglial CB_2 receptors in Aβ removal (Núñez *et al*., unpublished observations). It is known that one of the most important functions of microglial cells in AD is the removal of pathological proteinaceous deposits, such as neuritic plaques. Wyss-Coray *et al*. (2003) showed that adult mouse astrocytes may also participate in this process, suggesting a new way for therapeutic intervention. Our results indicate that the CB_2 agonist JWH-015 induces the removal of Aβ plaques from human AD tissue sections by human THP-1-derived macrophages, but not by other types of glioma cell lines. Furthermore, this effect was achieved at low concentrations (maximum effect at 5 nM) and was reversed by the CB_2 selective antagonist SR144528. Interestingly, Ehrhart *et al*. (2005) have recently shown that this same compound enhances Aβ phagocytosis by attenuating CD40-mediated inhibition in microglial cells. In light of these observations, we speculate that this effect could be related to the CB_2-mediated inhibition of the release of IL-1 and tumour necrosis factor-α by stimulated THP-1 cells (Klegeris *et al*., 2003). As shown by Koenigsknecht-Talboo and Landreth (2005), a pro-inflammatory milieu results in a marked interference with the ability of microglia to phagocytose Aβ. Therefore, CB_2 receptor activation might afford an additional advantage, as it could also decrease local neuroinflammation thus enhancing Aβ removal *in situ*.

In summary, CB_2 activation could provide beneficial effects in AD through several mechanisms, including a decrease in local, microglia-mediated inflammation and an enhancement of Aβ removal.

HIV-induced encephalitis

As mentioned before, the presence of CB_2 receptors in the healthy human brain is rather limited. In fact, we have only detected significant levels of CB_2 immunoreactivity in a discrete population of perivascular cells (Núñez *et al*., 2004). These cells were identified as perivascular macrophages. Although considered a part of the blood brain barrier, these cells of myeloid origin exhibit distinct properties from other cell types in the CNS (for a comprehensive review, see Williams and Hickey, 2002). Their selective location embracing the external wall of the blood vessels places them in a privileged position to participate in the control of the entry of exogenous elements into the CNS. In addition, they are considered transient CNS residents, as they are continuously replaced by bone marrow-derived monocytes.

It was this selective CB_2 receptor expression that turned our attention to the study of their possible relevance in the encephalitis induced by the type 1-human immunodeficiency virus. This inflammatory process underlies the clinical paradigm of acquired immuno deficiency syndrome dementia that affects at least 5–7% of acquired immuno deficiency syndrome patients per year, in Europe alone (Mollace *et al*., 2001). These patients exhibit a myriad of cognitive and motor symptoms, including leg weakness, memory loss, apathy, social withdrawal and personality changes. In its more advanced and severe form, the disease ultimately leads the patient progressing to a vegetative state (González-Scarano and Martín-García, 2005). Interestingly, children's brains seem to be more vulnerable, probably due to the immaturity of the blood brain barrier when they get infected.

It is currently thought that the entry of the HIV-1 into the CNS follows a 'Trojan horse' strategy (Kaul *et al*., 2001). According to this line of reasoning, peripheral infected monocytes committed to replace perivascular macrophages act as carriers of the virus. Thus, all the virus found in the brain is probably initially derived from monocytes that have differentiated into perivascular macrophages (González-Scarano and Martín-García, 2005). Once inside the CNS, productively infected macrophages constitute a source of virus that acts on microglia. Neurons themselves seem not to be directly infected. Thus, both pericytes and parenchymal microglia are the two main cell types involved in this process that leads to the formation of the so-called 'multinucleated giant cells'. Finally, several mechanisms (including oxidative stress, production and release of pro-inflammatory cytokines, and/or direct injury by viral proteins) lead to neurodegeneration and subsequent clinical symptoms (González-Scarano and Martín-García, 2005).

As with other types of G-protein-coupled receptors, the CB_2 is upregulated in perivascular macrophages as a consequence of the HIV-1-triggered inflammatory process. Specifically, chemokine receptors of the CCR3 and CCR5 type have been reported to be upregulated in Simian immunodeficiency virus-induced encephalitis and type 1-human immunodeficiency virus-induced encephalitis brains (Cartier *et al*., 2005). Using samples from macaque and human infected brains, we observed that only those samples from infected individuals with encephalitis showed high levels of CB_2 expression, in contrast with those from controls and infected individuals without encephalitis. The increases in CB_2 expression were especially evident in perivascular macrophages and microglial cuffs (Benito *et al*., 2005).

Interestingly, infiltrated T lymphocytes also show strong immunoreactivity for CB_2 receptors. The entry of these cells from the periphery into the CNS is an additional feature of type 1-human immunodeficiency virus-induced encephalitis, although their contribution to the virus pool in the CNS is not clear, mainly because genotypic and phenotypic analyses show that viruses from the brain are more similar to those from monocytes and macrophages than to those from T lymphocytes (González-Scarano and Martín-García, 2005). Ghosh *et al*. (2006) have recently shown that CB_2 activation inhibits the transendothelial migration of Jurkat T cells and human primary T lymphocytes by interfering with the CXCL12/CXCR4 system. This interaction seems to take place by cross-talk between their signal transduction routes. Very recently, a similar downstream interaction has been described for the CCR5 receptor (GA Cabral *et al*., personal communication).

Thus, CB_2 receptors might participate in the inflammatory response against viral infection of the brain by modifying the microglial production of inflammatory molecules and by modulating the entry of peripheral cells into the CNS.

Multiple sclerosis

MS is the major cause of neurological disability among young adults in North America and Europe (Noseworthy *et al*., 2000). Its aetiology is unknown but much evidence suggests that genetic and environmental factors may have an important role on MS susceptibility, although the possibility of a role for infectious agents has also been considered (Frohman *et al*., 2006). The neuropathology of this inflammatory and demyelinating disease of the CNS includes axonal degeneration, oligodendrocyte loss and subsequent induction of well-demarcated hypocellular and demyelinated areas (Frohman *et al*., 2006). Lymphocytes and monocytes infiltrate the white matter surrounding the blood vessels, destroying myelin, while axons are not directly damaged. Its characteristic symptoms (such as painful muscle spasms, tremor, ataxia, weakness or paralysis) are thought to be the result of new lesions and expansion of old lesions at the CNS level as a result of myelin phagocytic activity carried out by cells of monocytic origin (for a review, see Noseworthy *et al*., 2000).

The ECS constitutes a promising target for the development of new drugs for the treatment of MS (Pryce and Baker, 2005). Interest in the potential of cannabinoids as a treatment for the symptoms of MS is evidenced by results obtained in clinical trials performed during the last few years. A randomized, placebo-controlled trial in which stable MS and muscle spasticity patients were treated with cannabis extract or Δ^9-THC for 15 weeks showed that cannabinoids did not exhibit a beneficial effect on spasticity, as assessed with the Ashworth scale (Zajicek *et al*., 2003). However, these patients reported an improvement in pain relief as well as in mobility. In addition, patients who continued Δ^9-THC treatment for up to 12 months showed a small reduction of spasticity (Zajicek *et al*., 2005). The oromucosal spray Sativex (which contains Δ^9-THC and cannabidiol) tested in another clinical trial showed a significant reduction in pain and sleep disturbances in patients with central neuropathic pain syndromes due to MS (Rog *et al*., 2005).

These data are supported by results obtained in different animal models of MS. Thus, THC administration delayed the onset of the disease and markedly reduced CNS inflammation (Lyman *et al*., 1989) and synthetic cannabinoids ameliorated tremor and spasticity through a CB_1-mediated mechanism (Baker *et al*., 2000). In addition, they improved neurological deficits as a result of parallel reduction in CNS inflammation and extensive remyelination (Arevalo-Martin *et al*., 2003) and produced beneficial effects on motor activity, accompanied by a reduction of damage to axons, through CB_1 and CB_2 receptor activation (Docagne *et al*., 2007).

So far, the treatment of MS has focused on CB_1 activation. However, results from animals models (Arevalo-Martin *et al*., 2003; Docagne *et al*., 2007) and in human samples (Yiangou *et al*., 2006; Benito *et al*., 2007b) suggest that CB_2 receptors are potential therapeutic targets for the treatment of MS. Moreover, the probability of finding cannabinoid-based drugs devoid of undesirable psychotropic side effects for treating this disease now seems more likely.

The studies performed by Yiangou *et al*. (2006) and Benito *et al*. (2007b) in human spinal cord and brain MS samples, respectively, indicated the presence of strong CB_2-immunoreactivity in microglia/macrophages in areas of white matter, usually within active plaques or in the periphery of chronic lesions. These results confirm that CB_2 expression in glial cells in the human CNS is upregulated, as previously found in other neuroinflammatory conditions (Benito *et al*., 2003, 2005) and even in healthy human brains (Núñez *et al*., 2004). Importantly, we showed that a fraction of the CB_2-positive macrophages also contained myelin basic protein, indicating recent phagocytic activity and suggesting that CB_2 receptor expression in plaque-associated macrophages may be an early event in plaque evolution. Several *in vitro* studies have documented that microglia/macrophages are involved in phagocytosis of myelin debris in MS lesions and that this process triggers release of pro-inflammatory cytokines and nitric oxide (Williams *et al*., 1994; Mosley and Cuzner, 1996; van der Laan *et al*., 1996). Although little is known of the effects of cannabinoids on myelin phagocytosis, previous studies have shown that the activation of the ECS decreases the production of pro-inflammatory cytokines and levels of nitric oxide in macrophages/microglia. This process could account for the anti-inflammatory effect that seems to potentiate neuroprotection induced by cannabinoids (Mestre *et al*., 2005; Ortega-Gutierrez *et al*., 2005b). In addition, several characteristics of macrophages such as migration, presentation of peptide antigens or phagocytosis of foreign particles are also significantly influenced by cannabinoids (reviewed by Croxford and Yamamura, 2005).

Our immunohistochemical study performed on human tissue sections shows highest levels of CB_2 receptor immunoreactivity in microglia (Benito *et al*., 2007b). Further, the distribution of these CB_2-positive cells correlated with that of major histocompatibility complex type II-positive cells. The similarity between CB_2-positive cells and microglia and the colocalization of CB_2-receptors with D-region related human leukocyte-associated antigen led us to suggest the CB_2 receptor as a marker for the identification of MS plaques as they may indicate the evolution grade of demyelination areas. These major histocompatibility complex type II-positive cells are used as defining markers of plaque subtype depending on their localization and abundance in MS lesions (Trapp *et al*., 1999). Thus, major histocompatibility complex type II-positive cells are abundant throughout the entire extension of acutely active plaques, but are restricted to the periphery of chronic ones.

We also demonstrated CB_2 receptor expression in perivascular T lymphocytes. The myelin-reactive T lymphocytes are thought to be involved in the demyelinating process and cause inflammation (Frohman *et al*., 2006). It is important to note that cannabinoids decrease $CD4^+$ infiltration into the spinal cord in an animal model of MS through CB_1 and CB_2 activation (Arevalo-Martin *et al*., 2003). Thus, the presence of cannabinoid receptors in T lymphocytes is suggestive of a possible role of the ECS in MS-linked, T-cell-mediated neuroinflammation.

Surprisingly, the CB_2 receptor has also been found to be expressed in white matter astrocytes (Benito *et al*., 2007b), while not to being expressed by astrocytes in other pathologies such as AD (Benito *et al*., 2003; Ramírez *et al*., 2005), type 1-human immunodeficiency virus-induced

encephalitis or Simian immunodeficiency virus-induced encephalitis (Benito *et al*., 2005). There are few data about the role of CB_2 receptors in astrocytes, although studies *in vitro* suggest that they may modulate the production of different inflammatory mediators (Ortega-Gutierrez *et al*., 2005a, b; Sheng *et al*., 2005). More recently, Docagne *et al*. (2007) proposed a neuroprotective effect in an MS animal model as a result of the concomitant activation of CB_1 receptors in neurons and CB_2 in astrocytes.

In summary, cannabinoids are known to have a therapeutic effect in MS. New evidence suggests that the CB_2 receptor could also be a pharmacological target, as its expression is increased in several cell types known to be directly involved in the pathogenesis of MS. The activity of microglia, astrocytes and infiltrated lymphocytes could be modified by the activation of CB_2 receptors.

Conclusions

CB_2 receptors have been found to be present in the CNS of several animal species (Benito *et al*., 2003; Maresz *et al*., 2005; Van Sickle *et al*., 2005), thus offering new opportunities for the pharmacological use of cannabinoid agents. Furthermore, the fact that their expression is increased by inflammatory stimuli suggests that they may be involved in the pathogenesis and/or in the endogenous response to injury. Data obtained *in vitro* and in animal models show that CB_2 receptors may be part of the general neuroprotective action of the ECS by decreasing glial reactivity. Neuropathological findings in human brains (summarized in Table 3) suggest that the upregulation of CB_2 receptors is a common pattern of response against different types of chronic injury of the human CNS. In addition, their selective presence in microglial cells is highly suggestive of an important role in disease-associated neuroinflammatory processes. The anti-inflammatory effects triggered by the activation of the CB_2 receptor make it an attractive target for the development of novel anti-inflammatory therapies. In any case, further research is needed to corroborate the potential usefulness of cannabinoid-based treatments devoid of undesired psychoactive effects.

Conflict of interest

The authors state no conflict of interest.

References

Aisen PS (2002). The potential of anti-inflammatory drugs for the treatment of Alzheimer's disease. *Lancet Neurol* **1**: 279–284.

Arevalo-Martin A, Vela JM, Molina-Holgado E, Borrell J, Guaza C (2003). Therapeutic action of cannabinoids in a murine model of multiple sclerosis. *J Neurosci* **23**: 2511–2516.

Ashton JC, Rahman RMA, Nair SM, Sutherland BA, Glass M, Appleton I (2007). Cerebral hypoxia–ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. *Neurosci Lett* **412**: 114–117.

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Huffman JW *et al*. (2000). Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature* **404**: 84–87.

Beltramo M, Bernardini N, Bertorelli R, Campanella M, Nicolussi E, Fredduzzi S *et al*. (2006). CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. *Eur J Neurosci* **23**: 1530–1538.

Benito C, Kim WK, Chavarria I, Hillard CJ, Mackie K, Tolon RM *et al*. (2005). A glial endogenous cannabinoid system is upregulated in the brains of macaques with simian immunodeficiency virus-induced encephalitis. *J Neurosci* **25**: 2530–2536.

Benito C, Núñez E, Pazos MR, Tolón RM, Romero J (2007a). The endocannabinoid system and Alzheimer's disease. *Mol Neurobiol* (in press).

Benito C, Nunez E, Tolon RM, Carrier EJ, Rabano A, Hillard CJ *et al*. (2003). Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci* **23**: 11136–11141.

Benito C, Romero JP, Tolón RM, Clemente D, Docagne F, Hillard CJ *et al*. (2007b). Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis. *J Neurosci* **27**: 2396–2402.

Benito C, Tolón RM, Núñez E, Pazos MR, Romero J (2007c). Neuroinflammation and the glial endocannabinoid system. In: Koffalvi A (ed). *Cannabinoids and the Brain*. Springer Verlag, USA, pp 314–334.

Boje KM, Arora PK (1992). Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. *Brain Res* **587**: 250–256.

Cabral GA, Harmon KN, Carlisle SJ (2001). Cannabinoid-mediated inhibition of inducible nitric oxide production by rat microglial cells: evidence for CB1 receptor participation. *Adv Exp Med Biol* **493**: 207–214.

Cabral GA, Marciano-Cabral F (2005). Cannabinoid receptors in microglia of the central nervous system: immune functional relevance. *J Leukoc Biol* **78**: 1192–1197.

Carlisle SJ, Marciano-Cabral F, Staab A, Ludwick C, Cabral GA (2002). Differential expression of the CB_2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. *Int Immunopharmacol* **2**: 69–82.

Carrier EJ, Kearn CS, Barkmeier AJ, Breese NM, Yang W, Nithipatikom K *et al*. (2004). Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. *Mol Pharmacol* **65**: 999–1007.

Cartier L, Hartley O, Dubois-Dauphin M, Krause KH (2005). Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. *Brain Res Rev* **48**: 16–42.

Chao CC, Hu S, Molitor TW, Shaskan EG, Peterson PK (1992). Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. *J Immunol* **149**: 2736–2741.

Chopp M, Zhang RL, Chen H, Li Y, Jiang N, Rusche JR (1994). Postischemic administration of an anti-Mac-1 antibody reduces ischemic cell damage after transient middle cerebral artery occlusion in rats. *Stroke* **25**: 869–875.

Croxford JL, Yamamura T (2005). Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? *J Neuroimmunol* **166**: 3–18.

Docagne F, Muneton V, Clemente D, Ali C, Loria F, Correa F *et al*. (2007). Excitotoxicity in a chronic model of multiple sclerosis: neuroprotective effects of cannabinoids through CB1 and CB2 receptor activation. *Mol Cell Neurosci* **34**: 551–561.

Dusart I, Schwab ME (1994). Secondary cell death and the inflammatory reaction after dorsal hemisction of the rat spinal cord. *Eur J Neurosci* **6**: 712–724.

Ehrhart J, Obregon D, Mori T, Hou H, Sun N, Bai Y *et al*. (2005). Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J Neuroinflammation* **2**: 29–39.

Eljaschewitsch E, Witting A, Mawrin C, Lee T, Schmidt PM, Wolf S *et al*. (2006). The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron* **49**: 67–79.

Facchinetti F, Del Giudice E, Furegato S, Passarotto M, Leon A (2003). Cannabinoids ablate release of TNFalpha in rat microglial cells stimulated with lypopolysaccharide. *Glia* **41**: 161–168.

Fernández-López D, Martínez-Orgado J, Nuñez E, Romero J, Bonet B, Lorenzo P *et al*. (2006). Characterization of the neuroprotective effect of the cannabinoid agonist WIN-55212 in an *ex vivo* model of hypoxic–ischemic brain damage in newborn rats. *Pediatr Res* **60**: 169–173.

Fernández-Ruiz JJ, Gonzalez S, Romero J, Ramos JA (2005). Cannabinoids in neurodegeneration and neuroprotection. In: Mechoulam R (ed). *Cannabinoids as Therapeutics*. Birkhäuser Verlag: Switzerland, pp 79–109.

Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M *et al*. (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet* **366**: 2112–2117.

Franklin A, Stella N (2003). Arachidonylcyclopropylamide increases microglial cell migration through cannabinoid CB2 and abnormal-cannabidiol-sensitive receptors. *Eur J Pharmacol* **474**: 195–198.

Frohman EM, Racke MK, Raine CS (2006). Multiple sclerosis. The plaque and its pathogenesis. *New Eng J Med* **354**: 942–955.

Gendelman HE, Genis P, Jett M, Zhai QH, Nottet HS (1994). An experimental model sustem for HIV-1-induced brain injury. *Adv Neuroimmunol* **4**: 189–193.

Ghosh S, Preet A, Groopman JE, Ganju R (2006). Cannabinoid receptor CB2 modulates the CXCL12/CXCR4-mediated chemotaxis of T-lymphocytes. *Mol Immunol* **43**: 2169–2179.

Giulian D (1999). Microglia and the immune pathology of Alzheimer's disease. *Am J Hum Genet* **65**: 13–18.

Goni F, Sigurdsson EM (2005). New directions towards safer and effective vaccines for Alzheimer's disease. *Curr Opin Mol Ther* **7**: 17–23.

González-Scarano F, Martín-García J (2005). The neuropathogenesis of AIDS. *Nat Rev Immunol* **5**: 69–81.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA *et al*. (2002). International union of pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* **54**: 161–202.

Jin KL, Mao XO, Goldsmith PC, Greenberg DA (2000). CB1 cannabinoid receptor induction in experimental stroke. *Ann Neurol* **48**: 257–261.

Kaul M, Garde GA, Lipton SA (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature* **410**: 988–994.

Klegeris A, Bissonnette CJ, McGeer PL (2003). Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. *Br J Pharmacol* **139**: 775–786.

Klein TW (2005). Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol* **5**: 400–411.

Klein TW, Lane B, Newton CA, Friedman H (2000). The cannabinoid system and cytokine network. *Proc Soc Exp Biol Med* **225**: 1–8.

Koenigsknecht-Talboo J, Landreth G (2005). Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. *J Neurosci* **25**: 8240–8249.

Kreutz S, Koch M, Ghadban C, Korf HW, Dehghani F (2007). Cannabinoids and neuronal damage: differential effects of THC, AEA and 2-AG on activated microglial cells and degenerating neurons in excitotoxically lesioned rat organotypic hippocampal slice cultures. *Exp Neurol* **203**: 246–257.

Liu B, Hong JS (2003). Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J Pharmacol Exp Ther* **304**: 1–7.

Lleó A, Greenberg SM, Growdon JH (2006). Current pharmacotherapy for Alzheimer's disease. *Annu Rev Med* **57**: 513–533.

Lyman WD, Sonett JR, Brosnan CF, Elkin R, Bornstein MB (1989). Delta 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *J Neuroimmunol* **23**: 73–81.

Lynn AB, Herkenham M (1994). Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. *J Pharmacol Exp Ther* **268**: 1612–1623.

Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ, Dittel BN (2005). Modulation of the cannabinoid CB receptor in microglial cells in response to inflammatory stimuli. *J Neurochem* **95**: 437–445.

Martino G, Adorini L, Rieckmann P, Hillert J, Kallmann B, Comi G *et al*. (2002). Inflammation in multiple sclerosis the good, the bad, and the complex. *Lancet Neurol* **1**: 499–509.

Mc Guire SO, Ling ZD, Lipton JW, Sortwell CE, Collier TJ, Carvey P (2001). Tumor necrosis factor alpha is toxic to embryonic mesencephalic dopamine neurons. *Exp Neurol* **169**: 219–230.

McGeer PL, Rogers J (1992). Anti-inflammatory agents as a therapeutic approach to Alzheimer's disease. *Neurology* **42**: 447–449.

McGeer PL, Yasojima K, McGeer EG (2001). Inflammation in Parkinson's disease. *Adv Neuro* **86**: 83–89.

Mestre L, Correa F, Arevalo-Martin A, Molina-Holgado E, Valenti M, Ortar G *et al*. (2005). Pharmacological modulation of the endocannabinoid system in a viral model of multiple sclerosis. *J Neurochem* **92**: 1327–1339.

Molina-Holgado E, Vela JM, Arevalo-Martin A, Almazan G, Molina-Holgado F, Borrell J *et al*. (2002a). Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *J Neurosci* **22**: 9742–9753.

Molina-Holgado F, Lledo A, Guaza C (1997). Anandamide suppresses nitric oxide and TNF-alpha responses to Theiler's virus or endotoxin in astrocytes. *Neuroreport* **8**: 1929–1933.

Molina-Holgado F, Molina-Holgado E, Guaza C (1998). The endogenous cannabinoid anandamide potentiates interleukin-6 production by astrocytes infected with Theiler's murine encephalomyelitis virus by a receptor-mediated pathway. *FEBS Lett* **433**: 139–142.

Molina-Holgado F, Molina-Holgado E, Guaza C, Rothwell NJ (2002b). Role of CB1 and CB2 receptors in the inhibitory effects of cannabinoids on lipopolysaccharde-induced nitric oxide release in astrocytes cultures. *J Neurosci Res* **67**: 829–836.

Molina-Holgado F, Pinteaux E, Moore JD, Molina-Holgado E, Guaza C, Gibson RM *et al*. (2003). Endogenous interleukin-1 receptor antagonist mediates anti-inflammatory and neuroprotective actions of cannabinoids in neurons and glia. *J Neurosci* **23**: 6470–6474.

Mollace V, Nottet HSLM, Clayette P, Turco MC, Muscoli C, Salvemini D *et al*. (2001). Oxidative stress and neuroAIDS: triggers, modulators and novel antioxidants. *Trends Neurosci* **24**: 411–416.

Mosley K, Cuzner ML (1996). Receptor-mediated phagocytosis of myelin by macrophages and microglia: effect of opsonization and receptor blocking agents. *Neurochem Res* **21**: 481–487.

Mrak RE, Griffin WST (2005). Glia and their cytokines in progression of neurodegeneration. *Neurobiol Aging* **26**: 349–354.

Mukhopadhyay S, Das S, Williams EA, Moore D, Jones JD, Zahm DS *et al*. (2006). Lypopolysaccharide and cyclic AMP regulation of CB2 cannabinoid receptor levels in rat brain and mouse RAW 264.7 macrophages. *J Neuroimmunol* **181**: 82–92.

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG (2000). Multiple sclerosis. *N Engl J Med* **343**: 938–952.

Núñez E, Benito C, Pazos MR, Barbachano A, Fajardo O, Gonzalez S *et al*. (2004). Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. *Synapse* **53**: 208–213.

Ortega-Gutierrez S, Molina-Holgado E, Arevalo-Martin A, Correa F, Viso A, Lopez-Rodriguez ML *et al*. (2005a). Activation of the endocannabinoid system as therapeutic approach in a murine model of multiple sclerosis. *FASEB J* **19**: 1338–1340.

Ortega-Gutierrez S, Molina-Holgado E, Guaza C (2005b). Effect of anandamide uptake inhibition in the production of nitric oxide and in the release of cytokines in astrocyte cultures. *Glia* **52**: 163–168.

Pazos MR, Nunez E, Benito C, Tolon RM, Romero J (2005). Functional neuroanatomy of the endocannabinoid system. *Pharmacol Biochem Behav* **81**: 239–247.

Pryce G, Baker D (2005). Emerging properties of cannabinoid medicines in management of multiple sclerosis. *Trends Neurosci* **28**: 272–276.

Puffenbarger RA, Boothe AC, Cabral GA (2000). Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. *Glia* **29**: 58–69.

Ramírez BG, Blazquez C, Gomez DP, Guzman M, De Ceballos ML (2005). Prevention of Alzheimer's disease pathology by cannabinoids:

neuroprotection mediated by blockade of microglial activation. *J Neurosci* **25**: 1904–1913.

Rog DJ, Nurmikko TJ, Friede T, Young CA (2005). Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. *Neurology* **65**: 812–819.

Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K *et al*. (2001). Early and progressive accumulation of reactive microglia in the Huntington disease brain. *J Neuropathol Exp Neurol* **60**: 161–172.

Sheng WS, Hu S, Min X, Cabral GA, Lokensgard JR, Peterson P (2005). Synthetic cannabinoid WIN55,212-2 inhibits generation of inflammatory mediators by IL-1beta-stimulated human astrocytes. *Glia* **49**: 211–219.

Shohami E, Gallily R, Mechoulam R, Bass R, Ben-Hur T (1997). Cytokine production in the brain following closed head injury: dexanabinol (HU-211) is a novel TNF-alpha inhibitor and an effective neuroprotectant. *J Neuroimmuno* **72**: 169–177.

Stella N (2004). Cannabinoid signaling in glial cells. *Glia* **48**: 267–277.

Trapp BD, Bo L, Mork S, Chang A (1999). Pathogenesis of tissue injury in MS lesions. *J Neuroimmunol* **98**: 49–56.

Van der Laan LJ, Ruuls SR, Weber KS, Lodder IJ, Dopp EA, Dijkstra CD (1996). Macrophage phagocytosis of myelin *in vitro* determined by flow cytometry: phagocytosis is mediated by CR3 and induces production of tumor necrosis factor-alpha and nitric oxide. *J Neuroimmunol* **70**: 145–152.

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al*. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**: 329–332.

Volicer L, Stelly M, Morris J, McLaughlin J, Volicer BJ (1997). Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. *Int J Geriatr Psychiatry* **12**: 913–919.

Walsh DM, Selkoe DJ (2004). Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* **44**: 181–193.

Walter L, Franklin A, Witting A, Wade C, Xie Y, Kunos G *et al*. (2003). Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J Neurosci* **23**: 1398–1405.

Walther S, Mahlberg R, Eichmann U, Kunz D (2006). Delta-9-tetrahydrocannabinol for nighttime agitation in severe dementia. *Psychopharmacology (Berlin)* **185**: 524–528.

Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M (1994). Cannabinoid receptor binding and messenger RNA expression in human brain: an *in vitro* receptor autoradiography and *in situ* hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience* **63**: 637–652.

Williams K, Ulvestad E, Waage A, Antel JP, McLaurin J (1994). Activation of adult human derived microglia by myelin phagocytosis *in vitro*. *J Neurosci Res* **38**: 433–443.

Williams KC, Hickey WF (2002). Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. *Annu Rev Neurosci* **25**: 537–562.

Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F *et al*. (2003). Adult mouse astrocytes degrade amyloid-beta *in vitro* and *in situ*. *Nat Med* **9**: 453–457.

Wyss-Coray T, Mucke L (2002). Inflammation in neurodegenerative disease-A double-edged sword. *Neuron* **35**: 419–432.

Yiangou Y, Facer P, Durrenberger P, Chessell IP, Naylor A, Bountra C *et al*. (2006). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol* **6**: 12–18.

Zajicek J, Fox P, Sanders H, Wright D, Vickery J, Nunn A *et al*. (2003). Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. *Lancet* **362**: 1517–1526.

Zajicek JP, Sanders HP, Wright DE, Vickery PJ, Ingram WM, Reilly SM *et al*. (2005). Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up. *J Neurol Neurosurg Psychiatry* **76**: 1664–1669.

Zhang J, Hoffert C, Vu HK, Groblewski T, Ahmad S, O'Donnell D (2003). Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. *Eur J Neurosci* **17**: 2750–2754.