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REVIEW

CB_2 receptor-mediated migration of immune cells: it can go either way

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Though many studies have examined the role of CB_2 receptors in immune cell migration, it has been difficult to form definitive conclusions about the physiopathological role of these receptors in regulating immune responses and how this might be pharmacologically targeted for therapy. Do cannabinoids promote inflammation through the recruitment of immune cells, or reduce inflammation by interfering with the action of other chemoattractants? Is therapeutic intervention with an agonist or antagonist more appropriate for the reduction of inflammation? In this review, we will summarize the progress that has been made in answering these questions and outline current hypotheses.
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Abbreviations: 2-AG, 2-arachidonoylglycerol; abn-CBD, abnormal cannabidiol; anandamide, *N*-arachidonoylethanolamine; CBD, cannabidiol; Δ^9-THC, Δ^9-tetrahydrocannabinol; DEA, docosatetraenylethanolamide; eCBs, endocannabinoids; fMLP, formyl-methionyl-leucyl-phenylalanine; HEA, homo-γ-linolenylethanolamide

For centuries, *Cannabis sativa* has been recognized for its medicinal and psychoactive properties, but only in the last decade have we begun to unravel the molecular mechanisms of these effects. The cloning of two cannabinoid receptors, CB_1 and CB_2, has brought about the exciting possibility of harnessing the therapeutic, non-psychotropic effects of cannabinoid compounds. Indeed, the mechanisms underlying the psychotropic effects have been clearly attributed to CB_1 receptors (Huestis *et al*., 2001), while many of the medicinal properties, especially those related to immune system modulation, are likely mediated by CB_2. This dichotomy is based largely on the tissue distribution of these receptors: CB_1 is abundantly expressed by the vast majority of neurons, while CB_2 is abundantly expressed by immune cells and only by a restricted population of neurons in the brain stem (Van Sickle *et al*., 2005).

Cannabinoid ligands acting on CB_2 receptors expressed by immune cells inhibit cytokine production (Ehrhart *et al*., 2005), decrease antigen presentation (Buckley *et al*., 2000) and modulate cell migration—the subject of this review. The rank order of CB_2 mRNA expression in immune cells is: B lymphocytes > natural killer (NK) cells >> monocytes > polymorphonuclear neutrophil cells > $CD8^+$ T cells > $CD4^+$ T cells (Galiegue *et al*., 1995). Before outlining how cannabinoids affect the migration of each type of immune cell, we will introduce the basic pharmacology of these compounds.

Cannabinoids, the ligands that engage cannabinoid receptors, fall into three main classes. The endogenous ligands—or endocannabinoids (eCBs)—are long-chain fatty acid derivatives. Two eCBs, *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG), have been extensively studied and shown to be *bona fide* lipid transmitters (Piomelli, 2005). eCB candidates have also been identified, such as homo-γ-linolenylethanolamide (HEA) and docosatetraenylethanolamide (DEA), but so far these lipids have only been shown to bind to and activate CB_1 receptors (Hanus *et al*., 1993) and whether they also act on CB_2 receptors is not known. The second class of cannabinoids, the phytocannabinoids, consists of more than 60 compounds derived from the *Cannabis* plant family, several of which are bioactive. The most well-known bioactive phytocannabinoid is Δ^9-tetrahydrocannabinol (Δ^9-THC), which produces the psychotropic effects associated with marijuana use. Another bioactive phytocannabinoid, cannabidiol (CBD), regulates important physiological functions, such as immune responses and blood pressure (Pertwee, 2005). These effects are achieved in the absence of psychotropic side effects, since CBD does not bind to CB_1 receptors. The third class of ligands consists of synthetic compounds that act with varying degrees of efficacy and selectivity at cannabinoid receptors. For example, the non-selective

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agonists CP55940 and WIN55212-2 act at both CB_1 and CB_2 receptors with nanomolar affinities, whereas JWH-015 and JWH-133 selectively activate CB_2, also with nanomolar affinities. When administered near their respective K_d for the corresponding cannabinoid receptor, several antagonists are quite selective; notable examples include the CB_1 antagonist SR141716A and the CB_2 antagonists SR144528 and AM630. However, caution must be taken because at higher concentrations, these antagonists are capable of binding to both CB_1 and CB_2 (see Table 1). All these cannabinoids will be further discussed in this review.

In addition to CB_1 and CB_2, it is also important to note the existence of additional putative cannabinoid receptors. These receptors have been pharmacologically characterized either through studies performed in cannabinoid receptor-knockout mice, or using ligands that have little to no affinity for CB_1 and CB_2 (Mackie and Stella, 2006). At least three novel receptors have been identified. The first, identified in the brain of $CB_1^{-/-}$ mice, is activated by anandamide and WIN55212-2 (Breivogel *et al*., 2001). A second receptor, demonstrated in the vasculature, is activated by abnormal cannabidol (abn-CBD). This ligand, a structural analogue of plant-derived CBD, does not have a measurable affinity for either CB_1 or CB_2 (Showalter *et al*., 1996). Interestingly, a putative CB_1 selective agonist, arachidonylcyclopropylamide (Hillard *et al*., 1999), has recently been shown to also interact with this novel abn-CBD-sensitive receptor (Franklin and Stella, 2003). The third putative cannabinoid receptor was shown to reduce pain associated with inflammation (Calignano *et al*., 1998; Jaggar *et al*., 1998). This receptor is activated by palmitoylethanolamide (PEA), a putative endogenous cannabinoid. Although many effects of cannabinoids on migration can be attributed to CB_1 and CB_2, in some cases the involvement of these putative receptors has been demonstrated or at least cannot be ruled out.

Recent work has shown that the three classes of cannabinoid ligands can differentially modulate second messenger pathways (a phenomenon also known as 'agonist trafficking') (Shoemaker *et al*., 2005). For example, low concentrations of 2-AG stimulate the Erk kinase pathway without affecting adenylyl cyclase activity, while low concentrations of CP55940 inhibit adenylyl cyclase activity without affecting the Erk kinase pathway (Shoemaker *et al*., 2005). This appears to have relevance for CB_2-mediated modulation of immune cell migration, since we found many examples in the literature of 2-AG-induced migration of CB_2-expressing immune cells, but very few of CP55940-induced migration. Since 'agonist trafficking' is likely to be relevant when assessing cannabinoid-induced migration, we will emphasize the actual drug that was used and its concentrations when reviewing the effect of cannabinoids on the migration of each immune cell type.

Cell migration is important in a variety of physiological and pathological processes, including development, cancer metastasis and autoimmune diseases. Chemotaxis, the process by which cells migrate according to specific concentration gradients, is a mechanism of cell-to-cell communication whereby resident cells recruit additional cells through the release of a chemotactic agent, classically a chemokine. This process involves three interrelated modalities (Van Haastert and Devreotes, 2004). First, in the absence of a chemoattractant, cells randomly extend pseudopodia, which probe their environment for directional cues. This process is mediated by the spontaneous polymerization of actin filaments. Second, the presence of a chemotactic gradient serves a directional sensing function, increasing the probability of pseudopod formation and driving cells in the direction of highest chemokine concentration. Third, polarization occurs when both front and rear-specific signalling cascades occur. Although the interplay of these three modalities varies between cell types, the general pathway by which polarization occurs and migration is initiated often relies on the activation of seven-transmembrane $G_{i/o}$ protein-coupled receptors. For example, activated G_i-$\beta\gamma$ subunits trigger signalling cascades that modulate Rho family small GTPases and Erk kinase (Ridley *et al*., 2003). The outcomes of these signalling cascades often culminate in protrusion and adhesion of the leading edge, and retraction of the posterior tail of the cell.

The molecules stimulating chemotaxis can be either peptides (for example, chemokines), small molecules (for example, neurotransmitters and nucleotides) or lipids (Murphy *et al*., 2000; Straub *et al*., 2000; Honda *et al*., 2001). Understanding lipid-induced migration presents a unique challenge. While it is easy to imagine water-soluble peptides and small molecules diffusing throughout the intracellular milieu and forming concentration gradients, this concept is more difficult to comprehend when considering lipids, which dissolve much less readily into aqueous solutions. However, several examples exist in which lipids are capable of mediating cell migration, including lysophosphatidic acid (LPA), which induces chemotaxis in many different cell types (Gerrard *et al*., 1980; Jin *et al*., 2003; Bian *et al*., 2004; Hama *et al*., 2004; Idzko *et al*., 2004). eCBs are also lipids that modulate cell migration through specific receptors and we will now review their effects with regards to each type of immune cell.

Macrophages

Macrophages play an important role in immunity: they migrate toward diseased tissue, engulf damaged cells and invading pathogens, and present antigens to the adaptive immune system. Over 30 years of research has been conducted to try and understand how cannabinoids regulate the migration of macrophages and their progenitors, monocytes. Reports published in the 1970s showed that Δ^9-THC inhibited the migration of leukocytes isolated from human blood (Schwartzfarb *et al*., 1974). Purified Δ^9-THC had less inhibitory activity than total *Cannabis* extract, suggesting a role for other phytocannabinoids. An additional early finding showed that Δ^9-THC decreased the inhibitory effect of migration inhibition factor, thus resulting in net increase in macrophage migration (Gaul and Mellors, 1975).

In recent years, the cloning of the cannabinoid receptors and development of specific ligands to target each receptor subtype has opened a new round of investigation into the effects of cannabinoids on macrophages. In 2000, it was reported that CP55940 inhibited the spontaneous migration

Table 1 Cannabinoid receptor ligands discussed in this review

Ligand	*Abbreviations*	*CB_1 K_i (nM)*	*CB_2 K_i (nM)*	*Reference*	*Species*	*Radioligand*[a]	*Inhibitor*[b]
Endocannabinoids and derviates							
N-arachidonoylethanolamine	Anandamide	61	1930	Lin *et al.*, 1998	Rat	CP55940	PMSF
		78.2	1926	Khanolkar *et al.*, 1996	Rat	CP55940	PMSF
		89	371	Showalter *et al.*, 1996	Human	CP55940	PMSF
		543	1940	Felder *et al.*, 1995	Human	CP55940	None
		71.7	279	Hillard *et al.*, 1999	Human	CP55940	PMSF
		252	581	Mechoulam *et al.*, 1995	Not specified	HU-243	None
		ND	>10 000	Griffin *et al.*, 2000	Rat	CP55940	PMSF
		ND	1480	Griffin *et al.*, 2000	Mouse	CP55940	PMSF
		ND	306	Griffin *et al.*, 2000	Human	CP55940	PMSF
2-arachidonoylglycerol	2-AG	472	1400	Mechoulam *et al.*, 1995	Not specified	HU-243	None
		58.3	145	Ben-Shabat *et al.*, 1998	Not specified	HU-243	None
Homo-γ-linolenylethanolamide	HEA	53.4	ND	Hanus *et al.*, 1993	Rat	HU-243	None
Docosatetraenylethanolamide	DEA	34.4	ND	Hanus *et al.*, 1993	Rat	HU-243	None
Arachidonylcyclopropylamide	ACPA	2.2	715	Hillard *et al.*, 1999	Human	CP55940	PMSF
2-arachidonoylglycerol ether	2-AG ether	21.2	>3000	Hanus *et al.*, 2001	rCB_1/hCB_2	HU-243	None
Phytocannabinoids							
Δ^9-tetrahydrocannabinol	Δ^9-THC	53.3	75.3	Felder *et al.*, 1995	Human	CP55940	None
		39.5	40	Bayewitch *et al.*, 1996	rCB_1/hCB_2	HU-243	None
		40.7	36.4	Showalter *et al.*, 1996	Human	CP55940	None
		80.3	32.2	Rhee *et al.*, 1997	rCB_1/hCB_2	HU-243	None
		35.3	3.9	Rinaldi-Carmona *et al.*, 1994	Rat	CP55940	None
		13.5	6.80	Iwamura *et al.*, 2001	Rat	CP55940	None
		8.33	1.73	Iwamura *et al.*, 2001	Mouse	CP55940	None
		5.05	3.13	Iwamura *et al.*, 2001	Human	CP55940	None
		ND	28.3	Griffin *et al.*, 2000	Rat	CP55940	None
		ND	27.3	Griffin *et al.*, 2000	Mouse	CP55940	None
		ND	44.9	Griffin *et al.*, 2000	Human	CP55940	None
Cannabidiol	CBD	4350	2860	Showalter *et al.*, 1996	Human	CP55940	None
		>10 000	>10 000	Bisogno *et al.*, 2001	rCB_1/hCB_2	HU-243	None
Abnormal-cannabidiol	abn-CBD	>10 000	>10 000	Showalter *et al.*, 1996	Human	CP55940	None
Synthetic nonselective agonists							
CP55940		5	1.8	Ross *et al.*, 1999	Human	CP55940	None
		3.72	2.55	Felder *et al.*, 1995	Human	CP55940	None
		1.37	1.37	Rinaldi-Carmona *et al.*, 1994	Rat	CP55940	None
		0.58	0.69	Showalter *et al.*, 1996	Human	CP55940	None
		0.5	2.8	Hillard *et al.*, 1999	Human	CP55940	PMSF
		ND	0.64	Griffin *et al.*, 2000	Rat	CP55940	None
		ND	0.73	Griffin *et al.*, 2000	Mouse	CP55940	None
		ND	0.88	Griffin *et al.*, 2000	Human	CP55940	None
WIN55212-2		9.94	16.2	Rinaldi-Carmona *et al.*, 1994	Rat	CP55940	None
		4.4	1.2	Hillard *et al.*, 1999	Human	CP55940	PMSF
		1.89	0.28	Showalter *et al.*, 1996	Human	CP55940	None
		62.3	3.30	Felder *et al.*, 1995	Human	CP55940	None
		123	4.1	Shire *et al.*, 1996	Human	CP55940	None
		0.14	1.30	Iwamura *et al.*, 2001	Rat	CP55940	None
		0.41	0.56	Iwamura *et al.*, 2001	Mouse	CP55940	None
		9.87	0.29	Iwamura *et al.*, 2001	Human	CP55940	None
		ND	10.4	Griffin *et al.*, 2000	Rat	CP55940	None

Table 1 *Continued*

Ligand	*Abbreviations*	*CB_1 K_i (nM)*	*CB_2 K_i (nM)*	*Reference*	*Species*	*Radioligand*[a]	*Inhibitor*[b]
		ND	9.46	Griffin *et al.*, 2000	Mouse	CP55940	None
		ND	1.19	Griffin *et al.*, 2000	Human	CP55940	None
HU-210		0.0608	0.524	Felder *et al.*, 1995	Human	CP55940	None
		0.1	0.17	Rhee *et al.*, 1997	rCB_1/hCB_2	HU-243	None
		0.73	0.22	Showalter *et al.*, 1996	Human	CP55940	None
		ND	3.2	Lunn *et al.*, 2006	Human	CP55940	None
CB_2-selective agonists							
JWH-015		383	13.8	Showalter *et al.*, 1996	Human	CP55940	None
		ND	269	Griffin *et al.*, 2000	Rat	CP55940	None
		ND	431	Griffin *et al.*, 2000	Mouse	CP55940	None
		ND	98.4	Griffin *et al.*, 2000	Human	CP55940	None
JWH-133		677	3.4	Huffman *et al.*, 1999	rCB_1/hCB_2	CP55940	None
CB_1-selective antagonists/inverse agonists							
SR141716A		11.8	13 200	Felder *et al.*, 1998	Human	CP55940	None
		11.8	973	Felder *et al.*, 1995	Human	CP55940	None
		12.3	702	Showalter *et al.*, 1996	Human	CP55940	None
		5.6	>1000	Rinaldi-Carmona *et al.*, 1994	Rat	CP55940	None
		1.98	>1000	Rinaldi-Carmona *et al.*, 1994	Rat	CP55940	None
		1.8	514	Ruiu *et al.*, 2003	Mouse	CP55940	None
		ND	2596	Lunn *et al.*, 2006	Human	CP55940	None
AM251		7.49	2290	Lan *et al.*, 1999	rCB_1/mCB_2	CP55940	None
CB_2-selective antagonists/inverse agonists							
SR144528		437	0.6	Rinaldi-Carmona *et al.*, 1998	Human	CP55940	None
		305	0.3	Rinaldi-Carmona *et al.*, 1998	Rat	CP55940	None
		>10 000	5.6	Ross *et al.*, 1999	Human	CP55940	None
		70	0.28	Ruiu *et al.*, 2003	Mouse	CP55940	None
		27.6	0.24	Iwamura *et al.*, 2001	Rat	CP55940	None
		20.1	0.04	Iwamura *et al.*, 2001	Mouse	CP55940	None
		50.3	1.99	Iwamura *et al.*, 2001	Human	CP55940	None
		ND	0.3	Griffin *et al.*, 2000	Rat	CP55940	None
		ND	0.05	Griffin *et al.*, 2000	Mouse	CP55940	None
		ND	0.32	Griffin *et al.*, 2000	Human	CP55940	None
		ND	14.9	Lunn *et al.*, 2006	Human	CP55940	None
AM630		5152	31.2	Ross *et al.*, 1999	Human	CP55940	None
Sch.336		ND	1.8	Lunn *et al.*, 2006	Human	CP55940	None
abn-CBD receptor antagonist							
O-1918		>30 000	>30 000	Offertaler *et al.*, 2003	Mouse	CP55940	None

Cannabinoid receptor ligands discussed in this review.
[a]K_i values were calculated based on competition with the radioligand listed in this column.
[b]In some experiments, binding was carried out in the presence of an inhibitor of endocannabinoid hydrolysis listed in this column.

of murine primary macrophages (Sacerdote *et al.*, 2000). The induced chemotaxis toward the potent chemoattractant peptide formyl-methionyl-leucyl-phenylalanine (fMLP), which mimics the action of bacterial peptides, was also inhibited. This effect appeared to involve CB_2 receptors, since SR144528 potently blocked the effect of CP55940 on both spontaneous and fMLP-induced migration. SR141716A partially blocked the effect of CP55940 on spontaneous migration but had no effect on fMLP-induced chemotaxis. However, the concentrations of SR141716A needed ($\geqslant$100 nM) and the only partial blockade of CP55940-induced motility leave two possibilities open: there may be a minute role for CB_1 in regulating macrophage motility, or the effect may be due to the higher concentration of SR141716A now also acting on CB_2 receptors.

In 2003, the laboratory of T Sugiura showed that 2-AG induces the migration of HL-60 cells—the human monocytic cell line from which CB_2 receptor was originally cloned (Munro *et al.*, 1993; Kishimoto *et al.*, 2003). Specifically, in HL-60 cells differentiated into macrophage-like cells, migration toward 2-AG was blocked by SR144528. This effect was replicated in differentiated U937 and THP-1 cells, as well as human peripheral blood monocytes. In addition, migration in differentiated HL-60 cells was decreased by the mitogen-activated protein/extracellular signal-regulated kinase inhibitor PD98059 and the Rho kinase inhibitor Y-27632. Finally, other cannabinoid ligands, such as anandamide, CP55940 and WIN55212-2, had little—if any—effect on migration.

CBD also modulates macrophage migration. Specifically, CBD does not affect basal migration of peritoneal macrophages, but inhibits fMLP-induced migration (Sacerdote *et al.*, 2005). The authors suggested the involvement of CB_2 receptors in this CBD response, since it was blocked by SR144528, but this pharmacology does not correspond to what is known about recombinant CB_2 receptors (Showalter *et al.*, 1996). The effect of CBD occurred at concentrations as low as 10 nM, which is well below the affinity of CBD for CB_2. Is this an example of CBD trafficking differently between cell types (CHO cells versus macrophages), or is it the action of an uncloned cannabinoid receptor?

In 2005, Steffens and co-workers reported that low doses of Δ^9-THC—below its psychoactive concentration—inhibited the migration of murine peritoneal macrophages to monocyte chemoattractant protein-1 (Steffens *et al.*, 2005). This chemokine is produced by endothelial cells and macrophages associated with atherosclerotic plaques and contributes to atheroma growth by recruiting additional monocytes and macrophages (Charo and Taubman, 2004). The inhibitory effect of Δ^9-THC on macrophage migration was blocked by SR144528 and absent in $CB_2^{-/-}$ macrophages, strongly implicating CB_2 as the receptor mediating this effect.

In 2006, Schering-Plough Research showed that a CB_2 inverse agonist that they developed—Sch.336—blocked macrophage recruitment in an *in vivo* model of inflammation (Lunn *et al.*, 2006). Specifically, a sponge soaked with monocyte chemoattractant protein-1 was implanted in the peritoneum of mice and recovered 18 h later. In animals treated with Sch.336, migration of macrophages (as well as granulocytes and lymphocytes) into the sponge was reduced. Another intriguing finding was that when the sponge was soaked with the non-selective, highly efficacious cannabinoid agonist HU-210, the number of infiltrating cells was significantly increased. This is the only example of a synthetic cannabinoid agonist stimulating immune cell migration that we found while preparing this review.

A recent report has shed some light on the discrepancy between the actions of 2-AG and synthetic ligands on the CB_2 receptor (Oka *et al.*, 2006). Injection of either 12-*O*-tetradecanoylphorbol 13-acetate or 2-AG into the mouse ear provoked swelling, presumably through the recruitment of leukocytes. This swelling was reduced not only by WIN55212-2 and CP55940, but also by SR144528. Thus, the agonist and antagonist had identical effects. In transwell migration assays using HL-60 cells differentiated into macrophage-like cells, WIN55212-2 blocked chemotaxis toward 2-AG. Finally, in calcium imaging experiments, the investigators found a rapid return in the ability of 2-AG-pretreated cells to respond to subsequent pulses of 2-AG, whereas WIN55212-2-pretreated cells did not recover the ability to respond to 2-AG. A possible explanation for these results is that WIN55212-2 desensitizes the receptor, whereas 2-AG does not. This could be due to these ligands having differential agonist trafficking potential or to 2-AG being rapidly inactivated (Beltramo and Piomelli, 2000).

Substantial evidence has implicated cannabinoids, likely acting through CB_2 receptors, in the modulation of migration as well as other aspects of macrophage activity, including phagocytosis and antigen presentation. Exciting future work should reveal whether the power of these receptors might be harnessed to either bolster or temper macrophage-dependent immune responses.

Microglia

Microglial cells are the resident macrophage of the CNS. In healthy brain, they are in a resting state, but pathological conditions induce their activation. This allows them to execute a variety of neuroimmune-related functions, including migration, cytokine production, phagocytosis and antigen presentation. Constitutive CB_2 receptor expression is quite restricted in the CNS, being localized to specific nuclei of the brain stem (Van Sickle *et al.*, 2005). Nevertheless, while resting microglia express little—if any—CB_2, as proven by reverse transcription-PCR of healthy brain parenchyma (for review, see Stella, 2004), the expression of this receptor is induced under specific pathological conditions, including multiple sclerosis and amyotrophic lateral sclerosis (Yiangou *et al.*, 2006). Several reports have addressed the role of CB_2 receptors in activated microglia, which includes regulation of cytokine release (Ehrhart *et al.*, 2005) and cell proliferation (Carrier *et al.*, 2004), and our laboratory has published two reports on the role of CB_2 receptors in regulating microglial cell migration.

Using BV-2 cells, a mouse microglial cell line, we screened several cannabinoids for their ability to elicit cell migration (Franklin and Stella, 2003; Walter *et al.*, 2003). The most efficacious ligand was 2-AG (250% of basal), while anandamide and the two putative eCBs HEA and DEA produced

smaller responses (150–200% of basal). 2-AG stimulated both chemokinesis and chemotaxis, as determined by checkerboard analysis. Additionally, the 2-AG response was blocked by PD98059, a potent inhibitor of ERK phosphorylation. Of special interest was the finding that 2-AG-induced migration was blocked not only by SR144528 but also by an antagonist to the abn-CBD receptor (O-1918) (Offertaler *et al*., 2003), indicating that activation of both of these receptors is required for 2-AG-dependent recruitment of microglial cells. Whether this is also true for other cell types that migrate in response to 2-AG has not yet been determined. Furthermore, the CB_1 agonist arachidonylcyclopropylamide also induced migration, but this response was antagonized by SR144528 and CBD, indicating the involvement of the CB_2 and abn-CBD-sensitive receptor, rather than CB_1 receptors, in this response (Franklin and Stella, 2003). Thus, we can conclude that eCBs may recruit activated microglial cells, and that this action relies on the activation of CB_2 receptors and at least one other 'uncloned' cannabinoid receptor.

T lymphocytes

T lymphocytes are a central player in adaptive immune responses. Their migration into tissues is important for their role in mediating inflammation through cytokine release (in the case of $CD4^+$ cells) and killing diseased cells ($CD8^+$ cells). Although T cells express the least CB_2 mRNA relative to the other major leukocyte subsets, their migration is still tightly regulated by these receptors. Three laboratories have reported that activation of CB_2 receptors by various ligands inhibits T-cell migration in response to CXCL12, a well-characterized and highly efficacious chemokine important for T-cell recruitment (Bleul *et al*., 1996).

In 2004, Joseph and co-workers reported that anandamide reduces by approximately 50% the CXCL12-induced migration of isolated human $CD8^+$ T lymphocytes (Joseph *et al*., 2004). This inhibitory effect was mimicked by the CB_2 agonist JWH-133, whereas DEA had no effect. Additionally, both anandamide and JWH-133 had no effect on motility in the absence of CXCL12. Whether these effects result from the direct modulation of chemokine receptors by CB_2 or an indirect mechanism remains unclear.

A second study tested the effects of the non-selective agonists WIN55212-2 and CP-55940 on CXCL12-induced chemotaxis and *trans*-endothelial migration of Jurkat T cells, as well as primary $CD4^+$ and $CD8^+$ T lymphocytes (Ghosh *et al*., 2006). In transwell migration assays, cannabinoid ligands inhibited the chemotaxis of all three cell types by as much as 75%. These effects were mediated—at least in part—by CB_2 since the effect was also elicited by JWH-015 and partially blocked by AM630. However, the high concentrations of JWH-015 required (10–40 μM) and only partial inhibition by AM630 call the specificity of the effect into question.

Recent work used isolated human T lymphocytes to further investigate the role of CB_2 in inhibiting chemotactic responses to CXCL12 (Coopman *et al*., 2007). A key difference in this study was that the investigators used cultured cells activated by a superantigen (Staphylococcal enterotoxin B), while the previous studies used directly isolated cells. While no CB_2 receptor expression was detected by immunoblotting immediately after isolation, its induction peaked 5 days after superantigen treatment. These cells were then subjected to a chemotaxis assay, where both 2-AG and JWH-133 inhibited chemotaxis toward CXCL12.

Taken together, these results indicate that although T cells express low levels of CB_2 mRNA, the receptor is an active regulator of T-cell migration. Although not yet determined *in vivo*, eCB signalling *via* CB_2 may temper inflammatory responses by inhibiting CXCL12-induced T-cell recruitment. Whether CB_2 also affects other chemokine-induced migration remains an important open question.

B lymphocytes

Like T lymphocytes, B lymphocytes also migrate toward sites of inflammation, where they engulf pathogens and carry out antibody-mediated actions. B lymphocytes express large amounts of CB_2 mRNA and the receptor indeed regulates their functions, including migration. In 2002, Jorda and co-workers isolated cells from mouse spleen and found CB_2-dependent migration toward 2-AG (Jorda *et al*., 2002). Flow cytometry analysis showed that these cells were $B220^+$, $CD19^+$, IgM^+ and IgD^+ and thus confirmed that they were B lymphocytes. In a later study, the same group showed that Raji B lymphoma cells also migrate toward 2-AG and that this response is greatly enhanced by the costimulatory molecule CD40, suggesting crosstalk between these two pathways (Rayman *et al*., 2004). These studies have demonstrated functional CB_2 regulation of B-cell migration and open the door for future studies to determine the physiological relevance of such findings.

Myeloid leukaemia cells

One laboratory examined the migration of CB_2-expressing murine myeloid leukaemia cells as part of a larger, very elegant study investigating the role of CB_2 as a putative oncogene. Their first report implicated 2-AG as the major signalling molecule regulating CB_2-mediated chemotaxis in these cells (Jorda *et al*., 2002). 2-AG induced robust chemotaxis and chemokinesis of a leukaemia cell line endogenously expressing high levels of CB_2 (NFS 78), as well as transfected 32D/G-CSF-R cells expressing the receptor. The peak effect was observed at a concentration of 300 nM; higher and lower concentrations caused smaller amounts of chemotaxis, which is reminiscent of the classic 'bell-shaped' dose–response obtained with chemokines. Importantly, migration to 300 nM 2-AG was completely blocked by SR144528, whereas SR141716A had no effect. Additionally, anandamide stimulated cell migration that was only 20% that of 2-AG, while WIN55212-2, CP-55 940 and Δ^9-THC had no effect. Furthermore, when co-applied with 2-AG, these compounds fully blocked 2-AG-induced chemotaxis, again illustrating the unique pharmacology of endogenous, natural and synthetic cannabinoids at the CB_2 receptor. Possible explanations for some of these findings are that anandamide

has been shown to only very weakly activate CB_2 (Hillard *et al*., 1999), while Δ^9-THC can function as a CB_2 antagonist (Bayewitch *et al*., 1996). A final interesting finding of this study was that IL-3 and G-CSF alone did not induce migration of CB_2-expressing 32D/G-CSF-R cells, but synergized in combination with 2-AG.

A follow-up study provided insight into the mechanism of CB_2-mediated migration of these cells (Alberich Jorda *et al*., 2004). CB_2 receptors were expressed with a mutated DRY motif, which regulates the conformation state and G protein coupling of some G protein-coupled receptors (Rovati *et al*., 2007). Under these conditions, 2-AG did not induce migration. The authors also determined that migration to 2-AG was pertussis toxin-sensitive and partially blocked by the addition of a cAMP analogue (dibutyryl cAMP), suggesting that migration to 2-AG elicited by CB_2 activation requires $G_{i/o}$ proteins and a decrease in cAMP levels. These results further demonstrate the unique pharmacology of CB_2 ligands, where both agonists and antagonists block the effect of eCBs.

Natural killer cells

NK cells are important mediators of innate immunity, playing a role in antitumor and antimicrobial responses. Relatively little has been published on the role of CB_2 receptors in these cells. A 2005 publication showed that both a NK cell line and primary human NK cells migrate toward 2-AG, a response blocked by SR144528 and Δ^9-THC (Kishimoto *et al*., 2005). This response already occurred at 10 nM and reached a maximum at 10 μM. A non-hydrolysable analogue of 2-AG, 2-AG ether, also induced migration, although to a lesser extent than 2-AG, while anandamide and arachidonic acid caused no response. This 2-AG response was pertussis toxin sensitive, in agreement with of CB_2 receptors coupling to $G_{i/o}$ proteins.

Eosinophils

Eosinophils play a special function in defined pathological processes such as allergy and asthma. Two groups have examined cannabinoid effects on eosinophil migration. The first report, in 2004, demonstrated by reverse transcription-PCR and northern blot that an eosinophilic leukaemia cell line and human peripheral blood eosinophils express CB_2 but not CB_1 receptors (Oka *et al*., 2004). These receptors were functional in that they mediated chemotaxis toward 2-AG, a response that was blocked by SR144528 and abolished by pretreatment with pertussis toxin. Anandamide did not stimulate migration of these cells.

A second study revealed that CB_2 receptors modulate eosinophil migration *in vivo* (Lunn *et al*., 2006). Using a model of allergic asthma, where mice are challenged with aerosolized ovalbumin, the investigators showed that the CB_2 inverse agonist Sch.336 decreased the number of eosinophils by approximately 40% in the bronchoalveolar lavage of mice subjected to ovalbumin, a response that was as efficacious as what was obtained with the PDE4 inhibitor rolipram.

Although only these two studies have examined the role of CB_2 receptors in eosinophil migration, both *in vitro* and *in vivo* evidence shows that this receptor is likely to play an important role in their recruitment, suggesting that CB_2 agents may hold promise to reduce hypersensitivity associated with allergy and asthma.

Neutrophils

Neutrophils are vital early responders to tissue injury, efficiently leaving the blood stream and rapidly migrating toward growing foci of inflammation. Conflicting evidence exists regarding the expression of CB_2 by neutrophils, as well as the role of cannabinoids in regulating their migration. In 2003, Deusch and co-workers found no effect of Δ^9-THC on neutrophil migration and indeed no CB_2 protein was detected by western blot (Deusch *et al*., 2003). A year later, similar findings were obtained by Oka *et al*. (2004), who showed that neutrophils do not express CB_2 receptors and 2-AG has no effect on their migration. Both studies examined human peripheral blood neutrophils.

Contrary evidence has been presented more recently (Kurihara *et al*., 2006). Indeed, cell surface CB_2 expression was detected by flow cytometry in human neutrophils and HL-60 cells differentiated into neutrophil-like cells. JWH-015 and 2-AG inhibited the migration of both cell types toward fMLP. This inhibition was prevented by co-application of SR144528, further demonstrating the specificity of the effect. Interestingly, cells treated with JWH-015 or 2-AG plus fMLP displayed motility and morphological changes but did not develop front/rear polarity. These cells had a significantly decreased migratory velocity compared to cells not treated with cannabinoids. Cannabinoid treatment blocked the increase in RhoA activity normally associated with fMLP stimulation. Thus, it appears that signalling through CB_2 receptors prevents the formation of a retracting tail in these cells, preventing them from becoming polarized and migrating.

This finding suggests that cannabinoid signalling could reduce acute inflammation by preventing neutrophil migration. An explanation that would resolve the aforementioned discrepancy is that CB_2 receptor expression by neutrophils is exceptionally plastic, to the extent that methods used to prepare these cells could affect CB_2 expression. Hopefully, future studies will resolve these conflicting data and verify these important findings.

CB_2 receptors in migration of other cell types

Solid tumour growth requires large amounts of blood and thus induces the development of new vasculature, a process called angiogenesis. Several studies have demonstrated the ability of cannabinoids to restrict tumour growth by inhibiting this process (Bifulco *et al*., 2007). One mechanism by which cannabinoids exert this effect is through the inhibition of endothelial cell migration. Using primary

human umbilical vein endothelial cells, the laboratory of Manuel Guzmán showed that both WIN55212-2 and JWH-133 inhibited LPA-induced migration in these cells (Blazquez *et al*., 2003). Human umbilical vein endothelial cells express CB_1 and CB_2 receptors, both of which are likely to play a role in the inhibitory effect. Specifically, the inhibitory effect of WIN55212-2 was prevented by either SR141716A or SR144528, while the inhibitory effect of the more selective CB_2 ligand JWH-133 was only blocked by SR144528. These findings support the growing body of evidence suggesting the use of cannabinoids as therapeutic agents for cancer.

CB_1 receptors in cell migration

Although beyond the scope of this review, it is important to note that CB_1 receptors also modulate cell migration. For example, HU-210, WIN55212-2 and anandamide induce migration in transfected HEK293 cells expressing CB_1 (Song and Zhong, 2000), while anandamide inhibited the migration of breast and colon carcinoma cells in culture (Joseph *et al*., 2004; Grimaldi *et al*., 2006). Additionally, eCBs guide the migration of interneurons and the targeting of axon growth cones in the developing central nervous system (Berghuis *et al*., 2005, 2007). Clearly, the pharmacology of ligands inducing migration through CB_1 differs from that of CB_2 receptors, as indicated by the ability of anandamide and certain synthetic ligands to stimulate cells to migrate through CB_1.

Involvement of putative non-CB_1 non-CB_2 receptors in migration

It is important not to discount the involvement of additional cannabinoid receptors in cell migration (for a review, see Mackie and Stella, 2006). Some evidences have already shown a role for one or more of these receptors, such as our finding that the abn-CBD receptor antagonist O-1918 blocked the 2-AG-induced migration of microglial cells (Franklin and Stella, 2003; Walter *et al*., 2003). Additionally, Nilsson *et al*. (2006) found that WIN55212-2 inhibited neutrophil migration induced by tumour necrosis factor α, and this effect was blocked by neither AM251 nor AM630. The cloning and further pharmacological characterization of these receptors will in the future give us a clearer picture of cannabinoid effects on migration.

Conclusions

Since drugs acting on CB_2 receptors modulate immune cell migration, they represent a promising pharmacological platform for developing anti-inflammatory therapeutics, but this possibility comes across as a daunting challenge. Indeed, we must first gain a better understanding of the differential pharmacology of endogenous, synthetic and plant-derived cannabinoids acting at CB_2 receptors, not to mention cell type-specific effects. In general, 2-AG stimulates immune cell migration and CB_2 synthetic ligands, whether agonists or antagonists, inhibit chemotaxis toward 2-AG, and in some cases toward other chemokines. In the case of 2-AG-directed migration, it is possible that the strong activation of CB_2 receptors by synthetic agonists desensitizes the receptors, rendering them unable to further respond to eCBs (Oka *et al*., 2006). The cannabinoid-mediated inhibition of other chemokine-induced migration may occur through heterologous desensitization of chemokine receptors, as has been shown for opioids (Grimm *et al*., 1998). Receptor dimers may also exist between CB_2 and chemokine receptors, whereby CB_2 ligands would affect the ability of the chemokine receptor to signal properly—such examples already exist for CB_1 receptors (Kearn *et al*., 2005; Rios *et al*., 2006). Alternatively, ligands acting on CB_2 receptors could sequester G proteins and thus prevent efficacious coupling of the chemokine receptor (Bouaboula *et al*., 1999). Finally, ligands known to interact with CB_2 receptors are likely to traffic differently, stimulating or inhibiting specific signal transduction pathways depending on the cell type. We now know that the mere expression of CB_2 receptors by a specific cell type is not sufficient to predict that a CB_2 agonist or antagonist will either increase or decrease cell migration, and be either pro- or anti-inflammatory. Will we find a common denominator for the CB_2-mediated regulation of immune cells that predicts the therapeutic potential of these valuable ligands? Or do we have to systematically test each molecule in each disease setting? Both of these possibilities are open.

Conflict of interest

The authors state no conflict of interest.

References

Alberich Jorda M, Rayman N, Tas M, Verbakel SE, Battista N, van Lom K *et al*. (2004). The peripheral cannabinoid receptor Cb2, frequently expressed on AML blasts, either induces a neutrophilic differentiation block or confers abnormal migration properties in a ligand-dependent manner. *Blood* **104**: 526–534.

Bayewitch M, Rhee MH, Avidor-Reiss T, Breuer A, Mechoulam R, Vogel Z (1996). Delta9-tetrahydrocannabinol antagonizes the peripheral cannabinoid receptor-mediated inhibition of adenylyl cyclase. *J Biol Chem* **271**: 9902–9905.

Beltramo M, Piomelli D (2000). Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. *Neuroreport* **11**: 1231–1235.

Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee MH, Vogel Z *et al*. (1998). An encourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol* **353**: 23–31.

Berghuis P, Dobszay MB, Wang X, Spano S, Ledda F, Sousa KM *et al*. (2005). Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. *Proc Natl Acad Sci USA* **102**: 19115–19120.

Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Urban GM *et al*. (2007). Hardwiring the brain: endocannabinoids shape neuronal connectivity. *Science* **316**: 1212–1216.

Bian D, Su S, Mahanivong C, Cheng RK, Han Q, Pan ZK *et al*. (2004). Lysophosphatidic acid stimulates ovarian cancer cell migration via a ras-MEK kinase 1 pathway. *Cancer Res* **64**: 4209–4217.

Bifulco M, Laezza C, Gazzerro P, Pentimalli F (2007). Endocannabinoids as emerging suppressors of angiogenesis and tumor invasion (review). *Oncol Rep* **17**: 813–816.

Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I *et al.* (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* **134**: 845–852.

Blazquez C, Casanova ML, Planas A, Del Pulgar TG, Villanueva C, Fernandez-Acenero MJ *et al.* (2003). Inhibition of tumor angiogenesis by cannabinoids. *FASEB J* **17**: 529–531.

Bleul CC, Fuhlbrigge RC, Casasnovas JM, Aiuti A, Springer TA (1996). A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J Exp Med* **184**: 1101–1109.

Bouaboula M, Desnoyer N, Carayon P, Combes T, Casellas P (1999). Gi protein modulation induced by a selective inverse agonist for the peripheral cannabinoid receptor CB2: implication for intracellular signalization cross-regulation. *Mol Pharmacol* **55**: 473–480.

Breivogel CS, Griffin G, Di Marzo V, Martin BR (2001). Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharmacol* **60**: 155–163.

Buckley NE, McCoy KL, Mezey E, Bonner T, Zimmer A, Felder CC *et al.* (2000). Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. *Eur J Pharmacol* **396**: 141–149.

Calignano A, La Rana G, Giuffrida A, Piomelli D (1998). Control of pain initiation by endogenous cannabinoids. *Nature* **394**: 277–281.

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

Charo IF, Taubman MB (2004). Chemokines in the pathogenesis of vascular disease. *Circ Res* **95**: 858–866.

Coopman K, Smith LD, Wright KL, Ward SG (2007). Temporal variation in CB2R levels following T lymphocyte activation: evidence that cannabinoids modulate CXCL12-induced chemotaxis. *Int Immunopharmacol* **7**: 360–371.

Deusch E, Kraft B, Nahlik G, Weigl L, Hohenegger M, Kress HG (2003). No evidence for direct modulatory effects of delta 9-tetrahydrocannabinol on human polymorphonuclear leukocytes. *J Neuroimmunol* **141**: 99–103.

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

Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, Fahey KJ *et al.* (1998). LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. *J Pharmacol Exp Ther* **284**: 291–297.

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O *et al.* (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol Pharmacol* **48**: 443–450.

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

Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P *et al.* (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* **232**: 54–61.

Gaul CC, Mellors A (1975). Delta-9 -tetrahydrocannabinol and decreased macrophage migration inhibition activity. *Res Commun Chem Pathol Pharmacol* **10**: 559–564.

Gerrard JM, Clawson CC, White JG (1980). Lysophosphatidic acids: III. Enhancement of neutrophil chemotaxis. *Am J Pathol* **100**: 609–618.

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

Griffin G, Tao Q, Abood ME (2000). Cloning and pharmacological characterization of the rat CB(2) cannabinoid receptor. *J Pharmacol Exp Ther* **292**: 886–894.

Grimaldi C, Pisanti S, Laezza C, Malfitano AM, Santoro A, Vitale M *et al.* (2006). Anandamide inhibits adhesion and migration of breast cancer cells. *Exp Cell Res* **312**: 363–373.

Grimm MC, Ben-Baruch A, Taub DD, Howard OM, Resau JH, Wang JM *et al.* (1998). Opiates transdeactivate chemokine receptors: delta and mu opiate receptor-mediated heterologous desensitization. *J Exp Med* **188**: 317–325.

Hama K, Aoki J, Fukaya M, Kishi Y, Sakai T, Suzuki R *et al.* (2004). Lysophosphatidic acid and autotaxin stimulate cell motility of neoplastic and non-neoplastic cells through LPA1. *J Biol Chem* **279**: 17634–17639.

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE *et al.* (2001). 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci USA* **98**: 3662–3665.

Hanus L, Gopher A, Almog S, Mechoulam R (1993). Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J Med Chem* **36**: 3032–3034.

Hillard CJ, Manna S, Greenberg MJ, DiCamelli R, Ross RA, Stevenson LA *et al.* (1999). Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). *J Pharmacol Exp Ther* **289**: 1427–1433.

Honda S, Sasaki Y, Ohsawa K, Imai Y, Nakamura Y, Inoue K *et al.* (2001). Extracellular ATP or ADP induce chemotaxis of cultured microglia through Gi/o-coupled P2Y receptors. *J Neurosci* **21**: 1975–1982.

Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET *et al.* (2001). Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch Gen Psychiatry* **58**: 322–328.

Huffman JW, Liddle J, Yu S, Aung MM, Abood ME, Wiley JL *et al.* (1999). 3-(1′,1′-Dimethylbutyl)-1-deoxy-delta8-THC and related compounds: synthesis of selective ligands for the CB2 receptor. *Bioorg Med Chem* **7**: 2905–2914.

Idzko M, Laut M, Panther E, Sorichter S, Durk T, Fluhr JW *et al.* (2004). Lysophosphatidic acid induces chemotaxis, oxygen radical production, CD11b up-regulation, Ca2+ mobilization, and actin reorganization in human eosinophils via pertussis toxin-sensitive G proteins. *J Immunol* **172**: 4480–4485.

Iwamura H, Suzuki H, Ueda Y, Kaya T, Inaba T (2001). *In vitro* and *in vivo* pharmacological characterization of JTE-907, a novel selective ligand for cannabinoid CB2 receptor. *J Pharmacol Exp Ther* **296**: 420–425.

Jaggar SI, Hasnie FS, Sellaturay S, Rice AS (1998). The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. *Pain* **76**: 189–199.

Jin Y, Knudsen E, Wang L, Maghazachi AA (2003). Lysophosphatidic acid induces human natural killer cell chemotaxis and intracellular calcium mobilization. *Eur J Immunol* **33**: 2083–2089.

Jorda MA, Verbakel SE, Valk PJ, Vankan-Berkhoudt YV, Maccarrone M, Finazzi-Agro A *et al.* (2002). Hematopoietic cells expressing the peripheral cannabinoid receptor migrate in response to the endocannabinoid 2-arachidonoylglycerol. *Blood* **99**: 2786–2793.

Joseph J, Niggemann B, Zaenker KS, Entschladen F (2004). Anandamide is an endogenous inhibitor for the migration of tumor cells and T lymphocytes. *Cancer Immunol Immunother* **53**: 723–728.

Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M (2005). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Mol Pharmacol* **67**: 1697–1704.

Khanolkar AD, Abadji V, Lin S, Hill WA, Taha G, Abouzid K *et al.* (1996). Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. *J Med Chem* **39**: 4515–4519.

Kishimoto S, Gokoh M, Oka S, Muramatsu M, Kajiwara T, Waku K *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. *J Biol Chem* **278**: 24469–24475.

Kishimoto S, Muramatsu M, Gokoh M, Oka S, Waku K, Sugiura T (2005). Endogenous cannabinoid receptor ligand induces the migration of human natural killer cells. *J Biochem (Tokyo)* **137**: 217–223.

Kurihara R, Tohyama Y, Matsusaka S, Naruse H, Kinoshita E, Tsujioka T *et al.* (2006). Effects of peripheral cannabinoid receptor ligands

on motility and polarization in neutrophil-like HL60 cells and human neutrophils. *J Biol Chem* **281**: 12908–12918.
Lan R, Liu Q, Fan P, Lin S, Fernando SR, McCallion D *et al.* (1999). Structure-activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. *J Med Chem* **42**: 769–776.
Lin S, Khanolkar AD, Fan P, Goutopoulos A, Qin C, Papahadjis D *et al.* (1998). Novel analogues of arachidonylethanolamide (anandamide): affinities for the CB1 and CB2 cannabinoid receptors and metabolic stability. *J Med Chem* **41**: 5353–5361.
Lunn CA, Fine JS, Rojas-Triana A, Jackson JV, Fan X, Kung TT *et al.* (2006). A novel cannabinoid peripheral cannabinoid receptor-selective inverse agonist blocks leukocyte recruitment *in vivo*. *J Pharmacol Exp Ther* **316**: 780–788.
Mackie K, Stella N (2006). Cannabinoid receptors and endocannabinoids: evidence for new players. *AAPS J* **8**: E298–E306.
Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR *et al.* (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**: 83–90.
Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**: 61–65.
Murphy PM, Baggiolini M, Charo IF, Hebert CA, Horuk R, Matsushima K *et al.* (2000). International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev* **52**: 145–176.
Nilsson O, Fowler CJ, Jacobsson SO (2006). The cannabinoid agonist WIN 55212-2 inhibits TNF-alpha-induced neutrophil transmigration across ECV304 cells. *Eur J Pharmacol* **547**: 165–173.
Offertaler L, Mo FM, Batkai S, Liu J, Begg M, Razdan RK *et al.* (2003). Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. *Mol Pharmacol* **63**: 699–705.
Oka S, Ikeda S, Kishimoto S, Gokoh M, Yanagimoto S, Waku K *et al.* (2004). 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, induces the migration of EoL-1 human eosinophilic leukemia cells and human peripheral blood eosinophils. *J Leukoc Biol* **76**: 1002–1009.
Oka S, Wakui J, Gokoh M, Kishimoto S, Sugiura T (2006). Suppression by WIN55212-2, a cannabinoid receptor agonist, of inflammatory reactions in mouse ear: interference with the actions of an endogenous ligand, 2-arachidonoylglycerol. *Eur J Pharmacol* **538**: 154–162.
Pertwee RG (2005). Pharmacological actions of cannabinoids. *Handb Exp Pharmacol* 1–51.
Piomelli D (2005). The challenge of brain lipidomics. *Prostaglandins Other Lipid Mediat* **77**: 23–34.
Rayman N, Lam KH, Laman JD, Simons PJ, Lowenberg B, Sonneveld P *et al.* (2004). Distinct expression profiles of the peripheral cannabinoid receptor in lymphoid tissues depending on receptor activation status. *J Immunol* **172**: 2111–2117.
Rhee MH, Vogel Z, Barg J, Bayewitch M, Levy R, Hanus L *et al.* (1997). Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. *J Med Chem* **40**: 3228–3233.
Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G *et al.* (2003). Cell migration: integrating signals from front to back. *Science* **302**: 1704–1709.
Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C *et al.* (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* **350**: 240–244.
Rinaldi-Carmona M, Barth F, Millan J, Derocq JM, Casellas P, Congy C *et al.* (1998). SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther* **284**: 644–650.
Rios C, Gomes I, Devi LA (2006). mu opioid and CB1 cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. *Br J Pharmacol* **148**: 387–395.
Ross RA, Brockie HC, Stevenson LA, Murphy VL, Templeton F, Makriyannis A *et al.* (1999). Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. *Br J Pharmacol* **126**: 665–672.
Rovati GE, Capra V, Neubig RR (2007). The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. *Mol Pharmacol* **71**: 959–964.
Ruiu S, Pinna GA, Marchese G, Mussinu JM, Saba P, Tambaro S *et al.* (2003). Synthesis and characterization of NESS 0327: a novel putative antagonist of the CB1 cannabinoid receptor. *J Pharmacol Exp Ther* **306**: 363–370.
Sacerdote P, Martucci C, Vaccani A, Bariselli F, Panerai AE, Colombo A *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*. *J Neuroimmunol* **159**: 97–105.
Sacerdote P, Massi P, Panerai AE, Parolaro D (2000). *In vivo* and *in vitro* treatment with the synthetic cannabinoid CP55, 940 decreases the *in vitro* migration of macrophages in the rat: involvement of both CB1 and CB2 receptors. *J Neuroimmunol* **109**: 155–163.
Schwartzfarb L, Needle M, Chavez-Chase M (1974). Dose-related inhibition of leukocyte migration by marihuana and delta-9-tetrahydrocannabinol (THC) *in vitro*. *J Clin Pharmacol* **14**: 35–41.
Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessegue B, Bonnin-Cabanne O *et al.* (1996). Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. *Biochim Biophys Acta* **1307**: 132–136.
Shoemaker JL, Ruckle MB, Mayeux PR, Prather PL (2005). Agonist-directed trafficking of response by endocannabinoids acting at CB2 receptors. *J Pharmacol Exp Ther* **315**: 828–838.
Showalter VM, Compton DR, Martin BR, Abood ME (1996). Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther* **278**: 989–999.
Song ZH, Zhong M (2000). CB1 cannabinoid receptor-mediated cell migration. *J Pharmacol Exp Ther* **294**: 204–209.
Steffens S, Veillard NR, Arnaud C, Pelli G, Burger F, Staub C *et al.* (2005). Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* **434**: 782–786.
Stella N (2004). Cannabinoid signaling in glial cells. *Glia* **48**: 267–277.
Straub RH, Mayer M, Kreutz M, Leeb S, Scholmerich J, Falk W (2000). Neurotransmitters of the sympathetic nerve terminal are powerful chemoattractants for monocytes. *J Leukoc Biol* **67**: 553–558.
Van Haastert PJ, Devreotes PN (2004). Chemotaxis: signalling the way forward. *Nat Rev Mol Cell Biol* **5**: 626–634.
Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al.* (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**: 329–332.
Walter L, Franklin A, Witting A, Wade C, Xie Y, Kunos G *et al.* (2003). Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J Neurosci* **23**: 1398–1405.
Yiangou Y, Facer P, Durrenberger P, Chessell IP, Naylor A, Bountra C *et al.* (2006). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol* **6**: 12.