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Emerging role of the cannabinoid receptor CB_2 in immune regulation: therapeutic prospects for neuroinflammation

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There is now a large body of data indicating that the cannabinoid receptor type 2 (CB_2) is linked to a variety of immune events. This functional relevance appears to be most salient in the course of inflammation, a process during which there is an increased number of receptors that are available for activation. Studies aimed at elucidating signal transduction events resulting from CB_2 interaction with its native ligands, and of the role of exogenous cannabinoids in modulating this process, are providing novel insights into the role of CB_2 in maintaining a homeostatic immune balance within the host. Furthermore, these studies suggest that the CB_2 may serve as a selective molecular target for therapeutic manipulation of untoward immune responses, including those associated with a variety of neuropathies that exhibit a hyperinflammatory component.

Cannabinoids are highly lipophilic molecules that have been shown to alter the functional activities of immune cells in vitro and in vivo. The term 'exogenous cannabinoids' describes cannabinoids that are extracted from the marijuana plant *Cannabis sativa* or are synthesised in the laboratory. The marijuana cannabinoids delta-9-tetrahydrocannabinol (Δ^9-THC), cannabinol (CBN) and cannabidiol (CBD) have been the most studied exogenous cannabinoids. Δ^9-THC acts as a partial receptor agonist: on binding to cannabinoid receptors it elicits a response that never reaches the maximum efficacy. This compound is the major psychoactive and immunomodulatory component in marijuana and primarily exerts immunosuppressive effects on immune cells at peripheral sites and within the central nervous system (CNS). Synthetic exogenous cannabinoids that have been used widely in research include CP55940, WIN55212-2, SR141716A and SR144528 (Fig. 1). CP55940 and WIN55212-2 are full receptor agonists: on binding to cannabinoid receptors they elicit responses that garner maximum efficacy.

Endocannabinoids ('endogenous cannabinoids') constitute a second group of cannabinoids; these are found natively in vertebrate systems and are

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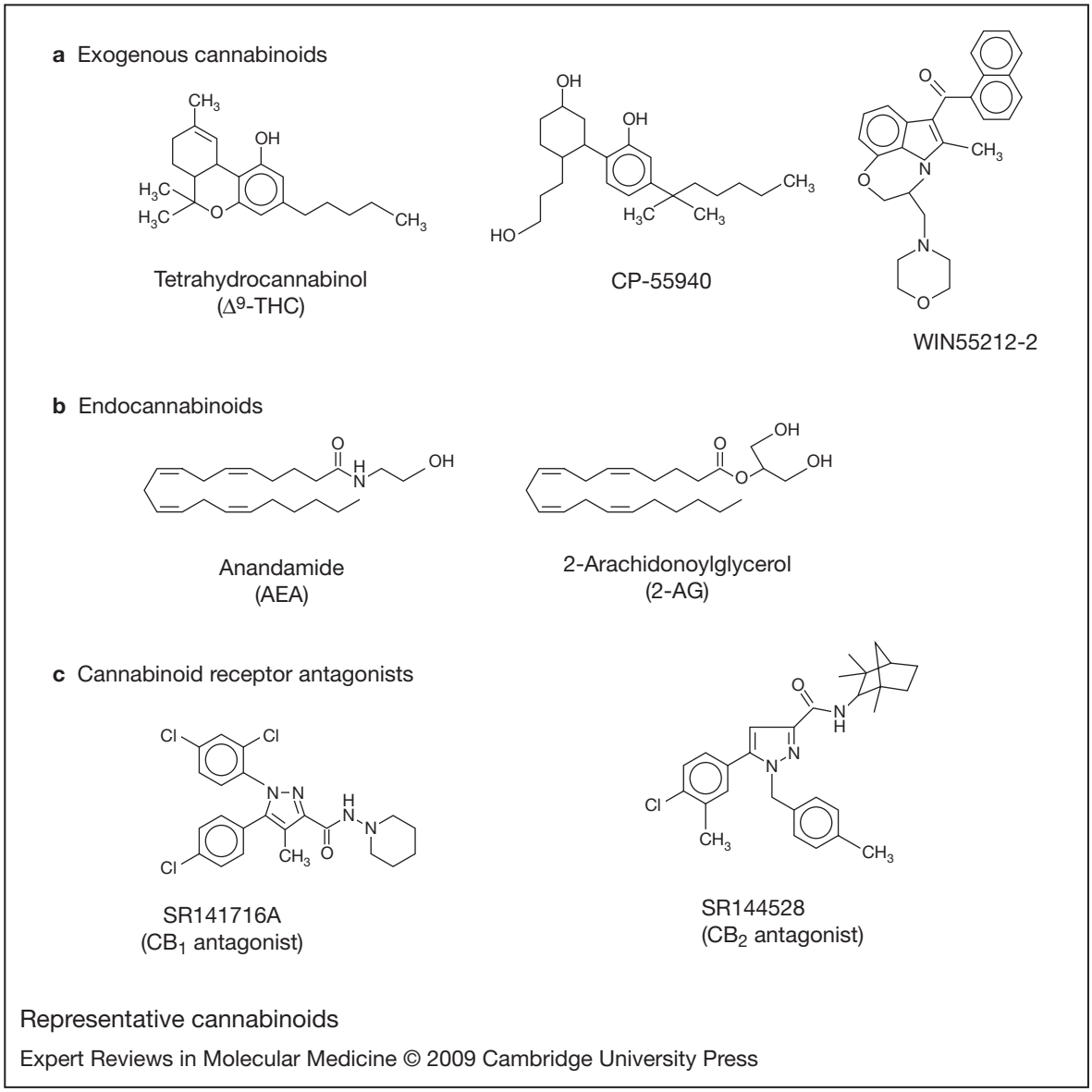

Figure 1. Representative cannabinoids. (a) Exogenous cannabinoids. Δ^9-THC is a partial agonist for CB_1 and CB_2; CP55940 and WIN55212-2 are full agonists for CB_1 and CB_2. (b) Endogenous cannabinoids. AEA and 2-AG show agonist behaviour at CB_1 and CB_2. (c) Cannabinoid receptor antagonists. SR141716A is an antagonist for CB_1; SR144528 is an antagonist for CB_2. Abbreviations: AEA, arachidonoylethanolamide (anadamide); CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CP55940, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; SR141716A, 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(1-piperidyl)pyrazole-3-carboxamide hydrochloride; SR144528, (1S-endo)-5-(4-Chloro-3-methylphenyl)-1-((4-methylphenyl)methyl)-N-(1,3,3-trimethylbicyclo(2.2.1)hept-2-yl)-1H-pyrazole-3-carboxamide; Δ^9-THC, delta-9-tetrahydrocannabinol; WIN55212-2, (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanone.

produced by a variety of cell types, including endothelial cells (Ref. 1), adipocytes (Ref. 2), glial cells (Ref. 3), macrophages (Ref. 4) and Purkinje cells (Ref. 5). These molecules are constituent elements of the 'endocannabinoid system', which also encompasses mediators responsible for their synthesis, metabolism and catabolism, as well as the cannabinoid receptors that serve as their

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molecular targets. Endocannabinoids are derivatives of integral components of cellular membranes and act as hydrophobic lipid messengers. As a result of their hydrophobicity, these molecules are not able to translocate in aqueous environments and, upon release, activate cannabinoid receptors locally or on nearby cells. Within the CNS, these bioactive lipids act as synaptic modulators (retrograde messengers); however, unlike other synaptic messengers such as the neurotransmitters acetylcholine and dopamine, endocannabinoids are not presynthesised and stored in vesicles but are produced 'on demand'.

The first endocannabinoid to be identified was arachidonoylethanolamide (AEA; anandamide), which was isolated from porcine brain (Ref. 6). AEA is the amide component of arachidonic acid and ethanolamine. The second endocannabinoid to be identified was 2-arachidonoylglycerol (2-AG), which was isolated from canine gut (Ref. 7). 2-AG is an ester derivative of arachidonic acid and glycerol, and is synthesised from the hydrolysis of 1,2-diacylglycerol (DAG) by a DAG lipase. In the brain, 2-AG is more bioactive and abundant than AEA. Both AEA and 2-AG are transported across the cell membrane before being degraded by fatty acid amide hydrolase (FAAH), although 2-AG can also be degraded by monoacylglycerol lipase (MGL), a serine hydrolase (Refs 8, 9, 10). Several reports suggest that transport across cellular membranes is facilitated by carrier proteins, which have yet to be fully identified (Refs 11, 12, 13, 14). In addition, while cannabinoid receptors serve as the molecular targets of AEA and 2-AG, it has been reported that these two compounds also can exert their effects in a receptor-independent fashion (Refs 15, 16, 17, 18).

Cannabinoid receptors

CB_1

The initial evidence for the existence of a cannabinoid receptor came from pharmacological studies. Treatment of neuroblastoma cells with Δ^9-THC, or with the synthetic compounds levonantradol and desacetyllevonantradol, resulted in inhibition of plasma membrane activity of adenylate cyclase, the enzyme that catalyses the conversion of ATP to cyclic AMP (cAMP) and pyrophosphate (Refs 19, 20). However, in contrast to levonantradol, dextronantradol was shown to have no effect on this activity, indicating that the inhibition was stereoselective – a requisite condition for a receptor-mediated action. Additional studies demonstrated that the putative cannabinoid receptor was coupled to an inhibitory guanine-nucleotide-binding complex ($G_{i/o}$), because treatment with pertussis toxin reversed the inhibitory effect on adenylate cyclase (Ref. 21). Radioligand binding assays and in situ mRNA hybridisation demonstrated that the receptor was distributed throughout the brain and was localised predominantly to the cerebellum, cerebral cortex, hippocampus, basal ganglia and spinal cord (Refs 6, 22, 23, 24). Subsequently, the gene encoding the receptor (*CNR1*) was isolated and cloned from a rat brain cDNA library (Ref. 22), revealing the sequence for a 473 amino acid long, seven-transmembrane G-protein-coupled protein with an extracellular, glycosylated N-terminus and an intracellular C-terminus (Fig. 2a). This receptor was referred to initially as the 'neuronal' or 'central' cannabinoid receptor and has since been designated cannabinoid receptor 1 (CB_1).

CB_1 inhibits the phosphorylation of A-type potassium channels through the coupled inhibitory G protein ($G_{i/o}$), as explained in further detail below in the cannabinoid receptor signalling section. Inhibition of the phosphorylation of A-type potassium channels results in continuous potassium currents that may prevent neurotransmission (Refs 25, 26). N-type calcium channels also are inhibited by CB_1 through direct interaction with the inhibitory G protein ($G_{i/o}$). CB_1-mediated restriction of neurotransmission via potassium and calcium channels accounts for cognitive impairment and sedative-like effects experienced by marijuana users (Refs 26, 27).

CB_2

Following the identification of CB_1, a 'peripheral' or 'non-neuronal' cannabinoid receptor was cloned from a human promyelocytic cell line (HL60) cDNA library, and was designated cannabinoid receptor 2 (CB_2) (Ref. 28). The gene for this receptor (*CNR2*) was shown to encode a 360 amino acid long, seven-transmembrane G-protein-coupled receptor (Fig. 2b) with, like CB_1, an extracellular, glycosylated N-terminus and an intracellular C-terminus. Unlike CB_1, there is a considerable level of sequence variation for CB_2 among human, mouse and rat species, particularly when comparing rat and human sequences. There is 81% amino acid identity between rat and human CB_2, as compared with

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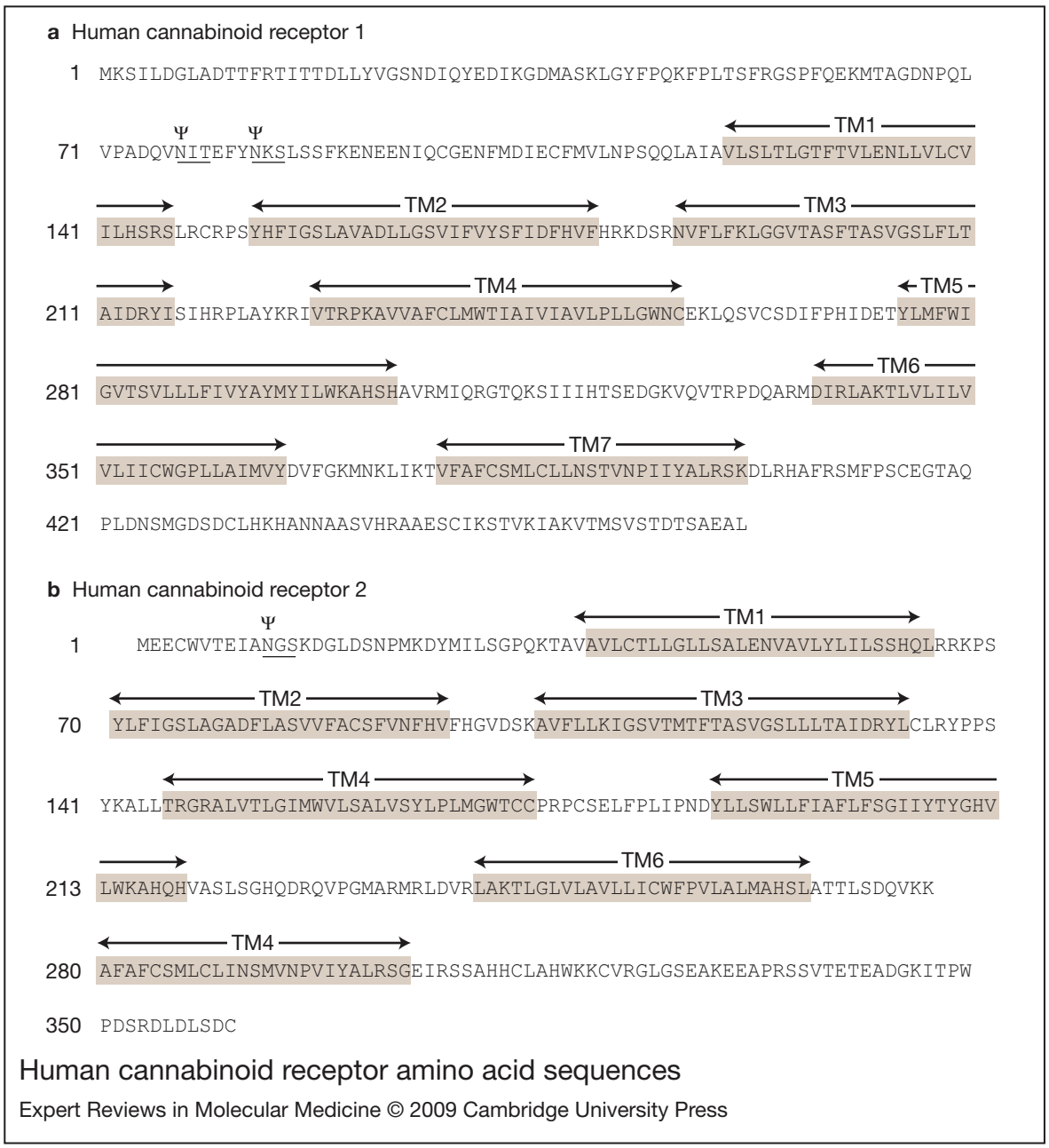

Figure 2. Human cannabinoid receptor amino acid sequences. (a) Amino acid sequence of the full-length human cannabinoid receptor 1 (CB_1). The putative asparagine-linked glycosylation sites are shown as Ψ. The seven transmembrane domains are highlighted and noted TM1 to TM7. The Genebank accession numbers are NM_016083, NM_001840 and NP_057167.2. (b) Amino acid sequence of the full-length human cannabinoid receptor 2 (CB_2). The putative asparagine-linked glycosylation site is shown as Ψ. The seven transmembrane domains are highlighted and noted TM1 to TM7. The Genebank accession numbers are NM_001841 and NP_001832.

93% amino acid identity between rat and mouse CB_2 (Ref. 29). The C-terminus in particular differs in rat as a result of intronic DNA (Ref. 30). Of note, it is the C-terminus of CB_2 that plays a critical role in regulating receptor desensitisation and internalisation (Ref. 31); thus, sequence variation within this region should be taken into consideration when investigating physiological,

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pharmacological and immunological responses of CB_2 in diverse species.

Another distinctive feature of CB_2 in comparison with CB_1 is that its distribution is predominantly in cells and tissues of the immune system, including the thymus, tonsils, B cells, T cells, macrophages, monocytes, natural killer (NK) cells and polymorphonuclear cells. B cells express the highest amounts of CB_2, followed by NK cells, macrophages and T cells, in that order (Refs 32, 33). Recent studies have demonstrated that CB_2 is also expressed within the CNS during various states of inflammation (Refs 34, 35, 36, 37), as discussed in detail later in the article. This expression of CB_2 has been localised primarily to microglia, the resident macrophages of the CNS. CB_2 expression is detected in these cells upon activation by various insults and stimuli, but measurable levels of CB_2 expression cannot be detected in resident, unstimulated microglia. In addition, during neuroinflammation, infiltrating immunocytes from peripheral non-neuronal sites that enter the brain as a result of breakdown of the blood–brain barrier contribute to the overall expression of CB_2. Table 1 lists select references for reports of the distribution of CB_1 and CB_2 in various immune tissues and cell types.

Other receptors

There is accumulating evidence that additional cannabinoid receptors exist, primarily from studies in which CB_1 knockout or CB_1–CB_2 double-knockout mice have been used to investigate the pharmacology and pharmacokinetics of Δ^9-THC, AEA and cannabinoid analogues. Recently, it has been suggested that the G-protein-coupled receptor GPR55, first cloned and identified in silico from an expressed sequence tags (ESTs) database (Refs 38, 39, 40), may be a novel cannabinoid receptor. Similar to CB_1 and CB_2, GPR55 has seven conserved transmembrane sequences and is activated by plantonic and synthetic exogenous cannabinoids such as Δ^9-THC, CBD, abnormal CBD, HU-210 and CP55940, and by the endogenous cannabinoids AEA, 2-AG and noladin ether (Ref. 41). Unlike CB_1 and CB_2, GPR55 is not activated by the synthetic agonist WIN55212-2, but is coupled to a $G_{\alpha 12/13}$ protein instead of a $G_{i/o}$ protein (Ref. 41) and has been shown to increase intracellular calcium levels upon activation (Ref. 42). GPR55 expression has been identified in a variety of tissues including spleen, gastrointestinal tissues and brain (Ref. 39). However, the physiological and pharmacological functional relevance of GPR55 has yet to be elucidated.

Another receptor reported to be a candidate cannabinoid receptor is the transient receptor potential vanilloid 1 (TRVP1) receptor, a ligand-gated cation channel and a member of the transient receptor potential channel family (Ref. 43). TRVP1 receptors are inherently activated by naturally occurring compounds such as capsaicin, vanilloids and resiniferatoxin (Ref. 44). Its implied role as a cannabinoid receptor is based on the ability of the endogenous cannabinoid AEA, shown to be structurally similar to capsaicin, to bind to and activate this receptor (Refs 45, 46, 47, 48). Nevertheless, despite the various speculative reports of additional cannabinoid receptor subtypes, a novel cannabinoid receptor that meets rigid criteria pharmacologically and functionally has yet to be identified (Refs 49, 50, 51, 52).

Cannabinoid receptor signalling

Both CB_1 and CB_2 are involved in activating signalling cascades that include adenylate cyclase and cAMP, mitogen-activated protein kinase (MAPK), and modulation of levels of intracellular calcium (Refs 26, 53, 54, 55, 56). Upon cannabinoid receptor interaction with its cognate ligand, the receptor-coupled G protein exchanges the inactive guanine nucleotide GDP for its active form GTP, and the heterotrimeric G protein dissociates into α and $\beta\gamma$ subunits (Fig. 3a). The $\beta\gamma$ subunits are thought to take part in signalling pathways distinct from those of the α subunit, such as the regulation of phospholipase C (PLC) isoforms and activation of the MAPK signalling network (Ref. 25) (Fig. 3b). CB_2 partly exerts its effects through initiation of phospholipase C (PLC) and inositol 1,4,5-trisphosphate (IP_3) signalling pathways that result in increased levels of intracellular calcium (Ref. 56). The α subunit binds to, and inhibits the activity of, adenylate cyclase, thereby preventing synthesis of the second messenger cAMP and negatively affecting downstream cAMP-dependent signalling events. As a decrease in cAMP production underlies a mechanism in which CB_1 prevents neurotransmitter release and maintains the homeostatic integrity of the CNS, decreased cAMP production also may represent a mode

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Table 1. Distribution of cannabinoid receptors in the immune system

Cell type/tissue	Species	Receptor	Refs
B cells	Human	CB_2	32, 78
T4 cells	Human	CB_2	32
T8 cells	Human	CB_2	32
Leukocytes	Human	CB_2	176
Macrophages	Human, mouse	CB_2	32, 105, 177, 178
Microglia	Rat	CB_1, CB_2	105, 167, 179
Mononuclear cells	Human, rat	CB_2	32, 86
Mast cells	Rat	CB_2	86
Natural killer cells	Human	CB_2	32
Peyer's patches	Rat	CB[a]	180
Spleen	Human, mouse, rat	CB_1, CB_2	28, 32, 86, 180, 181
Thymus	Human	CB_2	32
Tonsils	Human	CB_2	32
Lymph nodes	Rat	CB[a]	180

[a]Cannabinoid receptor type not specified.

by which CB_2 signalling in response to endocannabinoids maintains immunological homeostasis or, alternatively, in response to exogenous cannabinoids such as Δ^9-THC superimposes a perturbing immunosuppressive effect (Ref. 57).

Role of CB_2 in immune modulation

Effect of exogenous cannabinoids on host resistance and immunity

Exogenous cannabinoids have been shown to decrease host resistance to a variety of infectious agents. Administration of Δ^9-THC to mice lessened their ability to resist infection with the bacterial agent *Listeria monocytogenes* and herpes simplex virus 2 (Refs 58, 59). In mice and guinea pig models of genital herpes, treatment with Δ^9-THC increased incidence of viral lesions and recurrence (Refs 60, 61). In additon, cannabinoids compromised host resistance to *Legionella pneumophila*, *Staphylococcus albus*, *Treponema pallidum*, Friend leukaemia virus and *Acanthamoeba* (Refs 58, 62, 63, 64, 65, 66). These observations are consistent with the notion that exogenous cannabinoids can affect the activities of immune cells.

Indeed, in vitro studies using cells of human and rodent origin have demonstrated that cannabinoids alter the functionality of a diverse array of immune cells. Δ^9-THC and the synthetic cannabinoids CP55940 and HU-210 inhibited cell-contact-dependent cytolysis of tumour cells, which is mediated by macrophages and macrophage-like cells (Refs 67, 68). Δ^9-THC suppressed proliferation of B and T cells in response to cell-specific mitogens (Refs 67, 68, 69, 70), suppressed the cytolytic activity of NK cells (Ref. 69), and inhibited cell-killing activity, proliferation and maturation of cytotoxic T lymphocytes (CTLs) (Ref. 69). In addition, exogenous cannabinoids affected immune cell recruitment and chemotaxis to sites of infection and/or injury (Refs 71, 72). In murine models of granulomatous amoebic encephalitis (GAE) and atherosclerosis, macrophages and macrophage-like cells exposed to Δ^9-THC displayed less migration to sites of infection (Refs 73, 74, 75, 76). Thus, the collective data suggest that exogenous cannabinoids such as Δ^9-THC inhibit the functional activities of a variety of immunocytes – an outcome that is consistent with the role of these compounds in decreased host resistance to infectious agents.

Role of CB_2 in cell-mediated and humoral immunity

The recognition that select exogenous cannabinoids acted as anti-inflammatory agents and that immune cells also expressed cannabinoid receptors served as an impetus for studies aimed at defining a functional linkage between these two events. The preponderance of studies to date indicates that the cannabinoid receptor that is linked to modulation of the majority of immune functional responses is CB_2.

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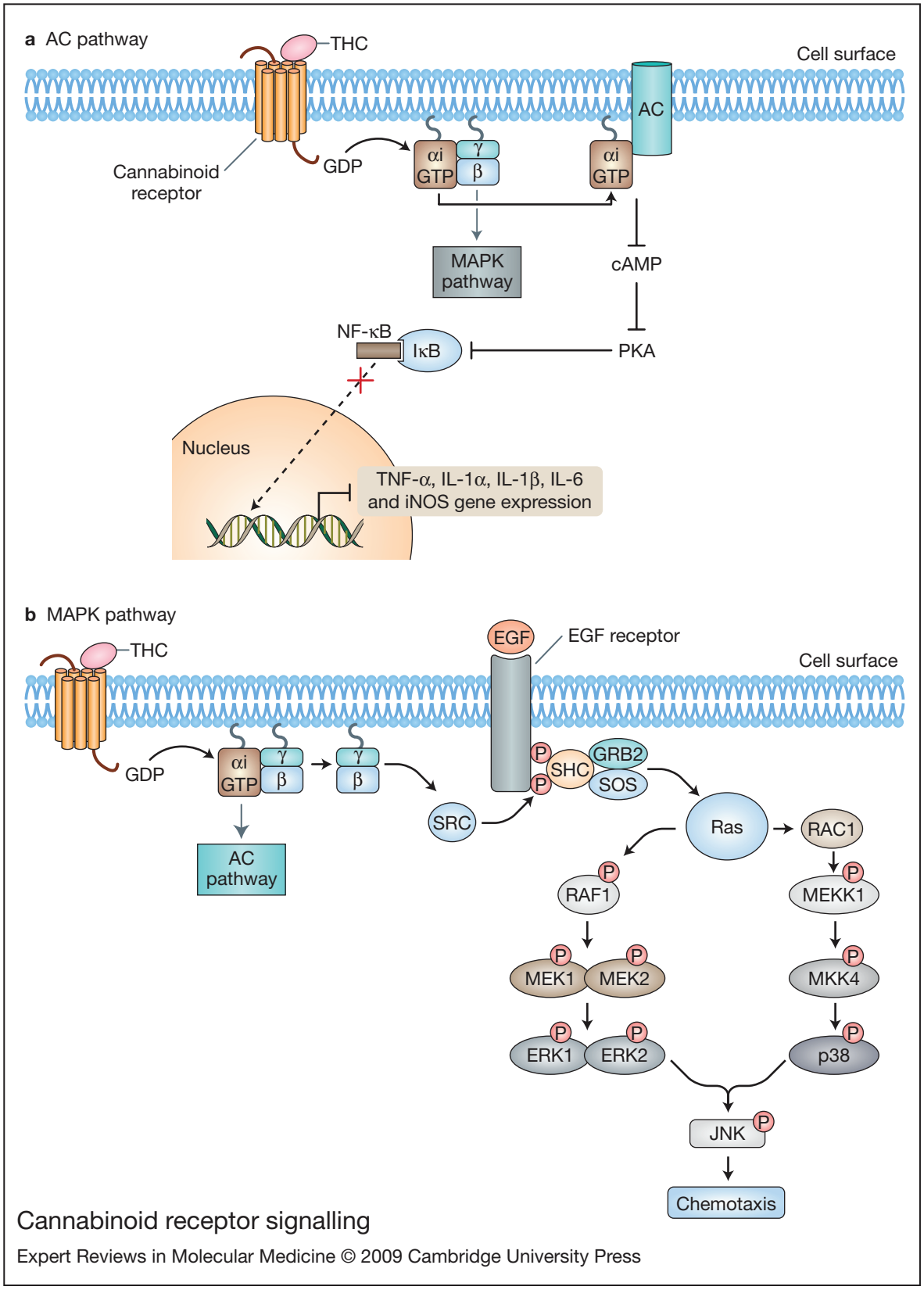

Cannabinoid receptor signalling

Figure 3. Cannabinoid receptor signalling. (*See next page for legend.*)

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Figure 3. Cannabinoid receptor signalling. (*Legend; see previous page for figure*.) (a) The adenylate cyclase (AC) pathway. $G_{i\alpha}$ signalling downregulates the pro-inflammatory immune response via adenylate cyclase. Upon cannabinoid receptor activation, the α subunit of the G_i protein interacts with adenylate cyclase to inhibit its activity. This results in a decrease of cAMP production, which leads to inactivation of protein kinase A (PKA). IκB-α remains unphosphorylated due to PKA inactivation, thus preventing activation, nuclear translocation and DNA binding of NF-κB and other transcription factors. These events ultimately lead to the downregulation of pro-inflammatory mediator gene expression. (b) The mitogen-activated protein kinase (MAPK) pathway. Following cannabinoid receptor activation, the $\beta\gamma$ subunits of the G_i protein induce Ras-dependent MAP kinase signalling pathways, culminating in chemotaxis. The SRC kinase is activated upon interaction with G_i $\beta\gamma$ subunits and phosphorylates tyrosine residues of the EGF receptor. The SHC–SOS–GRB2 trimer binds the EGF receptor via the SHC adaptor protein, while the guanine-nucleotide-exchange factor SOS activates Ras. Following Ras activation, two independent signalling pathways are initiated, leading to the activation of p38 (encoded by *MAPK14)* and ERK1 and 2 (encoded by the *MAPK1* and *MAPK2* genes, respectively). p38 as well as ERK1 and 2 can activate JNK, which induces chemotaxis of immune cells. Abbreviations: EGF, epidermal growth factor; ERK, extracellular-signal-regulated kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-jun N-terminal kinase; MEK1 and MEK2, MAPK kinase encoded by *MAP2K1* and *MAP2K2* genes, respectively; MEKK1, MAPK kinase kinase encoded by *MAP3K1* gene; MKK4, MAPK kinase 4 encoded by *MAP2K4* gene; RAC1, Ras-related C3 botulinum toxin substrate 1; RAF1, v-Raf-1 murine leukaemia viral oncogene homologue; SHC, Src homology 2 domain-containing; SOS, son of sevenless; TNF, tumour necrosis factor.

Effects of exogenous cannbinoids

The partial agonist Δ^9-THC, as well as the full cannabinoid agonists CP55940 and WIN55212-2, has been reported to enhance human tonsillar B cell growth when used at nanomolar concentrations (Ref. 77) by a CB_2-mediated mechanism. Consistent with these results, the CB_2-selective antagonist SR144528 reversed the stimulating effects of CP55940 on human tonsillar B cell activation (Ref. 78). By contrast, several reports have indicated that cannabinoids suppress the antibody response of humans and animals (Refs 79, 80), at least in part through inhibition of adenylate cyclase by a pertussis-toxin-sensitive G-protein-coupled mechanism (Ref. 81). Furthermore, CB_2 was shown to be downregulated at the mRNA and protein levels during B cell differentiation (Ref. 78). Together, these observations suggest that the CB_2 plays a stimulatory role in B cell activation and growth, while exerting a suppressive role in B cell differentiation and antibody production.

Cannabinoids also have been reported to suppress various activities of T cells, apparently through CB_2. For example, in vivo administration of Δ^9-THC to mice resulted in significant inhibition of NK cytolytic activity without affecting ConA-induced splenocyte proliferation (Ref. 82). Concomitant with this inhibition, levels of interferon γ (IFN-γ) were reduced significantly, and CB_1 and CB_2 antagonists reversed this effect. In view of these observations, it was suggested that both CB_1 and CB_2 were involved in the network that mediates NK cytolytic activity.

These and other studies have indicated that cannabinoids not only exert direct effects on immune cells but also have effects beyond the initial target cell in that they alter the expression of chemokines and cytokines– key mediators in a complex network of cross-signalling among immune cells for homeostatic balance between pro-inflammatory (T helper 1; Th1) and anti-inflammatory (T helper 2; Th2) activities. For example, Δ^9-THC treatment of BALB/c mice resulted in a decrease in levels of IFN-γ, interleukin (IL)-12 and IL-12 receptor β2 in response to *Legionella pneumophila* infection (Ref. 83). The use of cannabinoid receptor antagonists linked both CB_1 and CB_2 to decreased levels of IFN-γ and IL-12 and the suppression of Th1 immunity to *Legionella*. However, studies employing a tumour model implicated CB_2 as the receptor responsible for Δ^9-THC-mediated inhibition of immunity by a cytokine-dependent pathway (Ref. 84). In these studies, using a weakly immunogenic mouse lung cancer model, Δ^9-THC decreased tumour immunogenicity. At both the tumour site and in the spleens of Δ^9-THC-treated mice, levels of the immune-inhibitory Th2 cytokines IL-10 and transforming growth factor (TGF) were augmented, whereas levels of the immune-stimulatory Th1 cytokine IFN-γ were downregulated. In vivo administration of the CB_2 antagonist SR144528 blocked the effects of Δ^9-THC, suggesting that Δ^9-THC promoted tumour growth by inhibiting antitumour immunity through a CB_2-mediated, cytokine-

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dependent pathway. Collectively, the results from several studies suggest that exogenous cannabinoids elicit a shift from a Th1 pro-inflammatory cytokine expression profile to a Th2 anti-inflammatory profile, and that CB_2 may be linked to this effect.

Effects of endogenous cannabinoids

Endocannabinoids also have been reported to affect immune function, mostly though CB_2. The effects of AEA and palmitoylethanolamide, as well as Δ^9-THC, on the production of tumour necrosis factor (TNF)-α, IL-4, IL-6, IL-8, IL-10, IFN-γ, and p55 and p75 TNF-α soluble receptors have been examined (Ref. 85). AEA diminished production of IL-6 and IL-8 at low nanomolar concentrations and inhibited that of TNF-α, IFN-γ, IL-4 and p75 TNF-α soluble receptor at micromolar concentrations. Palmitoylethanolamide, at concentrations similar to those of AEA, inhibited the synthesis of IL-4, IL-6 and IL-8, and the production of p75 TNF-α soluble receptor. However, palmitoylethanolamide did not affect TNF-α and IFN-γ production. Neither AEA nor palmitoylethanolamide had an effect on IL-10 synthesis. Based on these observations, it was suggested that various endogenous fatty acid ethanolamides participated in the regulation of the immune response, and that the inhibitory properties of AEA and palmitoylethanolamide were due to activation of CB_2 since it has been shown that palmitoylethanolamide has a high affinity for the CB_2 receptor and does not bind the CB_1 receptor (Ref. 86). AEA also has been shown to exert an inhibitory effect on chemokine-elicited lymphocyte migration (Ref. 87). The inhibition of stromal derived factor 1 (SDF-1)-induced migration of $CD8^+$ T cells was found to be mediated through the CB_2. However, there also are reports that AEA can exert potentiating effects. AEA acted as a synergistic growth factor for primary murine marrow cells and haematopoietic growth factor (HGF)-dependent cell lines (Ref. 88). AEA also augmented production of IL-6 by astrocytes infected with Theiler's murine encephalomyelitis virus (Ref. 89). However, in these studies the enhancing effect of AEA was shown to be blocked by the CB_1 antagonist SR141716A, suggesting involvement of CB_1 rather than CB_2 in the elevation of levels of this pleiotropic cytokine.

By contrast to AEA, 2-AG has been associated primarily with augmentation of immune responses. 2-AG stimulated the release of nitric oxide (NO) from human immune and vascular tissues and from invertebrate immunocytes via CB_1 (Ref. 90), and haematopoietic cells expressing CB_2 migrated in response to 2-AG (Ref. 91). Distinct profiles for CB_2 expression in lymphoid tissues have been reported to be dependent on the state of receptor activation, and it has been proposed that cell migration constitutes a function of CB_2 upon stimulation with 2-AG (Ref. 92). Furthermore, 2-AG induced the migration of human peripheral blood monocytes and promyelocytic leukaemia HL60 cells that have been differentiated into macrophage-like cells (Ref. 76), apparently through a CB_2-dependent mechanism. Subsequent studies showed that 2-AG apparently accelerated production of chemokines by the HL-60 cells (Ref. 93). In addition, rat microglia have been reported to synthesise 2-AG in vitro, an event that has been linked to increased proliferation through a CB_2-dependent mechanism (Ref. 94).

Role of CB_2 in macrophages and macrophage-like cells

A major target of the action of exogenous and endogenous cannabinoids appears to be cells of macrophage lineage. Cannabinoids suppress macrophage functions such as phagocytosis, bactericidal activity, and spreading (Refs 79, 95), interfere with macrophage cell-contact-dependent lysis of tumour cells, herpesvirus-infected cells and amoebae, and deplete macrophage-elicited soluble tumouricidal activity (Refs 67, 96). These observations are consistent with reports that Δ^9-THC inhibits the synthesis of proteins associated with primed and activated macrophages (Ref. 97), alters cytokine secretion by activated macrophages (Refs 98, 99), and inhibits cytokine gene expression by microglia (Ref. 100). Cannabinoids also have been found to affect the production of NO by macrophages and macrophage-like cells (Refs 68, 101). Although it is now evident that cannabinoids exert a variety of effects on the activities of macrophage and macrophage-like cells, a picture is emerging as to the role of CB_2 in these processes and the state of cell activation under which it is functionally relevant.

Differential expression of CB_2

Macrophages and macrophage-like cells such as microglia undergo a maturation, differentiation and activation process characterised by

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differential gene expression and the acquisition of correlative distinctive functional capabilities (Refs 102, 103, 104). These cells can be driven sequentially in response to multiple signals from 'resting' to 'responsive', 'responsive' to 'primed', and 'primed' to 'fully activated' states, a process that mimics events in vivo. Using in vitro models, it has been shown that levels of CB_2 receptor mRNA and protein are modulated differentially in relation to cell activation state (Ref. 105). The CB_2 is not detected in resting cells, is present at high levels in responsive and primed cells, and is identified at greatly diminished levels in fully activated cells (Fig. 4). These observations suggest that the CB_2 is expressed 'on demand' and that the modulation of CB_2 levels is a feature common to cells of macrophage lineage as they participate in the inflammatory response and undergo differential gene expression and acquisition of distinctive functional properties. Furthermore, the relatively high levels of CB_2 recorded for macrophages when in responsive and primed states suggest that these cells, and possibly other immune cell types, exhibit a functionally relevant 'window' of CB_2 expression during which they are most susceptible to cannabinoid-mediated action. Furthermore, since CB_1 is expressed at low and constitutive levels in microglia as compared with CB_2, the kinetics of expression of the two receptors as linked to immune functional activities may be distinct.

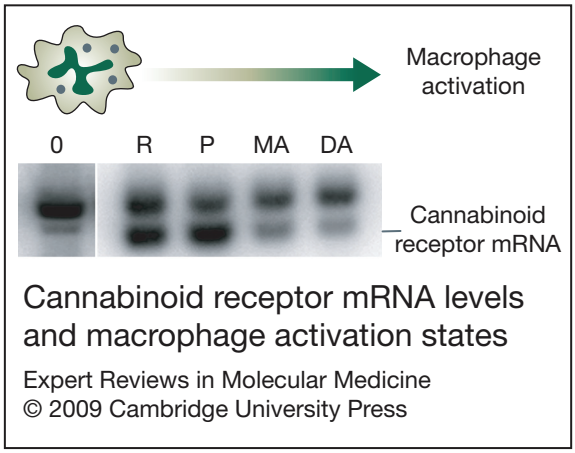

Figure 4. Cannabinoid receptor mRNA levels and macrophage activation state. Levels of cannabinoid receptor 2 (CB_2) mRNA are modulated differentially in relation to macrophage activation state, as reported in Ref. 105. CB_2 is detected at low levels in 'resting' cells (0), is present at high levels in 'responsive' (R) and 'primed' (P) cells, and is identified at greatly diminished levels in cells that have been subjected to multistep activation (MA) or direct activation (DA). The resting state was achieved by growing cells on an agar substratum, whereas the responsive state was obtained by growing cells on a plastic surface. Cells were treated with interferon (IFN)-γ (100 U/ml), IFN-γ (100 U/ml) plus lipopolysaccharide (LPS) (100 ng/ml), or LPS (100 ng/ml) to obtain prime, multistep activated, or directly activated states, respectively. Southern blot analysis was performed on mutagenic reverse transcriptase PCR (MRT-PCR) products of total RNA from mouse peritoneal macrophages (Ref. 92). The upper band of each doublet represents amplified genomic DNA used as an internal quantitative standard. The lower band of each doublet represents amplified product from mRNA.

Chemotaxis as a signature activity of 'responsive' macrophages

Chemotaxis and antigen presentation are signature activities of macrophages and macrophage-like cells when in responsive and primed states of activation – states that are associated with early stages of the inflammatory response. Chemotaxis describes the ability of cells to migrate towards an increasing concentration gradient of stimulating agent (Refs 106, 107, 108, 109) and is distinct from chemokinesis, which represents stimulus-dependent random cellular motion (Refs 110, 111, 112, 113). During chemotaxis, macrophage interaction with a chemoattractant results in the initiation of a rapid and directed movement that is associated with a complex array of cellular events that includes changes in ion fluxes, alterations in integrin avidity, production of superoxide anions, and secretion of lysosomal enzymes (Ref. 114). Classical chemoattractants include bacterial-derived *N*-formyl peptides, the complement fragment peptides C5a and C3a, and lipids such as leukotriene B_4 and platelet-activating factor (Refs 115, 116, 117, 118). Chemokines – cytokines of 8 to 17 kDa molecular mass that are selective for leukocytes in vitro and elicit accumulation of inflammatory cells in vivo – represent a second group of chemoattractants (Refs 119, 120, 121, 122). As in the case of cannabinoid binding to cannabinoid receptors, the specific effects of chemokines on target cells are mediated by G-protein-coupled receptors (Refs 114, 123). Ligation of chemokines to their cognate receptors initiates a series of signal transduction events

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that results in leukocyte trafficking during infection, inflammation, tissue injury and tumour development (Ref. 123).

The current data indicate that cannabinoids act through CB_2 to alter macrophage migration, with exogenous cannabinoids such as Δ^9-THC exerting an inhibitory effect and, conversely, endocannabinoids such as 2-AG eliciting a stimulatory effect. For example, in vivo and in vitro treatment of rat peritoneal macrophages with CP55940 resulted in decreased migration in vitro to the peptide formal-methionyl-leucine-phenylalanine (fMLP) in a mode that is linked primarily to CB_2 (Ref. 72). Also, the chemotactic response of mouse macrophages to fMLP was decreased by CBD (Ref. 124), which binds weakly to CB_2. CB_2 was implicated in this response since the CB_2-selective antagonist SR144528 prevented the decrease in migration. In contrast to events observed for Δ^9-THC, 2-AG triggered migration of microglia, and CB_2 was involved in this effect (Ref. 3). Recently, a combined pharmacological and genetic approach (employing macrophages from knockout mice) demonstrated that Δ^9-THC and CP55940 inhibited mouse peritoneal macrophage chemotaxis to CCL5 (RANTES) through CB_2 (Ref. 71). The Δ^9-THC and CP55940 deactivation of migratory responsiveness to the chemokine CCL5, an event that is mediated through activation of the cognate G-protein-coupled chemokine receptor CCR5, suggested that signalling through CB_2 leads to 'cross-talk' between that receptor and CCR5. These and other studies implicate the CB_2 in a network of G-protein-coupled receptor signal transduction systems, inclusive of chemokine receptors, that act co-ordinately to modulate macrophage migration.

Antigen processing as a signature activity of 'primed' macrophages

It has also been shown that CB_2 is involved in cannabinoid-mediated inhibition of processing of antigens by macrophages. In studies examining the effect of Δ^9-THC on the processing of intact lysozyme by macrophages, Δ^9-THC impaired the ability of a macrophage hybridoma to function as an antigen-presenting cell based on its ability to secrete IL-2 upon stimulation of a soluble-protein-antigen-specific helper T cell hybridoma (Ref. 70). Δ^9-THC exposure resulted in a significant reduction in the T cell response to the native form of lysozyme after pretreatment of the macrophages with nanomolar drug concentrations. However, Δ^9-THC did not affect IL-2 production when the macrophages presented a synthetic peptide of the antigen to T cells, suggesting that the drug interfered with antigen processing, not peptide presentation. The cannabinoid inhibition of the T cell response to native lysozyme was stereoselective, consistent with the involvement of a cannabinoid receptor: bioactive CP55940 diminished T cell activation whereas the relatively inactive stereoisomer CP56667 did not. The macrophage hybridoma expressed mRNA for CB_2 but not for CB_1. Furthermore, the CB_1-selective antagonist SR141716A did not reverse the suppression caused by Δ^9-THC, whereas the CB_2-selective antagonist SR144528 completely blocked the Δ^9-THC suppression of the T cell response. Collectively, these results implicated macrophages as the target of cannabinoid inhibition of antigen processing through CB_2.

CB_2 and neuroinflammation

CB_2 localisation in the CNS

Early studies to define the functional relevance of CB_1 and CB_2 suggested that CB_1 was compartmentalised to the CNS while the expression of CB_2 was limited to cells and tissues of the immune system. The development of phenotypically normal CB_2-knockout mice (Ref. 125) was a major breakthrough that contributed to elucidation of the role of CB_2 in immune modulation, including that in the CNS. In addition to the CB_2-knockout mouse strain developed by Buckley and colleagues (Ref. 125), Deltagen (San Mateo, CA, USA) developed a CB_2-knockout mouse strain that is commercially available through Jackson Laboratories (Bar Harbor, ME, USA) (Ref. 126); these CB_2-knockout mice strains have mutations in the C-terminus and the N-terminus of CB_2, respectively. The tissues from these mice have been employed extensively in studying CB_2 function and CB_2-mediated responses. Macrophage function in helper T cell activation in the knockout mouse developed by Buckley and colleagues is not sensitive to the inhibitory effects of Δ^9-THC, in contrast with macrophage function in wild-type counterparts (Ref. 125).

In addition, CB_2-knockout mice have been utilised to study the specificity of various CB_2

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antibodies. However, CB_2 localisation within the CNS has proven to be an elusive target. While some laboratories have reported detection of CB_2 in the brain (Refs 126, 127, 128), other laboratories have not been able to identify this protein, raising concern about the reliability and specificity of the CB_2 antibodies used. In the studies performed that identified the CB_2 protein in brainstem neurons, a polyclonal antibody against the C-terminus was used to detect the receptor, and the CB_2-knockout strain developed by Buckley and colleagues and wild-type mice were used as the knockout and positive controls, respectively, to confirm the specificity of the polyclonal CB_2 antibody (Refs 126, 127). The knockout control was appropriate for those experiments since this knockout strain has a deletion in the C-terminus of the CB_2 protein. In other studies, on brain of wild-type mice, an antibody that was raised against the C-terminus of the CB_2 protein was used to demonstrate receptor expression (Refs 126, 128, 129, 130). The investigators identified the CB_2 protein in various brain regions using an antibody specific for the N-terminus of the CB_2 protein; however, a knockout control using tissues from CB_2-knockout mice was not used to confirm the specificity of this antibody (Refs 126, 128). Similar potentially confounding issues have been raised for CB_1 antibodies. Grimsey and colleagues demonstrated that various CB_1-specific antibodies used in immunostaining and western blot analyses displayed a multitude of variability in expression profiles – an outcome that was attributed to possible conformational changes, dimerisation with other G-protein-coupled receptors, or post-translational modifications (Ref. 131). It was postulated that such factors, separately or combined, could result in epitope masking or insufficient binding of antibody (Ref. 131). Collectively, these studies highlight the importance of employing specific cannabinoid receptor antibodies whose specificity can be confirmed using appropriate knockout controls, particularly when investigating the complex arena of the CNS. While localisation of the CB_2 protein in the CNS may have been elusive, in vitro studies have shown that in addition to expression in microglia (Ref. 105), CB_2 has since been identified in neurons, oligodendrocytes and other glial cells in vitro and in intact tissue (Refs 34, 35, 36). This receptor can be induced on demand during early inflammatory events (Refs 35, 37) and is linked to attenuation of pro-inflammatory cytokine production by microglia, as discussed earlier (Refs 132, 133, 134, 135, 136).

Neuroinflammatory disorders: CB_2 as a therapeutic target?

Like macrophages at peripheral sites, microglia are able to phagocytose and process antigens, and upon activation produce pro-inflammatory factors including the cytokines IL-1, IL-6 and TNF-α (Refs 137, 138, 139). Pro-inflammatory mediators released from microglia are cytotoxic and also can secondarily activate astrocytes, leading to a further induction of the expression of inflammatory factors. The resultant 'storm' of pro-inflammatory mediators contributes to breakdown of the blood–brain barrier and plays a critical role in promoting influx into the CNS of immunocytes from peripheral non-neuronal sites that also express CB_2. Microglia are believed to play a major role in many neuropathogenic diseases and disorders such as Alzheimer disease, multiple sclerosis, amyotrophic lateral sclerosis, and HIV (human immunodeficiency virus) encephalitis.

Alzheimer disease

Alzheimer disease is the most common neurodegenerative disorder that causes senile dementia. The defining neuropathological features of the disease are the presence of extracellular neuritic amyloid plaques and intracellular neurofibrillary tangles in the brain; as neurodegeneration progresses, there is accelerated neurofibrillary tangle formation, neuroinflammation and neuronal loss.

It has been reported that cannabinoids can be neuroprotective in Alzheimer disease by inhibiting the activation of microglia (Ref. 35) induced by amyloid plaques consisting of extracellular aggregates of amyloid β (Aβ) peptides (Refs 140, 141). Recently, the CB_1/CB_2 agonist CP55940 and the CB_2 agonist JWH-015 were found to protect and rescue peripheral blood lymphocytes from Aβ- and H_2O_2-induced apoptosis by two alternative mechanisms (Ref. 142). A receptor-independent pathway was implicated through the demonstration that the reactive oxygen species dihydrorhodamine was not oxidised into fluorescent rhodamine 123 as a result of cannabinoid inhibition of Aβ-generated H_2O_2,

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while a receptor-dependent pathway was implicated through the activation of the transcription factor NF-κB and downregulation of the transcription factor p53, which can initiate apoptosis. The downregulation of p53 involved phosphoinositide 3-kinase, as demonstrated through the use of the phosphoinositide 3-kinase inhibitor LY294002 (Ref. 142). Collectively, these results suggested that cannabinoids have potential as neuroprotective compounds in Alzheimer disease.

Multiple sclerosis

Multiple sclerosis is a chronic, inflammatory demyelinating disease of the human CNS that primarily affects adults (Refs 143, 144, 145). The disease is characterised by T-cell-mediated degeneration of the myelin sheath that covers axons, resulting in an inflammatory process that stimulates other immune cells to secrete pro-inflammatory mediators and antibodies, breakdown of the blood–brain barrier, activation of macrophages, and production of 'cytotoxic' proteins such as metalloproteinases (Refs 146, 147).

A significantly greater density of CB_2-immunoreactive microglia/macrophages has been identified in affected regions of human multiple sclerosis post-mortem spinal cord (Ref. 148). However, most studies aimed at assessment of effects of cannabinoids on multiple sclerosis, and the role of CB_2 in this process have involved the use of mouse models, particularly the experimental autoimmune encephalomyelitis (EAE) model, which exhibits a $CD4^+$-T-cell-mediated autoimmune disease (Ref. 144). Δ^9-THC markedly inhibited neurodegeneration in the EAE model and reduced the associated induced elevated level of glutamate in cerebrospinal fluid (Ref. 149). CB_2 mRNA expression and protein internalisation were upregulated significantly in activated microglia of mice experiencing EAE, implicating CB_2 in this disease (Ref. 150). The cannabinoid WIN55212-2 ameliorated EAE and diminished cell infiltration of the spinal cord. WIN55212-2 was found to induce encephalitogenic T cell apoptosis, partly through CB_2 (Ref. 151). More recently, it has been proposed that CB_2 plays a protective role in EAE pathology by targeting myeloid progenitor trafficking, which may contribute to microglial activation in the CNS (Ref. 152). In Theiler's virus infection of murine CNS, another mouse model for human multiple sclerosis, improved neurological deficits, concomitant with reduced microglial activation, major histocompatibility complex class II expression and T cell infiltration, were observed following treatment of mice with the synthetic cannabinoids WIN55212-2, ACEA (a CB_1-selective agonist) and JWH-015 (a CB_2-selective agonist) (Refs 153, 154). In the Theiler's model of multiple sclerosis, clinical signs and axonal damage in the spinal cord were reduced by the AMPA (amino-3-hydroxy-5-methyl-4-isoxazolepropionate) glutamatergic receptor antagonist NBQX (Ref. 155). The cannabinoid HU-210 ameliorated symptoms, accompanied by a reduction of axonal damage. Furthermore, the HU-210-mediated reduction in AMPA-induced excitotoxicity in vivo and in vitro was linked to CB_1 and CB_2.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is characterised pathologically by progressive degeneration of cortical motor neurons (upper motor neurons) and clinically by muscle wasting, weakness and spasticity that progresses to complete paralysis (Ref. 156). A pathological hallmark of amyotrophic lateral sclerosis is neuroinflammmation, a process that is mediated by pro-inflammatory cytokines, prostaglandins and NO (Ref. 157).

CBN delayed the onset of symptoms in mice suffering from experimentally induced amyotrophic lateral sclerosis without affecting survival (Ref. 158), and treatment of mice with WIN55212-2 after onset of symptoms delayed overall disease progression (Ref. 159). Also, the CB_2 agonist AM-1241 prolonged survival in a G93A-SOD1 mutant transgenic mouse model of amyotrophic lateral sclerosis when administered at onset of disease symptoms (Ref. 160). mRNA and receptor binding of CB_2 were selectively upregulated in spinal cords of these mice in a fashion that paralleled disease progression. Daily injections of AM-1241 initiated at onset of symptoms increased the survival interval after disease onset by 56%. Collectively, the results suggested that the CB_2 agonist extended the interval for motor neuron degeneration and prolonged function in these affected mice.

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HIV encephalitis

HIV encephalitis, also known as acquired immune deficiency syndrome (AIDS)–dementia complex, results in progressive memory loss, intellectual deterioration, behavioural changes, and motor deficits (Ref. 161). The neuropathology of HIVE is characterised by neuronal loss, glial activation, presence of multinucleated giant cells, perivascular mononuclear infiltration, and in some cases vacuolar myelopathy and myelin pallor (Ref. 161). The production of pro-inflammatory cytokines such as TNF-α by activated monocytes and microglia, and neurotoxins such as glutamate and NO, is the primary cause of brain damage associated with this disorder. In addition, HIV-specific gene products such as the transactivator Tat and the envelope glycoprotein gp120 that are released from infected monocytes and microglia contribute to neuropathology.

The simian immunodeficiency model comes closest to replicating events that are associated with HIV infection of the human CNS. Examination of brains of macaques with simian immunodeficiency virus (SIV)-induced encephalitis has led to the suggestion that the endocannabinoid system participates in the development of HIV-induced encephalitis (Ref. 162). In this infectivity model, expression of CB_2 was induced in perivascular macrophages, microglial nodules, and T cells. It was proposed that activation of CB_2, expressed by perivascular macrophages, which play a critical role in viral entry into the CNS (Refs 163, 164), likely led to reduction of their antiviral response, thus favouring the entry of infected monocytes into the CNS (Ref. 162). In addition, the endogenous cannabinoid-degrading enzyme FAAH was reported as overexpressed in perivascular astrocytes as well as in astrocytic processes reaching cellular infiltrates (Ref. 162). Furthermore, activation of CB_2 resulted in inhibition of the transendothelial migration of Jurkat T cells and primary human T cells by interfering with the CXCL12–CXCR4 chemokine receptor system (Ref. 165). These observations suggest that activation of CB_2 can alter the activation of other G-protein-coupled receptors such as CXCR4, which functions as a coreceptor for T-lymphotropic HIV. A similar observation in terms of a linkage to CB_2 has been made for the chemokine receptor CCR5, which acts as the coreceptor for monotropic HIV (Ref. 71). Activation of CB_2 with Δ^9-THC, CP55940 or with the CB_2-selective compound O-2137 inhibited activation of CCR5 by its native chemokine ligand CCL5. Collectively, these results indicate that CB_2 as a $G_{i/o}$-protein-coupled receptor 'crosstalks' with several other G-protein-coupled receptors, especially chemokine receptors, to alter heterologous signal transduction pathways. Furthermore, these interactions might have implications for HIV infection, particularly for those receptors such as CXCR4 and CCR5 that act in a coreceptor capacity for HIV. Additionally, possible therapeutic implications of 'crosstalk' between cannabinoid receptors and other cellular receptors has come from studies suggesting that CB_2, along with CB_1, could play a role in linking the endocannabinoid sytem with the modulation of neural stem cell proliferation through bidirectional 'crosstalk' with TNF receptors (Ref. 166).

Summary of cannabinoid receptors in neuropathological disorders

In summary, cannabinoid receptors appear to play an important role in neuropathological diseases. CB_1 has been reported to be critical for the overall homeostatic balance and regulation of the CNS, while CB_2 has been implicated as playing a functionally relevant role during neuroinflammation. Microglia, as resident macrophages in the CNS, not only play a role in host defence and tissue repair but also have been implicated as contributive to, if not causative of, a variety of inflammatory neuropathological processes. In these cells CB_1 appears to be present at constitutive and relatively low levels while CB_2 is expressed inducibly during the inflammatory process and at relatively high levels. Immune responses during the early phase of neuropathological processes appear to involve preponderantly CB_2, and levels and functional relevance of this receptor may be amplified as disease progresses to later stages of inflammation. The recognition that immunocytes resident within the brain express CB_2, during the inflammatory process suggests the existence of a temporal window during which these cells may be susceptible to therapeutic manipulation through the use of CB_2-selective agonists. That is, selective targeting of the CB_2 could result in dampening of untoward immune responses such as elicitation of a chemokine/cytokine 'storm' within the CNS that would result in breakdown of the blood–brain barrier, influx of immunocytes

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from peripheral non-neuronal sites, and further inflammation.

Clinical implications/applications

Cannabinoids, as ligands that signal through cannabinoid receptors, may be particularly useful as agents for therapeutic manipulation of hyperinflammatory immune responses within the CNS. These compounds are highly lipophilic and thus readily penetrate the blood–brain barrier, a challenge that is posed to a variety of agents that have therapeutic potential. Furthermore, through the application of appropriately engineered molecules, it may be possible to specifically target CB_2, a condition that would obviate generation of untoward psychotropic effects that could be engendered if the CB_1 were activated also.

The principal potential cellular target in the CNS for these compounds – given its involvement in early stages of the inflammatory response in generation of a cascade of inflammatory factors and its expression of CB_2 – is the microglial cell. During activation, microglia, as macrophage-like cells, also upregulate an array of cell-surface receptors that may be critical in regeneration and/or degeneration of the CNS. Included among these are immunoglobulin (Ig) superfamily receptors, complement receptors, Toll-like receptors, cytokine/chemokine receptors, and opioid receptors. These cells, in addition to expressing both the CB_1 and the CB_2 in vitro (Refs 105, 167), also produce the endocannabinoids 2-AG as well as AEA, although the latter is generated in lesser quantities (Ref. 94). Thus, microglia appear to harbour a fully constituted system of endogenous cannabinoid ligands and cognate receptors. Activation of CB_2 on these cells appears to promote migration and proliferation. 2-AG induces migration of microglia and this occurs through the CB_2 and abnormal-CBD-sensitive receptors, which subsequently leads to activation of the extracellular-signal-regulated kinase (ERK)1 and 2 (encoded by the *MAPK1* and *MAPK2* genes, respectively) signal transduction pathway (Ref. 3). Furthermore, it has been shown that microglia express CB_2 at the leading edge of lamellipodia, consistent with an involvement in cell migration. There is accumulating evidence that CB_2 also is expressed in the CNS in vivo. The expression of CB_2 in microglial, astrocyte and neuronal subpopulations has been identified in a variety of neurodegenerative disease models (Ref. 37). This expression of CB_2 in vivo has been attributed, in large measure, to microglia. In several neurodegenerative diseases, upregulation of microglial CB_2 has been observed (Refs 148, 162, 168, 169, 170, 171). In studies investigating the expression profile of FAAH and the CB_2 in postmortem brain tissues from Alzheimer disease patients, it was observed that congregated microglia associated with neuritic plaques selectively overexpressed CB_2 (Ref. 172). In addition, CB_2-positive microglia have been identified dispersed within active multiple sclerosis plaques and in the periphery of chronic active plaques (Ref. 169).

The collective findings support the concept that, in addition to CB_1, CB_2 has a functionally relevant role in the CNS. This role appears to play out during the inflammatory process associated with a variety of neuropathies. In this context, it has been proposed that the role of the CB_2 in immunity in the CNS is primarily one that is anti-inflammatory (Ref. 173). Since microglia exhibit phenotypic and functional properties of macrophages and inducibly express CB_2 at maximal levels when in responsive and primed states, there may be a window of functional relevance for this receptor, comparable to that for macrophages at peripheral sites. That is, antigen processing and/or chemotaxis by these cells may also be susceptible to cannabinoids acting via CB_2.

Indeed, studies using a mouse model of GAE, a chronic progressive human infection of the CNS that is caused by the opportunistic pathogen *Acanthamoeba*, revealed a paucity of cells expressing the Mac-1 (CD11b) protein marker for macrophages and macrophage-like cells at focal sites containing *Acanthamoeba* in the brains of infected mice treated with Δ^9-THC as compared with vehicle-treated *Acanthamoeba*-infected controls (Ref. 65). These observations indicated that microglia (and possibly macrophages introduced from peripheral sites) either did not migrate to infected areas or were selectively targeted by the *Acanthamoeba* and destroyed. Treatment of neonatal rat cerebral cortex microglial cultures with Δ^9-THC resulted in inhibition of the migratory response to *Acanthamoeba*-conditioned medium, which harbours proteases and other factors released from amoebae that serve as chemotactic stimuli (Ref. 57). In addition, treatment with the potent CB_1/CB_2 agonist CP55940 resulted in a significant concentration-related decrease in microglial migration in response to conditioned

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medium. The highly selective CB_2 ligand O-2137 exerted a profound and significant inhibition in the microglial migratory response to conditioned medium while treatment with the CB_1-selective ligand ACEA had a minimal effect. Finally, treatment of microglia with the CB_1 antagonist SR141716A did not block the inhibitory effect of CP55940 while treatment with the CB_2-specific antagonist SR144528 resulted in a reversal of the inhibitory effect of CP55940. These collective results indicated that the cannabinoid-mediated inhibition of the conditioned-medium-stimulated microglial response to *A. culbertsoni* in mouse brain was linked, at least in part, to CB_2. The mode by which Δ^9-THC and other exogenous cannabinoids such as CP55940 signal through CB_2 to inhibit the chemotactic response of microglia to *Acanthamoeba* remains to be defined. However, it is known that *Acanthamoeba* produces proteases, phospholipases and other factors (Ref. 174) that may act on phospholipids in microglial membranes, generating cleavage products (Ref. 175). It is postulated that bioactive lipid mediators thus generated include the endocannabinoid 2-AG that serves to drive chemotaxis by autocrine and/or paracrine activation of CB_2. The exogenous cannabinoid Δ^9-THC may alter this chemotactic response, as well as chemotactic responses to other stimuli, by superimposing an inhibitory effect through activation of CB_2. That is, Δ^9-THC could inhibit the synthesis and/or, release of 2-AG or, by virtue of its relative long half-life as compared with that of 2-AG, pre-empt this endocannabinoid from ligating to CB_2.

Summary, research in progress, and outstanding research questions

There is currently a large body of data indicating that the CB_2 plays a functionally relevant role during inflammation. This role is especially evident for cells of myeloid lineage, including macrophages and macrophage-like cells, as well as microglia that are resident in the CNS. These latter cells are morphologically, phenotypically, and functionally related to macrophages. The CB_2 is differentially expressed by macrophages and macrophage-like cells, with highest levels detected when these cells are in responsive and primed states, suggesting the existence of a window of functional relevance during which activation of the CB_2 modulates macrophage activities. Signature activities of responsive and primed macrophages are chemotaxis and antigen processing, respectively. The endocannabinoid 2-AG, elicited from macrophages and microglia during the activation process, stimulates a chemotactic response from these cells through the CB_2. By contrast, exogenous cannabinoids such as Δ^9-THC and CP55940 inhibit the chemotactic response as well as antigen processing of antigens, through activation of the CB_2. Furthermore, exogenous cannabinoids such as Δ^9-THC may superimpose an inhibitory effect on pro-chemotactic endocannabinoids.

Although in recent years major advances have been made regarding the functional relevance of the CB_2, a number of outstanding research questions remains. Principal among these is definition of the mechanism through which exogenous cannabinoids such as Δ^9-THC superimpose an inhibitory effect on endocannabinoid-mediated immune functional activities. In this context, are differential signal transductional pathways involved following CB_2 activation by Δ^9-THC versus endocannabinoids? Do exogenous cannabinoids by virtue of their relatively long half-life as compared with endocannabinoids persist in cells so as to affect receptor-mediated endocytosis and recycling of receptor–ligand complexes? In addition, what is the extent of the ability of the CB_2 to 'crosstalk' with other G-protein-coupled receptors, especially chemokine receptors such as CXCR4 and CCR5, which also serve as coreceptors for HIV? Do the endocannabinoids AEA and 2-AG exert differential effects on immune function, thereby acting in an immune homeostatic role? That is, does AEA act in an anti-inflammatory capacity while 2-AG acts as a pro-inflammatory agent as is typical for other bioactive lipids such as select prostaglandins that exert pro-inflammatory versus anti-inflammatory activities? These are but a few of the salient questions that await resolution.

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References

1 Gauthier, K.M. et al. (2005) Endothelium-derived 2-arachidonylglycerol: an intermediate in vasodilatory eicosanoid release in bovine coronary arteries. American Journal of Physiology –

Accession information: doi:10.1017/S1462399409000957; Vol. 11; e3; January 2009

Heart and Circulatory Physiology 288, H1344-H1351
2 Gonthier, M-P. et al. (2007) Identification of endocannabinoids and related compounds in human fat cells. Obesity 15, 837-845
3 Walter, L. et al. (2003) Nonpsychotropic cannabinoid receptors regulate microglial cell migration. Journal of Neuroscience 23, 1398-1405
4 Di Marzo, V. et al. (1999) Biosynthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in circulating and tumoral macrophages. European Journal of Biochemistry 264, 258-267
5 Maejima, T. et al. (2001) Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. Neuron 31, 463-475
6 Devane, W.A. et al. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946-1949
7 Mechoulam, R. et al. (1995) Identification of an endogenous 2-monoglyceride present in canine gut, that binds to cannabinoid receptors. Biochemical Pharmacology 50, 83-90
8 Dinh, T.P. et al. (2002a) Brain monoglyceride lipase participating in endocannabinoid inactivation. Proceedings of the National Academy of Sciences of the United States of America 99, 10819-10824
9 Dinh, T.P. et al. (2002b) A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chemistry and Physics of Lipids 121, 149-158
10 Saario, S.M. et al. (2004) Monoglyceride lipase-like enzymatic activity is responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. Biochemical Pharmacology, 67, 1381-1387
11 Di Marzo, V. et al. (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372, 686-691
12 Beltramo, M. et al. (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 277, 1094-1097
13 Rakhshan, F. et al. (2000) Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. Journal of Pharmacology and Experimental Therapeutics 292, 960-967
14 Day, T.A. et al. (2001) Role of fatty acid amide hydrolase in the transport of the endogenous cannabinoid anandamide. Molecular Pharmacology 59, 1369-1375
15 Gardner, B. et al. (2003) Methanandamide increases COX-2 expression and tumor growth in murine lung cancer. FASEB Journal 17, 2157-2159
16 Hinz, B. et al. (2004) R(+)-methanandamide-induced cyclooxygenase-2 expression in H4 human neuroglioma cells: possible involvement of membrane lipid rafts. Biochemical and Biophysical Research Communications 324, 621-626
17 Oz, M. (2006) Receptor-independent actions of cannabinoids on cell membranes: Focus on endocannabinoids. Pharmacology and Therapeutics 111, 114-144
18 De Morrow, S. et al. (2007) Opposing actions of endocannabinoids on cholangiocarcinoma growth. Recruitment of Fas and Fas ligand to lipid rafts. Journal of Biological Chemistry 282, 13098-13113
19 Howlett, A.C. and Fleming, R.M. (1984) Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Molecular Pharmacology 26, 532-538
20 Howlett, A.C. (1985) Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. Molecular Pharmacology 27, 429-436
21 Howlett, A.C., Qualy, J.M. and Khachatrian, L.L. (1986) Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. Molecular Pharmacology 29, 307-313
22 Matsuda, L.A. et al. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561-564
23 Herkenham, M. et al. (1990) Cannabinoid receptor localization in brain. Proceedings of the National Academy of Sciences of the United States of America 87, 1932-1936
24 Westlake, T.M. et al. (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
25 Howlett, A.C. and Mukhopadhyay, S. (2000) Cellular signal transduction by anandamide and 2-arachidonoylglycerol. Chemistry and Physics of Lipids 108, 53-70
26 Demuth, D.G. and Molleman, A. (2006) Cannabinoid signalling. Life Sciences 78, 546-563
27 Gifford, A.N. et al. (2002) In vivo imaging of the brain cannabinoid receptor. Chemistry and Physics of Lipids 121, 65-72

Accession information: doi:10.1017/S1462399409000957; Vol. 11; e3; January 2009

28 Munro, S., Thomas, K.L. and Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61-65
29 Griffin, G. et al. (2000) Cloning and pharmacological characterization of the rat CB2 cannabinoid receptor. Journal of Pharmacology and Experimental Therapeutics 292, 888-894
30 Brown, S.M. et al. (2002) Cloning and molecular characterization of the rat CB2 cannabinoid receptor. Biochimica et Biophysica Acta 1576, 255-264
31 Bouaboula, M. et al. (1999) Regulation of peripheral cannabinoid receptor CB2 phosphorylation by the inverse agonist SR144528. Implications for receptor biological responses. Journal of Biological Chemistry 274, 20397-20405
32 Galiegue, S. et al. (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. European Journal of Biochemistry 232, 54-61
33 Schatz, A.R. et al. (1997) Cannabinoid receptors CB1 and CB2: a characterization of expression and adenylate cyclase modulation within the immune system. Toxicology and Applied Pharmacology 142, 278-287
34 Nunez, E. et al. (2004) Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. Synapse 53, 208-213
35 Ramirez, B.G. et al. (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. Journal of Neuroscience 25, 1904-1913
36 Cabral, G.A. and Marciano-Cabral, F. (2005) Cannabinoid receptors in microglia of the central nervous system: immune functional relevance. Journal of Leukocyte Biology 78, 1192-1197
37 Fernandez-Ruiz, J. et al. (2007) Cannabinoid CB2 receptor: a new target for controlling neural cell survival? Trends in Pharmacological Sciences 28, 39-45
38 Sawzdargo, M. et al. (1999) Identification and cloning of three novel human G protein-coupledreceptor genes GPR52, PsiGPR53 and GPR55: GPR55 is extensively expressed in human brain. Molecular Brain Research 64, 193-198
39 Baker, D. et al. (2006) *In silico* patent searching reveals a new cannabinoid receptor. Trends in Pharmacological Sciences 27, 1-4
40 Pertwee, R.G. (2007) GPR55: a new member of the cannabinoid receptor clan? British Journal of Pharmacology 152, 984-986
41 Ryberg, E. et al. (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. British Journal of Pharmacology 152, 1092-1101
42 Lauckner, J.E. et al. (2008) GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. Proceedings of the National Academy of Sciences of the United States of America 105, 2699-2704
43 Caterina, M.J. et al. (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816-824
44 Szallasi, A. (2002) Vanilloid (capsaicin) receptors in health and disease. American Journal of Clinical Pathology 118, 110-121
45 Melck, D. et al. (1999) Unsaturated long-chain N-acyl-vanillyl-amides (N-AVAMs): vanilloid receptor ligands that inhibit anandamide-facilitated transport and bind to CB1 cannabinoid receptors. Biochemical and Biophysical Research Communications 262, 275-284
46 Zygmunt, P.M. et al. (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400, 452-457
47 Smart, D. et al. (2000) The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). British Journal of Pharmacology 129, 227-230
48 Ross, R.A. (2003) Anandamide and vanilloid TRVP1 receptors. British Journal of Pharmacology 140, 790-801
49 Jarai, Z. et al. (1999) Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. Proceedings of the National Academy of Sciences of the United States of America 96, 14136-14141
50 Di Marzo, V. et al. (2000) Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. Journal of Neurochemistry 75, 2434-2444
51 Breivogel, C.S. et al. (2001) Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Molecular Pharmacology 60, 155-163
52 Wiley, J.L. and Martin, B.D. (2002) Cannabinoid pharmacology: implications for additional cannabinoid receptor subtypes. Chemistry and Physics of Lipids 121, 57-63

Accession information: doi:10.1017/S1462399409000957; Vol. 11; e3; January 2009

53 Bayewitch, M. et al. (1995) The peripheral cannabinoid receptor: adenylate cyclase inhibition and G protein coupling. FEBS Letters 375, 143-147
54 Slipetz, D.M. et al. (1995) Activation of the human peripheral cannabinoid receptor results in inhibition of adenylyl cyclase. Molecular Pharmacology 48, 352-361
55 Bouaboula, M. et al. (1996) Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. European Journal of Biochemistry 237, 704-711
56 Zoratti, C. et al. (2003) Anandamide initiates Ca(2+) signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. British Journal of Pharmacology 140, 1351-1362
57 Cabral, G.A. et al. (2008) CB2 receptors in the brain: role in central immune function. British Journal of Pharmacology 153, 240-251
58 Cabral, G.A. and Staab, A. (2005) Effects on the immune system. Handbook of Experimental Pharmacology 168, 385-423
59 Morahan, P.S. et al. (1979) Effects of cannabinoids on host resistance to *Listeria monocytogenes* and herpes simplex virus. Infection and Immunity 23, 670-674
60 Juel-Jensen, B.E. (1972) Cannabis and recurrent herpes simplex. British Medical Journal. 4, 296
61 Cabral, G.A. and Dove-Pettit, D.A. (1998) Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. Journal of Neuroimmunology 83, 116-123
62 Klein, T.W., Newton, C. and Friedman, H. (1998) Cannabinoid receptors and immunity. Immunology Today 19, 373-381
63 Arata, S. et al. (1992) Enhanced growth of *Legionella pneumophila* in tetrahydrocannabinol-treated macrophages. Proceedings of the Society for Experimental Biology and Medicine 199, 65-67
64 Arata, S. et al. (1991) Tetrahydrocannabinol treatment suppresses growth restriction of *Legionella pneumophila* in murine macrophage cultures. Life Sciences 49, 473-479
65 Marciano-Cabral, F. et al. (2001) Delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, exacerbates brain infection by *Acanthamoeba*. Journal of Eukaryotic Microbiology (Suppl), 4S-5S
66 Newton, C.A., Klein, T. and Friedman, H. (1994) Secondary immunity to *Legionella pneumophila* and Th1 activity are suppressed by delta-9-tetrahydrocannabinol injection. Infection and Immunity 62, 4015-4020
67 Burnette-Curley, D. and Cabral, G.A. (1995) Differential inhibition of RAW264.7 macrophage tumoricidal activity by delta 9tetrahydrocannabinol. Proceedings of the Society for Experimental Biology and Medicine 210, 64-76
68 Coffey, R.G. et al. (1996) Tetrahydrocannabinol inhibition of macrophage nitric oxide production. Biochemical Pharmacology 52, 743-751
69 Klein, T.W. et al. (1991) Marijuana components suppress induction and cytolytic function of murine cytotoxic T cells in vitro and in vivo. Journal of Toxicology and Environmental Health 32, 465-477
70 McCoy, K.L. et al. (1999) Cannabinoid inhibition of the processing of intact lysozyme by macrophages: evidence for CB2 receptor participation. Journal of Pharmacology and Experimental Therapeutics 289, 1620-1625
71 Raborn, E.S. et al. (2008) The cannabinoid delta-9-tetrahydrocannabinol mediates inhibition of macrophage chemotaxis to RANTES/CCL5: linkage to the CB(2) receptor. Journal of Neuroimmune Pharmacology 3, 117-129
72 Sacerdote, P. et al. (2000) In vivo and in vitro treatment with the synthetic cannabinoid CP55, 940 decrease the in vitro migration of macrophages in the rat: involvement of both CB1 and CB2 receptors. Journal of Neuroimmunology 109, 155-163
73 Ullrich, O. et al. (2007) Immune control by endocannabinoids - new mechanisms of neuroprotection? Journal of Neuroimmunology 184, 127-135
74 Cabral, G.A. and Marciano-Cabral, F. (2004) Cannabinoid-mediated exacerbation of brain infection by opportunistic amebae. Journal of Neuroimmunology 147, 127-130
75 Steffens, S. et al. (2005) Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. Nature 434, 782-786
76 Kishimoto, S. et al. (2003) 2-arachidonoylglycerol induces the migration of HL-60 cells differentiated into macrophage-like cells and human peripheral blood monocytes through the cannabinoid CB2 receptor-dependent mechanism. Journal of Biological Chemistry 278, 24469-24475
77 Derocq, J. et al. (1995) Cannabinoids enhance human B-cell growth at low nanomolar concentrations. FEBS Letters 369, 177-182
78 Carayon, P. et al. (1998) Modulation and functional involvement of CB2 peripheral cannabinoid receptors during B-cell differentiation. Blood 92, 3605-3615

Accession information: doi:10.1017/S1462399409000957; Vol. 11; e3; January 2009

79 Friedman, H. et al. (1991) Immunosuppression by marijuana components. In Psychoneuroimmunology (Ader, R., Felten, D.L. and Cohen, N., eds.), pp. 931-953, Academic Press
80 Klein, T. et al. (1998b) Marijuana, immunity and infection. Journal of Neuroimmunology 83, 102-115
81 Kaminski, N. et al. (1994) Suppression of the humoral immune response by cannabinoids is partially mediated through inhibition of adenylate cyclase by a pertussis toxin-sensitive G-protein coupled mechanism. Biochemical Pharmacology 48, 1899-1908
82 Massi, P. et al. (2000) Relative involvement of cannabinoid CB(1) and CB(2) receptors in the Delta(9)-tetrahydrocannabinol-induced inhibition of natural killer activity. European Journal of Pharmacology 387, 343-347
83 Klein, T.W. et al. (2000) Delta-9-tetrahydrocannabinol treatment suppresses immunity and early IFN-gamma, IL-12, and IL-12 receptor beta 2 responses to *Legionella pneumophila* infection. Journal of Immunology 164, 6461-6466
84 Zhu, L.X. et al. (2000) Delta-9-tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. Journal of Immunology 165, 373-380
85 Berdyshev, E.V. et al. (1997) Influence of fatty acid ethanolamides and delta9-tetrahydrocannabinol on cytokine and arachidonate release by mononuclear cells. European Journal of Pharmacology 330, 231-240
86 Facci, L. et al. (1995) Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proceedings of the National Academy of Sciences of the United States of America 92, 3376-3380
87 Joseph, J. et al. (2004) Anandamide is an endogenous inhibitor for the migration of tumor cells and T lymphocytes. Cancer Immunology, Immunotherapy 53, 723-728
88 Valk, P. et al. (1997) Anandamide, a natural ligand for the peripheral cannabinoid receptor is a novel synergistic growth factor for hematopoietic cells. Blood 90, 1448-1457
89 Molina-Holgado, F. et al. (1998) The endogenous cannabinoid anandamide potentiates interleukin-6 production by astrocytes infected with Theiler's murine encephalomyelitis virus by a receptor-mediated pathway. FEBS Letters 433, 139-142
90 Stefano, G.B. et al. (2000) 2-Arachidonyl-glycerol stimulates nitric oxide release from human immune and vascular tissues and invertebrate immunocytes by cannabinoid receptor 1. Pharmacological Research 42, 317-322
91 Jorda, M.A. et al. (2002) Hematopoietic cells expressing the peripheral cannabinoid receptor migrate in response to the endocannabinoid 2-arachidonoylglycerol. Blood 99, 2786-2793
92 Rayman, N. et al. (2004) Distinct expression profiles of the peripheral cannabinoid receptor in lymphoid tissues depending on receptor activation status. Journal of Immunology 172, 2111-2117
93 Kishimoto, S. et al. (2004) 2-Arachidonoylglycerol an endogenous cannabinoid receptor ligand, induces accelerated production of chemokines in HL-60 cells. Journal of Biochemistry (Tokyo) 135, 517-524
94 Carrier, E.J. et al. (2004) Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. Molecular Pharmacology 65, 999-1007
95 Klein, T.W. and Friedman, H. (1990) Modulation of murine immune cell function by marijuana components. In Drugs of Abuse and Immune Function (Watson, R., ed.), pp. 87-111, CRC Press
96 Burnette-Curley, D. et al. (1993) Delta-9-tetrahydrocannabinol inhibits cell contact-dependent cytotoxicity of *Bacillus* Calmette-Guerin-activated macrophages. International Journal of Immunopharmacology 15, 371-382
97 Cabral, G.A. and Mishkin, E.M. (1989) Delta-9-tetrahydrocannabinol inhibits macrophage protein expression in response to bacterial immunomodulators. Journal of Toxicology and Environmental Health 26, 175-182
98 Watzl, B. et al. (1991) Influence of marijuana components (THC and CBD) on human mononuclear cell cytokine secretion in vitro. In Advances in Experimental Medicine and Biology (Friedman, H., Specter, S. and Klein, T.W., eds), pp. 63-70, Plenum Press
99 Nakano, Y. et al. (1992) Modulation of interleukin 2 activity by delta 9-tetrahydrocannabinol after stimulation with concanavalin A, phytohemagglutinin, or anti-CD3 antibody. Proceedings of the Society for Experimental Biology and Medicine 201, 165-168
100 Puffenbarger, R.A. et al. (2000) Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. Glia 29, 58-69
101 Jeon, Y.J. et al. (1996) Attenuation of inducible nitric oxide synthase gene expression by delta

Accession information: doi:10.1017/S1462399409000957; Vol. 11; e3; January 2009

9-tetrahydrocannabinol is mediated through the inhibition of nuclear factor-κB/Rel activation. Molecular Pharmacology 50, 334-341
102 Adams, D.O. and Hamilton, T.A. (1984) The cell biology of macrophage activation. Annual Review of Immunology 2, 283-318
103 Hamilton, T.A. and Adams, D.O. (1987) Molecular mechanisms of signal transduction in macrophages. Immunology Today 8, 151-158
104 Hamilton, T.A. et al. (1986) Effects of bacterial lipopolysaccharide on protein synthesis in murine peritoneal macrophages: Relationship to activation for macrophage tumoricidal function. Journal of Cellular Physiology 128, 9-17
105 Carlisle, S. et al. (2002) Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. International Immunopharmacology 2, 69-82
106 Harris, H. (1953) Chemotaxis of monocytes. British Journal of Experimental Pathology 34, 276-279
107 Harris, H. (1954) Role of chemotaxis in inflammation. Physiological Reviews 34, 529-562
108 Jin, T. and Hereld, D. (2006) Moving toward understanding eukaryotic chemotaxis. European Journal of Cell Biology 85, 905-913
109 Kehrl, J.H. (2006) Chemoattractant receptor signaling and the control of lymphocyte migration. Immunologic Research 34, 211-227
110 Becker, E.L. (1977) Stimulated neutrophil locomotion: chemokinesis and chemotaxis. Archives of Pathology and Laboratory Medicine 101, 509-513
111 Keller, H.U. et al. (1978) Distinct chemokinetic and chemotactic responses in neutrophil granulocytes. European Journal of Immunology 8, 1-7
112 Lauffenburger, D.A. and Horwitz, A.F. (1996) Cell migration: a physically integrated molecular process. Cell 84, 359-369
113 Mitchison, T.J. and Cramer, L.P. (1996) Actin-based cell motility and cell locomotion. Cell 84, 371-379
114 Murdoch, C. and Finn, A. (2000) Chemokine receptors and their role in inflammation and infectious disease. Blood 95, 3032-3043
115 Schiffmann, E. et al. (1975) N-formyl-methionyl peptides as chemoattractants for leucocytes. Proceedings of the National Academy of Sciences of the United States of America 72, 1059-1062
116 Goldman, D.W. and Goetzl, E.J. (1982) Specific binding of leukotriene B4 to receptors on human polymorphonuclear leukocytes. Journal of Immunology 129, 1600-1604
117 Hanahan, D.J. (1986) Platelet activating factor: A biologically active phosphoglyceride. Annual Review of Biochemistry 55, 483-509
118 Gerard, C. and Gerard, N.P. (1994) C5A anaphylatoxin and its seven transmembrane-segment receptor. Annual Review of Immunology 12, 775-808
119 Baggiolini, M. et al. (1994) Interleukin-8 and related chemotactic cytokines CXC and CC chemokines. Advances in Immunology 55, 97-179
120 Baggiolini, M. et al. (1997) Human chemokines: an update. Annual Review of Immunology 15, 675-705
121 Kim, C.H. (2004) Chemokine-chemokine receptor network in immune cell trafficking. Current Drug Targets – Immune, Endocrine & Metabolic Disorders 4, 343-361
122 Le, Y. et al. (2004) Chemokines and chemokine receptors: their manifold roles in homeostasis and disease. Cell Molecular Immunology 1, 95-104
123 Charo, I.F. and Ransohoff, R.M. (2006) Mechanisms of disease: the many roles of chemokines and chemokine receptors in inflammation. New England Journal of Medicine 354, 610-621
124 Sacerdote, P. et al. (2005) The nonpsychoactive component of marijuana cannabidiol modulates chemotaxis and IL-10 and IL-12 production of murine macrophages both in vivo and in vitro. Journal of Neuroimmunology 159, 97-105
125 Buckley, N.E. et al. (2000) Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. European Journal of Pharmacology 396, 141-149
126 Buckley, N.E. (2008) The peripheral cannabinoid receptor knockout mice: an update. European Journal of Pharmacology 153, 309-318
127 Van Sickle, M.D. et al. (2005) Identification and functional characterization of brainstem cannabinoid CB_2 receptors. Science 310, 329-332
128 Gong, J-P. et al. (2006) Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. Brain Research 1071, 10-23
129 Onaivi, E.S. (2006) Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB_2 receptors in the brain. Neuropsychobiology 54, 231-246
130 Onaivi, E.S. et al. (2006) Discovery of the presence and functional expression of cannabinoid CB2 receptors in the brain. Annals of the New York Academy of Sciences 1074, 514-536
131 Grimsey, N.L. et al. (2008) Specific detection of CB1 receptors; cannabinoid CB1 receptor antibodies are not all created equal! Journal of Neuroscience Methods 171, 78-86

Accession information: doi:10.1017/S1462399409000957; Vol. 11; e3; January 2009

132 Shohami, E. et al. (1997) Cytokine production in the brain following closed head injury: dexanabinol (HU-211) is a novel TNF-alpha inhibitor and an effective neuroprotectant. Journal of Neuroimmunology 72, 169-177
133 Cabral, G.A. et al. (2001) Cannabinoid-mediated inhibition of inducible nitric oxide production by rat microglial cells: evidence for CB1 receptor participation. Advances in Experimental Medicine and Biology 493, 207-214
134 Molina-Holgado, F. et al. (2002) Role of CB1 and CB2 receptors in the inhibitory effects of cannabinoids on lipopolysaccharide-induced nitric oxide release in astrocyte cultures. Journal of Neuroscience Research 67, 829-836
135 Klegeris, A. et al. (2003) Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. British Journal of Pharmacology 139, 775-786
136 Sheng, W.S. et al. (2005) Synthetic cannabinoid WIN55,212-2 inhibits generation of inflammatory mediators by IL-1beta-stimulated human astrocytes. Glia 49, 211-219
137 Kreutzberg, G.W. (1996) Microglia: a sensor for pathological events in the CNS. Trends in Neurosciences 19, 312-318
138 Benveniste, E.N. (1997) Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. Journal of Molecular Medicine 75, 165-173
139 Giulian, D. and Baker, T.J. (1986) Characterization of ameboid microglia isolated from developing mammalian brain. Journal of Neuroscience 6, 2163-2178
140 Dickson, D.W. (1997) Neuropathological diagnosis of Alzheimer's disease: a perspective from longitudinal clinicopathological studies. Neurobiology of Aging 18, S21-S26
141 Selkoe, D.J. (1998) The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. Trends in Cell Biology 8, 447-453
142 Velez-Pardo, C. and Del Rio, M.J. (2006) Avoidance of Abeta[(25–35)]/(H_2O_2)-induced apoptosis in lymphocytes by the cannabinoid agonists CP55,940 and JWJ-015 via receptor-independent and PI3K-dependedent mechanisms: role of NF-kappaB and p53. Medicinal Chemistry 2, 471-479
143 Raine, C.S. and Wu, E. (1993) Multiple sclerosis: remyelination in acute lesions. Journal of Neuropathology and Experimental Neurology 52, 199-204
144 Racke, M.K. (2001) Experimental autoimmune encephalomyelitis (EAE). Current Protocols in Neuroscience 9, Unit 9.7
145 Calderon, T.M. et al. (2006) A role for CXCL12 (SDF-1alpha) in the pathogenesis of multiple sclerosis: regulation of CXCL12 expression in astrocytes by soluble myelin basic protein. Journal of Neuroimmunology 177, 27-39
146 Kieseier, B.C. et al. (1999) Matrix metalloproteinases exhibit different expression patterns in inflammatory demyelinating diseases of the central and peripheral nervous system. Annals of the New York Academy of Sciences 878, 567-570
147 Anthony, D.C. et al. (2000) The evidence for primary axonal loss in multiple sclerosis. Revue Neurologique 30, 1203-1208
148 Yiangou, Y. 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 Neurology 6, 12
149 Fujiwara, M. and Egashira, N. (2004) New perspectives in the studies on endocannabinoid and cannabis: abnormal behaviors associate with CB1 cannabinoid receptor and development of therapeutic application. Journal of Pharmacological Sciences 96, 362-366
150 Ehrhart, J. et al. (2005) Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. Journal of Neuroinflammation 2, 29
151 Sanchez, A.J. et al. (2006) R-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphtalenylmethanone (WIN-2) ameliorates experimental autoimmune encephalomyelitis and induces encephalitogenic T cell apoptosis: partial involvement of the CB(2) receptor. Biochemical Pharmacology 72, 1697-1706
152 Palazuelos, J. et al. (2008) The CB(2) cannabinoid receptor controls myeloid progenitor trafficking: involvement in the pathogenesis of an animal model of multiple sclerosis. Journal of Biological Chemistry 283, 13320-13329
153 Arevalo-Martin, A. et al. (2003) Therapeutic action of cannabinoids in a murine model of multiple sclerosis. Journal of Neuroscience 23, 2511-2516
154 Croxford, J.L. and Miller, S.D. (2003) Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R + WIN55,212. Journal of Clinical Investigation 111, 1231-1240

Accession information: doi:10.1017/S1462399409000957; Vol. 11; e3; January 2009

155 Docagne, F. et al. (2007) Excitotoxicity in a chronic model of multiple sclerosis: Neuroprotective effects of cannabinoids through CB1 and CB2 receptor activation. Molecular and Cellular Neurosciences 34, 551-561
156 Kuncl, R.W. et al. (1992) Motor neuron disease. In Diseases of the Nervous System (2nd edn) (Asbury, A.K., McKhann, G.M. and McDonald, W.I., eds), pp. 1179-1208, W. B. Saunders
157 Babu, G.N. et al. (2008) Elevated inflammatory markers in a group of amyotrophic lateral sclerosis patients from northern India. Neurochemical Research 33, 1145-1149
158 Weydt, P. et al. (2005) Cannabinol delays symptom onset in SOD1 (G93A) transgenic mice without affecting survival. Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders 6, 182-184
159 Bisland, L.G. et al. (2006) Increasing cannabinoid levels by pharmacological and genetic manipulation delay disease progression in SOD1 mice. FASEB Journal 20, 1003-1005
160 Shoemaker, J.L. et al. (2007) The CB2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset. Journal of Neurochemistry 101, 87-98
161 Spencer, D.C. and Price, R.W. (1992) Human immunodeficiency virus and the central nervous system. Annual Review of Microbiology 46, 655-693
162 Benito, C. et al. (2005) A glial endogenous cannabinoid system is upregulated in the brains of macaques with simian immunodeficiency virus-induced encephalitis. Journal of Neuroscience 25, 2530-2536
163 Williams, K. et al. (2001a) Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. Glia 36, 156-164
164 Williams, K.C. et al. (2001b) Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. Journal of Experimental Medicine 193, 905-915
165 Ghosh, S. et al. (2006) Cannabinoid receptor CB2 modulates the CXCL12/CXCR4-mediated chemotaxis of T lymphocytes. Molecular Immunology 43, 2169-2179
166 Rubio-Araiz, A. et al. (2008) The endocannabinoid system modulates a transient TNF pathway that induces neural stem cell proliferation. Molecular and Cellular Neurosciences 38, 374-380
167 Waksman, Y. et al. (1999) The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. Journal of Pharmacology and Experimental Therapeutics 288, 1357-1366
168 Zhang, J. et al. (2003) Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. European Journal of Neuroscience 17, 2750-2754
169 Benito, C. et al. (2007) Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis. Journal of Neuroscience 27, 2396-2402
170 Maresz, K. et al. (2005) Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. Journal of Neurochemistry 95, 437-445
171 Ashton, J.C. et al. (2007) Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. Neuroscience Letters 412, 114-117
172 Benito, C. et al. (2003) Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in alzheimer's disease brains. Journal of Neuroscience 23, 11136-11141
173 Carrier, E.J. et al. (2005) Endocannabinoids in neuroimmunology and stress. Current Drug Targets – CNS & Neurological Disorders 4, 657-665
174 Marciano-Cabral, F. and Cabral, G.A. (2003) *Acanthamoeba* spp. as agents of disease in humans. Clinical Microbiology Reviews 16, 273-307
175 Cabral, G.A. (2005) Lipids as bioeffectors in the immune system. Life Sciences 77, 1699-1710
176 Bouaboula, M. et al. (1993) Cannabinoid-receptor expression in human leukocytes. European Journal of Biochemistry 214, 173-180
177 Lee, S.F. et al. (2001a) Downregulation of cannabinoid receptor 2 (CB2) messenger RNA expression during in vitro stimulation of murine splenocytes with lipopolysaccharide. Advances in Experimental Medicine and Biology 493, 223-228
178 Lee, S.F. et al. (2001b) Differential expression of cannabinoid CB(2) receptor mRNA in mouse immune cell subpopulations and following B cell stimulation. European Journal of Pharmacology 423, 235-241

Accession information: doi:10.1017/S1462399409000957; Vol. 11; e3; January 2009

179 Sinha, D. et al. (1998) Expression of the CB1 cannabinoid receptor in macrophage-like cells from brain tissue: immunochemical characterization by fusion protein antibodies. Journal of Neuroimmunology 82, 13-21
180 Lynn, A.B. and 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. Journal of Pharmacology and Experimental Therapeutics 268, 1612-1623
181 Kaminski, N.E. et al. (1992) Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. Molecular Pharmacology 42, 736-742

Further reading, resources and contacts

References

Klein, T.W. (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. Nature Reviews Immunology 5, 400-411
This review article discusses the immunosuppressive and anti-inflammatory properties of cannabinoids, and the feasibility of employing cannabinoid-based drugs as therapeutics for chronic inflammatory diseases.

Ashton, J.C. and Glass, M. (2007) The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration. Current Neuropharmacology 5, 73-80
This review highlights both in vivo and in vitro studies of CB_2 expression in microglia during brain injury and inflammation as well as after injury.

Arevalo-Martin, A. et al. (2008) CB2 cannabinoid receptors as an emerging target for demyelinating diseases: from neuroimmune interactions to cell replacement strategies. British Journal of Pharmacology 153, 216-225
This review discusses the role of immune-modulatory cannabinoid compounds on demyelinating diseases (i.e. multiple sclerosis) induced by inflammation.

Websites

Relevant OMIM (Online Mendelian Inheritance in Man|) webpages:

http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=105400 (amyotrophic lateral sclerosis)
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=126200 (multiple sclerosis)
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=114610 (CB_1)
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=605051 (CB_2)

The National Institute of Drug Abuse website includes listings of clinical trials:

http://www.nida.nih.gov/funding/clintrials.html

Discussion of the potential use of cannabinoids for symptom management in multiple sclerosis can be found through The National Multiple Sclerosis Society website:

http://www.nationalmssociety.org/search-results/index.aspx?pageindex=0&pagesize=20&keywords=cannabinoids

Features associated with this article

Figures

Figure 1. Representative cannabinoids.
Figure 2. Human cannabinoid receptor amino acid sequences.
Figure 3. Cannabinoid receptor signalling.
Figure 4. Cannabinoid receptor mRNA levels and macrophage activation state.

Table

Table 1. Distribution of cannabinoid receptors in the immune system.

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