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MINI REVIEW

Cannabinoids and neuroinflammation

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Growing evidence suggests that a major physiological function of the cannabinoid signaling system is to modulate neuroinflammation. This review discusses the anti-inflammatory properties of cannabinoid compounds at molecular, cellular and whole animal levels, first by examining the evidence for anti-inflammatory effects of cannabinoids obtained using *in vivo* animal models of clinical neuroinflammatory conditions, specifically rodent models of multiple sclerosis, and second by describing the endogenous cannabinoid (endocannabinoid) system components in immune cells. Our aim is to identify immune functions modulated by cannabinoids that could account for their anti-inflammatory effects in these animal models.
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Abbreviations: 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; anandamide, arachidonoylethanolamide; CNS, central nervous system; DC, dendritic cells; EAE, experimental autoimmune encephalomyelitis; FAAH, fatty acid amide hydrolase; IFN-γ, interferon-gamma; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein 1; MGL, monoglyceride lipase; MHCII, major histocompatibility complex class II; MS, multiple sclerosis; NK cells, natural killer cells; NO, nitric oxide; PAF, platelet-activating factor; TGF-β, transforming growth factor β; T_H cells, T-helper cells; THC, Δ^9-tetrahydrocannabinol; TMEV, Theiler's murine encephalomyelitis virus; TNFα, tumor necrosis factor α

Introduction

Inflammation is an active defense reaction against diverse insults, designed to remove or inactivate noxious agents and to inhibit their detrimental effects. Although inflammation serves as a protective function in controlling infections and promoting tissue repair, it can also cause tissue damage and disease. Many cell types involved in this process express components of the cannabinoid signaling system that can be endogenously or pharmacologically controlled. Here, we propose to review this evidence; specifically, evidence showing that cannabinoids inhibit neuroinflammation and that immune cells express the entire machinery that constitutes a functional cannabinoid signaling system.

Two cannabinoid G protein-coupled receptors have been cloned thus far, CB_1 receptors (Matsuda *et al*., 1990), expressed primarily by neurons, and CB_2 receptors (Munro *et al*., 1993), expressed primarily by immune cells. Aside from these two receptors, evidence exists supporting the presence of yet uncloned cannabinoid receptors, a hypothesis predominantly based on pharmacological activity of cannabinoid compounds in CB_1 and CB_2 receptor-deficient mice or following the administration of 'selective' CB_1 and CB_2 receptor antagonists (Jarai *et al*., 1999; Di Marzo *et al*., 2000; Breivogel *et al*., 2001; Hajos & Freund, 2002; Begg *et al*., 2003). Here cannabinoids are defined as ingredients of the cannabis plant or other compounds that bind to and activate cannabinoid receptors, and Table 1 summarizes their pharmacological properties.

Following the cloning of CB_1 and CB_2 receptors, two endocannabinoid ligands were identified and characterized: arachidonoylethanolamide (anandamide) (Devane *et al*., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam *et al*., 1995; Sugiura *et al*., 1995). There are notable studies showing the anti-inflammatory properties of cannabinoid-like compounds, such as Δ^9-tetrahydrocannabinol (THC) metabolites and the endogenous compound palmitoylethanolamide. Since these compounds do not act through known cannabinoid receptors (Dajani *et al*., 1999; Lambert *et al*., 1999; Franklin *et al*., 2003; Zurier *et al*., 2003), we will only discuss them briefly here.

Cannabinoids have anti-inflammatory effects in animal models of neuroinflammation

CNS inflammation occurs in myelin degenerative disorders such as multiple sclerosis (MS) (reviewed in Martino *et al*., 2002) and also in neurodegenerative disorders such as Alzheimer's disease (McGeer & Rogers, 1992), HIV encephalopathy (Gendelman *et al*., 1994), ischemia (Chopp *et al*., 1994), and traumatic brain injury (Dusart & Schwab, 1994). In order to understand the anti-inflammatory potential of cannabinoids in clinical neuroinflammation, it is necessary to examine their anti-inflammatory effects in animal models. We chose to focus on MS because it is characterized by relapsing-remitting and chronic inflammation in the central nervous

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Table 1 Pharmacology of cannabinoid compounds

Compound	*CB_1 receptor activity*	*CB_2 receptor activity*	*References*
Plant cannabinoids			
THC	Partial agonist	Partial agonist	Gerard *et al.* (1991), Bayewitch *et al.* (1996)
Δ^8-THC	Partial agonist	Partial agonist	Matsuda *et al.* (1990), Gerard *et al.* (1991)
Cannabinol	Partial agonist	Partial agonist	Rhee *et al.* (1997), Condie *et al.* (1996)
Cannabidiol[a]	No activity	No activity	Showalter *et al.* (1996)
Endogenous cannabinoids			
Anandamide	Partial agonist	Partial agonist	Bayewitch *et al.* (1995), Rinaldi-Carmona *et al.* (1996)
2-AG	Agonist	Agonist	Stella *et al.* (1997), Sugiura *et al.* (2000)
Synthetic cannabinoids			
HU210	Agonist	Agonist	Slipetz *et al.* (1995), Song and Bonner (1996)
CP55940	Agonist	Agonist	Little *et al.* (1988)
WIN55212-2	Agonist	Agonist	Tao and Abood (1998)
JWH-015	No activity	Agonist	Showalter *et al.* (1996)
JWH-133	No activity	Agonist	Hanus *et al.* (1999)
ACEA	Agonist	No activity	Hillard *et al.* (1999)
Methanandamide	Agonist	Partial agonist	Lin *et al.* (1998), Berglund *et al.* (1998)
SR144528	No activity	Antagonist	Rinaldi-Carmona *et al.* (1998)
SR141716A	Antagonist	No activity[b]	Rinaldi-Carmona *et al.* (1994)
AM251	Antagonist	No activity	Gatley *et al.* (1997), Simonean *et al.* (2001)

[a]Cannabidiol acts as an antagonist on a presently uncloned cannabinoid receptor (Jarai *et al.*, 1999). [b]SR141716A binds to CB2 receptors at concentrations in the high nanomolar range and above (reviewed in Howlett *et al.*, 2002).

system (CNS), and cannabinoids have been shown to affect its pathogenesis.

Owing to the histological similarities with MS, experimental autoimmune encephalomyelitis (EAE) is a widely used animal model of this clinical disease (reviewed in Zamvil & Steinman, 1990). Initiation and maintenance of EAE result from T lymphocytes becoming sensitized to myelin proteins and eliciting a cell-mediated immune response. The subsequent pathology involves demyelination and a progression of inflammation in the CNS. To date, several independent studies performed on various rodent models of MS show that cannabinoids influence the course of disease progression.

The earlier studies focused on the effectiveness of cannabinoids in the treatment of rodents with EAE over a period of weeks. Lyman *et al.* (1989) administered THC or vehicle to guinea-pigs and rats once daily beginning either several days before or following inoculation with EAE. In animals given THC prior to inoculation, full clinical development of EAE was prevented, suggesting that THC suppressed the immune system (Lyman *et al.*, 1989). In animals given THC after inoculation, onset of symptoms was delayed and clinical index was lowered (Lyman *et al.*, 1989). Histological examination of spinal cords yielded significantly less inflammation in THC-treated animals (Lyman *et al.*, 1989). Wirguin *et al.* (1994) administered Δ^8-THC or vehicle daily to rats with EAE beginning several days prior to symptom onset. Δ^8-THC-treated animals had a delayed symptom onset, lowered incidence of EAE, and a shorter mean duration of EAE, but not a lower mean severity of disease (Wirguin *et al.*, 1994). Histological evaluation of microglia and astrocytes did not reveal any differences in the presence or distribution of these cells between treated and untreated animals (Wirguin *et al.*, 1994). The effects of cannabinoids on glial cell function in this model were not examined.

Later work on this topic examined cannabinoids as an acute treatment of symptoms associated with MS, that is, the immediate effects of cannabinoids on spasticity and tremor. In mice with EAE, WIN55212-2, THC, methanandamide, and JWH-133, but not cannabidiol, relieved these symptoms within 1 to 10 min of administration, and the effects lasted up to 1 h (Baker *et al.*, 2000). The effects of WIN55212-2 were reversed by treatment with cannabinoid receptor antagonists SR141716A and SR144528, and these two compounds administered alone worsened symptoms (Baker *et al.*, 2000). Here, since a CB_2 receptor agonist and a CB_2 receptor antagonist influenced these symptoms, these results again point towards an anti-inflammatory effect of cannabinoids because CB_2 receptors are expressed mainly on immune cells.

Theiler's murine encephalomyelitis virus (TMEV) infection of the CNS induces an immune-mediated demyelinating disease in susceptible mouse strains and serves as another model for human MS (dal Canto & Lipton, 1977). To examine the effects of cannabinoid compounds over several weeks in this model, Arevalo-Martin *et al.* (2003) administered cannabinoid agonists WIN55212-2, ACEA, and JWH-015 daily for 10 days following TMEV infection but prior to symptoms. These drugs improved motor function, decreased the number of activated microglia in the spinal cord, decreased major histocompatibility complex class II (MHCII) expression, decreased the number of CD4+ T cells in spinal cord, and promoted spinal cord remyelination (Arevalo-Martin *et al.*, 2003). Another group administered WIN55212-2 daily for 5 days to mice with TMEV beginning either prior to symptoms, at symptom onset, or several days after symptom onset (Croxford & Miller, 2003). Clinical disease symptoms were decreased under all these conditions (Croxford & Miller, 2003). WIN55212-2 increased susceptibility of mice to TMEV infection, suggesting an immunosuppressive effect, but it had no effect on splenic cell populations (Croxford & Miller, 2003). WIN55212-2 also decreased CNS mRNA encoding for proinflammatory cytokines tumor necrosis factor α (TNFα), interleukin (IL)-1β, and IL-6 in these mice (Croxford & Miller, 2003). (See Table 2 for a list of neuroinflammatory properties of cytokines.)

In EAE animals, there is evidence suggesting that changes in cannabinoid receptor expression and function occur. Berrendero *et al*. (2001) found, compared to control animals, rats with EAE had decreased CB_1 receptor binding in the cerebral cortex and caudate-putamen, and decreased CB_1 receptor mRNA levels in caudate-putamen of EAE animals but not other brain regions. However, there was increased CB_1 receptor activity in the cerebral cortex and caudate-putamen of EAE animals, suggesting that the remaining receptors in these areas may be more efficiently coupled to G protein-mediated signaling mechanisms (Berrendero *et al*., 2001).

Taking a more genetic approach to examining the cannabinoid system in EAE, Pryce *et al*. (2003) induced EAE in wild-type and CB_1 receptor-deficient mice. No difference in day of onset or peak clinical score was detected between wild-type and CB_1 receptor-deficient animals (Pryce *et al*., 2003). However, after EAE symptoms in wild-type animals had remitted, CB_1 receptor-deficient animals still had a high mean clinical score (Pryce *et al*., 2003). In addition, disability was worse in the CB_1 receptor-deficient animals. Spinal cord neurofilament levels were lower and spinal cord caspase-3 activity was higher in CB_1 receptor-deficient animals (Pryce *et al*., 2003). These findings imply a role of cannabinoid receptors in ameliorating the progression of and symptoms associated with neuroinflammation.

The combined results of these studies show that cannabinoid agonists ameliorate symptoms both acutely and over several weeks in EAE and TMEV models of MS. In addition, CB_1 receptor expression and function change in EAE and the absence of CB_1 receptors worsens symptoms of EAE. Although these studies provide appealing insights into the effects of cannabinoids on neuroinflammation, a model of how these compounds work is still incomplete due to differences in compounds, animal models of MS, rodent species, routes of drug administration, and dosing schedules used. To gain a more complete understanding of the cells targeted by cannabinoid compounds and the downstream effects of cannabinoids on these cells, we must turn to studies performed at the cellular level. Do cells involved in neuroinflammation express cannabinoid receptors? What immune cell functions are regulated by cannabinoid receptor activation?

Glial cells express cannabinoid receptors

Cells in healthy brain do not express CB_2 cannabinoid receptors (Munro *et al*., 1993; Griffin *et al*., 1999; Zimmer *et al*., 1999; Buckley *et al*., 2000). However, immune cells such as microglial cells, the macrophages of the brain, frequently alter levels of gene expression and express new gene products when stimulated with antigens and other bioactive substances. Chronic pain models associated with peripheral nerve injury induce CB_2 receptor expression in the spinal cord, coinciding with the appearance of activated microglia (Zhang *et al*., 2003). An investigation on the presence of CB_2 receptors in inflamed brain or spinal cord in a mouse model of neuroinflammation such as MS remains to be carried out.

Microglia regulate the initiation and progression of immune responses in the CNS (reviewed in Carson & Sutcliffe, 1999). Primary cultures of rat and mouse microglia express both CB_1 and CB_2 receptor mRNA and protein (Sinha *et al*., 1998; Carlisle *et al*., 2002; Facchinetti *et al*., 2003; Walter *et al*., 2003a). Human microglia also express CB_2 receptor mRNA (Klegeris *et al*., 2003). Primary mouse microglia express CB_2 receptors at the leading edges of lamellipodia and microspikes (Walter *et al*., 2003a), suggesting a function in motility, discussed below. The proinflammatory cytokine interferon-gamma (IFN-γ), which is produced by T_H1 T cells and natural killer (NK) cells in MS and EAE (reviewed in Popko *et al*., 1997), increases CB_2 receptor mRNA and protein in rat microglia (Carlisle *et al*., 2002).

Astrocytes, the main glial cell type in the brain, help regulate aspects of inflammation in the CNS (reviewed in Dong & Benveniste, 2001) and may be involved in the pathogenesis of MS (reviewed in De Keyser *et al*., 2003). While evidence for CB_1 receptor expression by astrocytes has been found by some groups (Bouaboula *et al*., 1995; Sanchez *et al*., 1998; Moldrich & Wenger, 2000; Abood *et al*., 2001; Rodriguez *et al*., 2001; Molina-Holgado *et al*., 2002b; Salio *et al*., 2002), it has not been found by others (Sagan *et al*., 1999; Walter & Stella, 2003b). These conflicting results may indicate variations in CB_1 receptor expression due to differences in species, culture systems, CNS structures from which cultures are derived, ages of cultures, or activation levels of cells; CB_2 receptor expression by astrocytes has not been found (Walter & Stella, 2003b). Oligodendrocytes, which undergo degeneration in MS and EAE (reviewed in Kornek & Lassman, 2003), also express CB_1 and CB_2 receptors (Molina-Holgado *et al*., 2002a).

Examples of all major types of glial cells expressing cannabinoid receptors exist and this may account for some of the anti-inflammatory effects seen with cannabinoids in rodent models of MS. While it is known that cannabinoid receptor expression is modulated by cytokines in microglial cells (Carlisle *et al*., 2002), it is not yet known if cannabinoid receptor expression is modulated in astrocytes or oligodendrocytes.

Table 2 Inflammatory properties of selected cytokines

Cytokine	*Neuroinflammatory property*	*References*
IFN-γ	Proinflammatory	Benveniste (1998), Popko *et al*. (1997)
IL-1β	Proinflammatory	Loddick *et al*. (1998), Rothwell and Luheshi (2000)
IL-4	Anti-inflammatory	Chao *et al*. (1993), Kitamura *et al*. (2000)
IL-6	Pro- or anti-inflammatory[a]	Campbell (1998), Raivich *et al*. (1999)
IL-8	Proinflammatory	Cuzner and Opdenakker (1999)
IL-12	Proinflammatory	Segal *et al*. (1998), Constantinescu *et al*. (1998)
MCP-1	Proinflammatory	Mahad and Ransohoff (2003)
TGFβ	Anti-inflammatory	Wyss-Coray *et al*. (2001), Bright and Sriram (2001)
TNFα	Proinflammatory	Bezzi *et al*. (2001), Lenzlinger *et al*. (2001)

[a]IL-6 can have pro- or anti-inflammatory outcomes likely determined by the simultaneous presence of other cytokines.

Glial cell function is modulated by cannabinoid compounds

Neuroinflammation induces a complex and dynamic change in glial cell phenotypes. One of the first cell types to respond is microglial cells, which retract their processes and migrate towards the site of injury, where they release proinflammatory cytokines such as IL-1β, TNFα, and IL-6 (Kreutzberg, 1996; Bruce-Keller, 1999; Becher *et al*., 2000). In primary cultures of mouse microglia, 2-AG induces cell migration, and this is reversed by SR144528, cannabinol, and cannabidiol (Walter *et al*., 2003a). Perhaps under neuroinflammatory conditions, neurons or astrocytes produce endocannabinoids as a means of recruiting microglia (Walter *et al*., 2002; 2003a; Walter & Stella, 2003b).

Nitric oxide (NO) production by glial cells is also associated with immune-mediated cellular cytotoxicity and pathogenesis of MS and EAE (reviewed in Parkinson *et al*., 1997). CP55940 inhibits NO production in IFN-γ- and lipopolysaccharide-(LPS, a bacterial cell wall molecule) stimulated rat microglia (Waksman *et al*., 1999; Cabral *et al*., 2001). Primary cultures of rat microglia activated by LPS release TNFα, and this is inhibited by anandamide, 2-AG, WIN55212-2, CP55940, and HU210, but SR141716A, AM251, and SR144528 do not alter WIN55212-2 effects (Facchinetti *et al*., 2003). THC reduces IL-1β, IL-6, and TNFα production in LPS-stimulated rat microglia (Puffenbarger *et al*., 2000). JWH-015 treatment reduces toxicity of human microglia towards neurons (Klegeris *et al*., 2003). Taken together, these results suggest that cannabinoid agonists decrease neurotoxicity and release of proinflammatory cytokines from microglia. However, whether these effects are mediated through cannabinoid receptors or other mechanisms is unknown.

Less is known about how cannabinoids influence the function of astrocytes and oligodendrocytes. Anandamide enhances the release of IL-6 from astrocytes infected with TMEV, the virus that elicits a mouse model of MS, and this is blocked by SR141716A (Molina-Holgado *et al*., 1998). Anandamide inhibits the release of NO and TNFα in LPS- or TMEV-stimulated astrocytes (Molina-Holgado *et al*., 1997). CP55940 and anandamide inhibit the release of NO in LPS-activated mouse astrocytes, and this is blocked by treatment with SR141716A (Molina-Holgado *et al*., 2002b). Anandamide and THC increase arachidonic acid (AA) release from rat astrocytes, and these effects are reversed by SR141716A (Shivachar *et al*., 1996). In mouse mixed glial cultures, HU210 and CP55940 increase LPS-induced production of IL-1 receptor antagonist, an anti-inflammatory cytokine that blocks the actions of IL-1β, and SR141716A and SR144528 lower this response (Molina-Holgado *et al*., 2003). In oligodendrocytes, ACEA, HU210, and WIN55212-2 enhance cell survival, and these effects are sensitive to SR141716A, suggesting possible CB_1 receptor involvement (Molina-Holgado *et al*., 2002a).

In summary, it is clear that microglia, astrocytes, and oligodendrocytes respond to cannabinoids, but this is an area that deserves further study to fully understand the cellular and tissue responses of CNS immune cells. Nevertheless, these studies suggest that some of the positive effects of cannabinoids in rodent models of MS may be due to an inhibition of proinflammatory mediator production from glia, an inhibition of microglial migration, and an enhancement of oligodendrocyte survival. It remains to be shown whether these specific cellular effects occur *in vivo*.

Peripheral immune cells express cannabinoid receptors

Peripheral immune cells also participate in the neuroinflammatory response (reviewed in Carson & Sutcliffe, 1999). CB_2 receptor mRNA is expressed, in decreasing rank order, by human B cells, NK cells, monocytes, neutrophils, and T cells at levels 10 to 100 times higher than CB_1 receptor mRNA (Galiegue *et al*., 1995). Cells of the immune system express mRNA for the CB_1 receptor, but at lower levels than cells of the CNS (Galiegue *et al*., 1995). In human peripheral blood cells, CB_1 receptor mRNA and protein are expressed, in decreasing rank order, by B cells, NK cells, neutrophils, CD8+ T cells, monocytes, and CD4+ T cells (Galiegue *et al*., 1995).

CB_1 and CB_2 receptors are expressed by human peripheral blood cell-derived dendritic cells (DCs) (Matias *et al*., 2002), and mRNA for both CB_1 and CB_2 receptors is expressed in mouse bone marrow-derived DC (Klein *et al*., 2003). Mouse peritoneal macrophages and rodent macrophage cell lines express CB_2 receptor mRNA, and this message is more abundant than the message for CB_1 (Carlisle *et al*., 2002). Rat peritoneal macrophages also express CB_2 receptors (Carlisle *et al*., 2002). In peripheral macrophages, like in their CNS counterpart, CB_2 receptor expression may be modulated by the activation state of the cell. IFN-γ increases CB_2 receptor protein in mouse macrophages (Carlisle *et al*., 2002). Levels of CB_2 receptors in cells of macrophage lineage undergo changes correlating with cell activation, and inflammatory and primed macrophages express higher levels of CB_2 receptor, so the functions of macrophages in these states of activation may be the most sensitive to the actions of cannabinoids (Carlisle *et al*., 2002).

Other peripheral immune cells also modulate their cannabinoid receptor expression. CD40 is expressed by both macrophages and DC and can regulate T- and B-cell responses in MS (reviewed in Laman *et al*., 1998). CB_2 receptor expression in human B cells increases following the activation by anti-CD40 antibody (Carayon *et al*., 1998). There is a clear role of mature B cells in MS and EAE, as clonal B-cell accumulation in the CSF or lesions of MS patients occurs (reviewed in Cross *et al*., 2001). Differentiation of B cells is accompanied by decreased expression of CB_2 receptor mRNA and protein (Carayon *et al*., 1998). Transforming growth factor β (TGF-β), an anti-inflammatory cytokine produced by glial and neural cells that influences the function and survival of glial cells in MS (reviewed in Pratt & Mcpherson, 1997), leads to a decrease in CB_2 receptor expression in human peripheral blood lymphocytes (Gardner *et al*., 2002).

In summary, peripheral immune cells that participate in neuroinflammation express cannabinoid receptors. The expression level of CB_2 receptors depends on whether and how these cells are activated. These data suggest a physiological role of the endocannabinoid system in the functions of immune cells with respect to inflammation, and point to cannabinoid receptors as pharmacological targets aimed at treating neuroinflammation, perhaps explaining some of the benefits seen with cannabinoids in rodent models of MS. However, it

remains to be shown if cannabinoid receptor expression in immune cells is altered during MS.

Peripheral cells involved in neuroinflammation respond to cannabinoid compounds

During inflammation, T cells and macrophages secrete cytokines that stimulate recruitment and activation of leukocytes to eliminate a perceived infectious agent. CB_2 receptor activation typically inhibits the functions of immune cells (reviewed in Parolaro, 1999), likely *via* the known CB_2 receptor intracellular signaling mechanisms: inhibition of adenylyl cyclase activity by $G_{i/o}$ proteins and activation of mitogen-activated protein kinase (Bouaboula *et al*., 1993; Bayewitch *et al*., 1995; Felder *et al*., 1995; Wartmann *et al*., 1995). The following subsections discuss in more detail the effects of endogenous, plant, and synthetic cannabinoid compounds on peripheral immune cells.

Proliferation and chemotaxis

Cannabinoids may suppress the immune response, and hence the inflammatory response, by modulating proliferation or inducing apoptosis in lymphocytes. An increase in lymphocyte cell number is crucial for an inflammatory response to occur. THC induces apoptosis in macrophages (Zhu *et al*., 1998). Cannabidiol causes a dose-dependent suppression of lymphocyte proliferation (Malfait *et al*., 2000). Δ^8-THC, CP55940, and anandamide also suppress T- and B-cell proliferation (Schwarz *et al*., 1994). Interestingly, another report showed that CP55940 enhances proliferation of B cells and this is blocked by SR144528 (Carayon *et al*., 1998).

Myeloid leukemia cells expressing CB_2 receptors display chemotaxis and chemokinesis in response to 2-AG, but not in response to other cannabinoid compounds (Jorda *et al*., 2002). CP55940 causes chemotaxis and chemokinesis of HL60 human leukemia cells expressing CB_2 receptors (Derocq *et al*., 2000), but CP55940 inhibits rat macrophage migration (Sacerdote *et al*., 2000).

Cytokine production

T-helper (T_H) cells regulate cell-mediated (T_H1) and humoral (T_H2) adaptive immunity. A shift towards T_H1 has been associated with disease progression, while polarizing T-cell responses towards a T_H2 phenotype has been associated with therapeutic benefit in MS and EAE, although some evidence suggests that this model may be more complex (reviewed in Hemmer *et al*., 2002). THC suppresses T_H1 immunity by inhibiting the production of IFN-γ and proinflammatory IL-12 as well as the expression of IL-12 receptors, and it increases the expression of anti-inflammatory IL-4, a T_H2 cytokine, all through CB_1 and CB_2 receptors (Newton *et al*., 1994; Klein *et al*., 2000; Yuan *et al*., 2002). These results may explain some of the longer-term effects of cannabinoids on ameliorating EAE.

Monocytes and macrophages are critical to the progression of MS and EAE. Anandamide inhibits proinflammatory TNFα production, inhibits cytokine soluble receptors, and inhibits IL-6 and IL-8 in LPS-stimulated monocytes (Berdyshev *et al*., 1997). 2-AG inhibits TNFα production from mouse macrophages (Gallily *et al*., 2000). JWH-015 added to THP-1 macrophages before stimulation with LPS and IFN-γ reduces the secretion of proinflammatory IL-1β and TNFα and the toxicity of culture supernatants to human neuroblastoma cells, and this latter effect is reversed by SR144528 but not SR141716A (Klegeris *et al*., 2003), suggesting that this effect could be mediated by CB_2 receptors. Although these results indicate an anti-inflammatory effect of cannabinoids, there are other results that suggest that cannabinoids may in some cases be proinflammatory. Indeed, THC was shown to increase release of IL-1β from macrophages (Zhu *et al*., 1998). In addition, CP55940, but not anandamide or THC, induces proinflammatory IL-8 and monocyte chemotactic protein 1 (MCP-1) gene expression in unstimulated HL60 human leukemia cells, due to the activation of CB_2 receptors (Jbilo *et al*., 1999).

NO production

THC inhibits NO production in LPS/IFN-γ-stimulated mouse macrophages (Coffey *et al*., 1996a, b) and in LPS-stimulated RAW 264.7 macrophages (Jeon *et al*., 1996). WIN also inhibits the LPS-induced release of NO in macrophages, an effect blocked by SR144528 (Ross *et al*., 2000). CP55940 reduces NO production from IFN-γ/LPS-stimulated feline macrophages and this is reversed by either SR141716A or SR144528 (Ponti *et al*., 2001).

Although plant and synthetic cannabinoids inhibit NO production from immune cells, endogenous cannabinoids induce it. Anandamide increases NO production in human monocytes (Stefano *et al*., 1996) and macrophages (Stefano *et al*., 1998). 2-AG increases NO in human monocytes, and this is blocked by SR141716A, but not SR144528 (Stefano *et al*., 2000). 2-AG does not alter NO from mouse macrophages (Gallily *et al*., 2000). Why plant and synthetic cannabinoid agonists induce the opposite response to endocannabinoids is an open question.

AA release

Increased AA levels may be associated with MS (reviewed in Greco *et al*., 2000). Indeed, it is the precursor of the class of bioactive molecules consisting of the proinflammatory eicosanoids. Anandamide stimulates AA release in monocytes (Berdyshev *et al*., 1997). Anandamide induces AA release from J774 mouse macrophages, and this is blocked by pertussis toxin, an inhibitor of $G_{i/o}$ proteins (Di Marzo *et al*., 1997). However, anandamide also induces AA release in cells that do not express CB_1 or CB_2 receptors (Felder *et al*., 1993; Felder *et al*., 1995). THC induces AA release from RAW 264.7 mouse macrophages, and this is likely mediated by the CB_2 receptor (Hunter & Burstein, 1997). The effects of cannabinoids on AA release imply a proinflammatory influence on peripheral immune cells.

Several other cannabinoid-like compounds may have anti-inflammatory potential. Ajulemic acid, an analog of a THC metabolite (reviewed in Burstein, 2000), has been shown to elicit anti-inflammatory properties in transfected human embryonic kidney HEK293 cells in culture (Liu *et al*., 2003). Palmitoylethanolamide is a powerful anti-inflammatory agent (reviewed in Lambert *et al*., 2002), and its action is blocked by the antagonist SR144528 in a rodent model of peripheral

inflammation (Calignano *et al*., 1998). The endogenous compound oleoylethanolamide is structurally related to palmitoylethanolamide, does not bind to CB_1 or CB_2 receptors (Lin *et al*., 1998), and has an anti-inflammatory molecular target (Fu *et al*., 2003). Noladin ether, a putative endocannabinoid, can bind to the CB_1 receptor but not the CB_2 receptor (Hanus *et al*., 2001). However, its natural presence in tissue is controversial since Fezza *et al*. (2002) quantified noladin ether in rat brain, but Oka *et al*. (2003) did not detect this compound in rat, mouse, hamster, guinea-pig, or pig brain. The possibility for these compounds to influence the course of neuroinflammation remains to be discovered.

To summarize this section, peripheral immune cells involved in inflammation respond to endogenous, plant, and synthetic cannabinoids by altering the production of pro- and anti-inflammatory mediators, migrating, and decreasing proliferation. This information points towards possible explanations of the effects seen with cannabinoids in rodent models of MS. As with the cannabinoid effects on glial cells, it also remains to be seen whether cannabinoids elicit these effects on peripheral immune cells *in vivo*.

Cells involved in inflammation produce and degrade endocannabinoids

Synthesis of endocannabinoids occurs *via* hydrolysis of membrane lipid precursors (reviewed in Piomelli, 2003). Anandamide is formed *via* cleavage of its precursor *N*-arachidonoyl phosphatidylethanolamine by phospholipase D, whereas 2-AG is formed *via* cleavage of its precursor diacylglycerol by diacylglycerol lipase (reviewed in Di Marzo *et al*., 1999a). Anandamide (Willoughby *et al*., 1997) and 2-AG (Jarai *et al*., 2000) are both degraded *in vivo* into AA. The main hydrolyzing enzyme for anandamide is fatty acid amide hydrolase (FAAH) (Cravatt *et al*., 1996), and the main hydrolyzing enzyme for 2-AG is monoglyceride lipase (MGL) (Dinh *et al*., 2002). In addition, there is evidence suggesting specific transporters for endocannabinoids exist (reviewed in Hillard & Jarrahian, 2000; Fowler & Jacobsson, 2002), although this topic is subject to some controversy (Glaser *et al*., 2003).

Many cell types, including immune cells, are capable of producing and degrading endocannabinoids (reviewed in Di Marzo *et al*., 1999a). Although the complete list of mediators capable of influencing endocannabinoid production and degradation is not known, basal production and degradation of endocannabinoids has been characterized in immune cells, and some modulators have been identified.

Production

One group measured endocannabinoid levels in mice with EAE. Baker *et al*. (2001) found that brains and spinal cords of spastic animals contained elevated levels of anandamide and 2-AG compared with control animals. Interestingly, exogenous administration of anandamide and 2-AG ameliorated spasticity within minutes (Baker *et al*., 2001). These results show that inflamed CNS tissues produce endocannabinoids and that augmenting this production may have a beneficial effect. The specific cellular sources of endocannabinoids are discussed below.

Primary cultures of mouse microglia produce anandamide and 2-AG, and 2-AG production increases in response to ATP (Walter *et al*., 2003a). BV-2 mouse microglial cells also produce anandamide and 2-AG, and 2-AG increases in response to ionomycin, a calcium ionophore (Walter *et al*., 2003a), reinforcing the notion that endocannabinoid production is calcium dependent. In addition, mouse astrocytes produce anandamide and 2-AG, and levels of these endocannabinoids increase in response to the vasoconstrictor endothelin-1 (Walter *et al*., 2003a; Walter & Stella, 2003b).

Like their CNS counterparts, J774 mouse macrophages also increase anandamide and 2-AG in response to ionomycin (Di Marzo *et al*., 1999b). LPS induces anandamide and 2-AG in rat circulating macrophages (Wagner *et al*., 1997; Di Marzo *et al*., 1999b) and 2-AG in rat platelets (Varga *et al*., 1998). In RAW 264.7 mouse macrophages, LPS (Pestonjamasp & Burstein, 1998; Liu *et al*., 2003) and platelet-activating factor (PAF) (Pestonjamasp & Burstein, 1998) induce anandamide. PAF also increases 2-AG in these cells (Liu *et al*., 2003). LPS has been shown to increase anandamide in human lymphocytes (Maccarrone *et al*., 2001) and 2-AG in human DCs (Matias *et al*., 2002).

Degradation

Baker *et al*. (2001) also used mice with EAE to examine the potential for pharmacologically targeting the FAAH enzyme as a treatment of neuroinflammation. A pharmacological inhibitor of FAAH, as well as inhibitors of the putative anandamide transporter, reduced spasticity within minutes, and effects were blocked by the administration of SR141716A in conjunction with SR144528 (Baker *et al*., 2001). The specific cells involved in neuoinflammation that are capable of degrading endocannabinoids are discussed below.

In the CNS, the majority of FAAH is expressed by neurons, but one study showed that astrocytes also express FAAH (Romero *et al*., 2002). Outside the CNS, human T cells (Maccarrone *et al*., 2003) and human DC (Matias *et al*., 2002) express FAAH. RBL-2H3 rat basophilic leukemia cells have FAAH activity (Bisogno *et al*., 1997) and also express FAAH (Day *et al*., 2001). Rat macrophages express FAAH mRNA (Di Marzo *et al*., 1999b), and LPS induces FAAH mRNA expression in RAW 264.7 macrophages (Liu *et al*., 2003). J774 macrophages rapidly inactivate anandamide (Bisogno *et al*., 1997). In addition, U937 human lymphoma cells are sensitive to FAAH inhibitors (Maccarrone *et al*., 2000b), and HMC-1 human mast cells hydrolyze anandamide (Maccarrone *et al*., 2000a).

RBL-2H3 rat basophilic leukemia cells hydrolyze 2-AG (Di Marzo *et al*., 1998). J774 mouse macrophages also rapidly inactivate 2-AG (Di Marzo *et al*., 1999b), and LPS downregulates enzymatic 2-AG hydrolysis in rat macrophages (Di Marzo *et al*., 1999b).

In summary, cells and tissues involved in neuroinflammation produce and degrade endocannabinoids, and anandamide and 2-AG levels are differentially regulated in cells. Glial and immune cells increase endocannabinoid production under selective conditions. It is possible that injured tissues produce endocannabinoids and consequently induce immune cells to migrate towards them. Enzymes responsible for the production and degradation of endocannabinoids may be suitable targets for pharmacological therapeutics against inflammation, as

they are expressed both in the CNS and in the periphery. It would be of interest to identify the specific cell types responsible for the consequences of inhibiting FAAH in the mouse model of MS. Animals genetically engineered to lack the primary metabolizing enzyme of anandamide (Cravatt *et al*., 2001) and 2-AG will help to further determine the role of the cannabinoid system in inflammation.

Conclusion

Cells involved in neuroinflammation express functional cannabinoid receptors and produce and degrade endocannabinoids, suggesting that the endocannabinoid signaling system has a regulatory function in the inflammatory response. Specifically, during neuroinflammation, there is an upregulation of components involved in the cannabinoid signaling system. This suggests that the cannabinoid signaling system participates in the complex development of this disease, which includes a tight orchestration of the various immune cells involved. If this is the case, the cannabinoid signaling machinery may provide ideal targets, since these would be more susceptible to pharmacological effects than those in the same system under healthy conditions. In line with this, cannabinoid compounds alter the functions of these cells, generally by eliciting anti-inflammatory effects. In the case of MS, neuroinflammation is accompanied by autoimmunity and suppressing the immune response may halt or even prevent associated symptoms. As seen in rodent models of MS, cannabinoids ameliorate the progression of and symptoms associated with neuroinflammation. Future experiments into the components that alter endocannabinoid production and degradation, cannabinoid receptor expression, and effects of cannabinoid receptor agonists on immune cells will provide the necessary information to design more effective treatments for neuroinflammation.

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