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Review

Regulation of inflammation by cannabinoids, the endocannabinoids 2-arachidonoyl-glycerol and arachidonoyl-ethanolamide, and their metabolites

Caroline Turcotte, François Chouinard, Julie S. Lefebvre, and Nicolas Flamand[1]

Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec (IUCPQ), Département de Médecine, Faculté de Médecine, Université Laval, Québec City, QC, Canada

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ABSTRACT

2-Arachidonoyl-glycerol (2-AG) and arachidonyl-ethanolamide (AEA) are endocannabinoids that have been implicated in many physiologic disorders, including obesity, metabolic syndromes, hepatic diseases, pain, neurologic disorders, and inflammation. Their immunomodulatory effects are numerous and are not always mediated by cannabinoid receptors, reflecting the presence of an arachidonic acid (AA) molecule in their structure, the latter being the precursor of numerous bioactive lipids that are pro- or anti-inflammatory. 2-AG and AEA can thus serve as a source of AA but can also be metabolized by most eicosanoid biosynthetic enzymes, yielding additional lipids. In this regard, enhancing endocannabinoid levels by using endocannabinoid hydrolysis inhibitors is likely to augment the levels of these lipids that could regulate inflammatory cell functions. This review summarizes the metabolic pathways involved in the biosynthesis and metabolism of AEA and 2-AG, as well as the biologic effects of the 2-AG and AEA lipidomes in the regulation of inflammation.
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Introduction

The use of cannabis for recreational purposes is widespread among the population. Indeed, ingesting the plant or its extracts has considerable psychotropic effects, as documented by Baudelaire in the beginning of the 19th century [1]. In addition, cannabis has been used as a medicine for millennia [2]. With the technological advancement over the past decades, key cellular and molecular mechanisms by which cannabinoids and their endogenous counterparts exert their effects have been identified. In this regard, the cannabinoid system has been linked with several disorders—notably, obesity, metabolic syndrome, pain, and multiple sclerosis [3–5].

The structural elucidation of the main psychoactive substance found in *Cannabis sativa* in 1964, THC, and the chemical synthesis of the THC analog CP 55940 in the mid 1980s, paved the way for cannabinoid research [6–9]. These compounds were key chemical probes for the pharmacological characterization of a rat receptor localized in the brain and its cloning from rats and humans. This brain receptor, referred to as CB_1, is primarily found in the brain and testes [10–12]. Another receptor, CB_2, was rapidly cloned from the human promyelocytic cell line HL-60 [13]. In sharp contrast to CB_1, CB_2 is mainly expressed in the periphery in lymphoid tissues and by myeloid cells [10–13]. Both the CB_1 and the CB_2 receptors belong to the GPCR superfamily, and their activation triggers $G\alpha_{i/o}$ signaling events, such as adenylyl cyclase inhibition and MAPK activation (reviewed in ref. 14). GPR55 is another GPCR that has been shown to bind THC and CP 55940 [15]. However, subsequent studies have indicated that GPR55 responds better to lysophosphatidylinositol than to classic cannabinoid receptor agonists [16, 17].

After the cloning of the CB_1 and CB_2 receptors in 1990 and 1993, respectively, their endogenous ligands, the endocannabinoids, were rapidly identified. Using porcine brain extracts and the radiolabeled synthetic cannabinoid canbisol (HU-243), Devane and colleagues [18] defined AEA (**Fig. 1**), as a high-affinity CB_1 receptor agonist (K_i = 39 ± 5 nM). They initially termed AEA anandamide, which is derived from the Sanskrit term ananda (meaning bliss and referring to its psychoactive effects) and the amide nature of the molecule. The cloning of the CB_2 receptor by Munro and colleagues [13] further established AEA as a potent endocannabinoid and led to the identification of other endogenous ligands. In this respect, 2-AG (**Fig. 2**) was subsequently identified by Mechoulam and colleagues [19] in 1995. They isolated 2-AG from the canine gut,

Abbreviations: 2-AG = 2-arachidonoyl-glycerol, 5,8,11-ETI = 5,8,11-eicosatrienoic acid, AA = arachidonic acid, ABHD = α/β hydrolase domain, AEA = arachidonyl-ethanolamide, AM251 = *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole 3-carboxamide, AM281 = 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide, AM630 = 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-

(*continued on next page*)

1. Correspondence: Centre de Recherche de l'IUCPQ, 2725 Chemin Sainte-Foy, Québec City, QC G1V 4G5, Canada. E-mail: nicolas.flamand@criucpq.ulaval.ca

characterized its binding properties to CB receptors using CB_1- and CB_2-transfected COS-7 cells, and demonstrated that intravenously injected AEA or 2-AG mimics the effect of THC (decreased nociception, spontaneous activity, and rectal temperature) in mice [19]. In parallel, Sugiura and colleagues [20] isolated 2-AG from rat brain extracts and showed that it competes against CP 55940 in binding assays of rat synaptosomal membranes. Of note, the identification of 2-AG as a CB_1 and CB_2 ligand took place more than 10 years after its biosynthesis was observed by Prescott and Majeurus [21] in thrombin-stimulated platelets.

Several other endocannabinoids, such as noladin ether [22], *O*-arachidonyl-ethanolamine [23], and *N*-arachidonyl-dopamine [24], also activate the CB_1 and CB_2 receptors. To date, numerous endogenous molecules have been documented as CB receptor ligands. Their biosynthesis and biologic effects have recently been [25–27] reviewed and will not be documented in this article.

ENDOCANNABINOID BIOSYNTHESIS

A limited number of studies have documented endocannabinoid biosynthesis by inflammatory cells. Usually, AEA and 2-AG biosynthesis can be observed after cell stress or stimulation with either GPCR agonists or Ca^{2+} ionophores (**Table 1**).

(*continued from previous page*)

indol-3-yl](4-methoxyphenyl)-methanone, baicalein = 5,6,7-trihydroxyflavone, COX = cyclooxygenase, CP 55940 = (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol, DAG = diacyl-glycerol, DFU = [5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-*2*(5*H*)-furanone], EET = epoxyeicosatrienoyl, EP = prostaglandin E2, FAAH = fatty acid amid hydrolase, GPCR = G-protein-coupled receptor, GW627368X = 4-(4,9-diethoxy-1,3-dihydro-1-oxo-2*H*-benz[f]isoindol-2-yl)-*N*-(phenylsulfonyl)-benzeneacetamide, GW6471 = [(2*S*)-2-[[(1*Z*)-1-methyl-3-oxo-3-[4-(trifluoromethyl)phenyl]-1-propenyl]amino]-3-[4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy]phenyl]propyl]-carbamic acid ethyl ester, GW9662 = 2-chloro-5-nitro-*N*-phenylbenzamide, HpETE = hydroperoxyeicosatetraenoyl, HU-201 = (6a*R*)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol, HU-243 = [(3*H*)HU 243,3-dimethylheptyl-11-hydroxyhexahydrocannabinol, IL = interleukin, JWH-015 = (2-methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone, JWH-133 = (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran, LOX = lipoxygenase, LTB_4 = leukotriene B_4, MAFP = (5*Z*,8*Z*,11*Z*,14*Z*)-5,8,11,14-eicosatetraenyl-methyl ester phosphonofluoridic acid, MAGL = monoacylglycerol lipase, NAPE = *N*-acyl-phosphatidyl-ethanolamine, NS398 = *N*-[2-cyclohexyloxy-4-nitrophenyl]methanesulfonamide, OMDM1-2 = (9*Z*)-*N*-[1-((*R*)-4-hydroxbenzyl)-2-hydroxyethyl]-9-octadecenamide, OTFP = 3-octylthio-1,1,1-trifluoropropan-2-one, PF3845 = *N*-3-pyridinyl-4-[[3-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenyl]methyl]-1-piperidine carboxamide, PLA_2 = phospholipase A_2, PLC = phospholipase C, PLD = phospholipase D, PPAR, proliferating peroxisome activating receptor, SC-19920 = 8-chloro-dibenz[*b*,*f*][1,4]oxazepine-10(11*H*)-carboxy-(2-acetyl)hydrazide, SR144528 = 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1*S*, 2*S*,4*R*)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1*H*-pyrazole-3- carboxamide, SR144716 = 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-1-piperidinyl-1*H*-pyrazole-3-carboxamide, T0070907 = 2-chloro-5-nitro-*N*-4-pyridinyl-benzamide, THC = (−)-Δ9-tetrahydrocannabinol, TX = thromboxane, UCM707 = (5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(3-furanylmethyl)-5,8,11,14-eicosatetraenamide, URB597 = (3′-(aminocarbonyl)[1,1′-biphenyl]-3-yl)-cyclohexylcarbamate, URB602 = [1,1′-biphenyl]-3-yl-carbamic acid, cyclohexyl ester, VDM11 = (5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(4-hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide, WIN55-212 = (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone

AEA biosynthesis

Although AEA can be obtained from the reverse reaction of fatty acid amide hydrolase (FAAH) [43], its levels are increased in FAAH-deficient mice, indicating that FAAH is mostly involved in its hydrolysis and that other enzymes are responsible for AEA biosynthesis [44]. In fact, the main precursor of AEA is *N*-acyl-phosphatidyl-ethanolamine (NAPE), which is obtained from the acylation of the ethanolamine portion of phosphatidyl-ethanolamines by Ca^{2+}-dependent and -independent *N*-acyl-transferases [45, 46]. Four pathways have been described for the metabolism of NAPE into AEA (Fig. 1): *1*) a 1-step hydrolysis of NAPE by at least 2 distinct phospholipases type D (PLD) that are specific for NAPE [47–50]; *2*) a 2-step process involving the hydrolysis of NAPE by a PLC, generating a phospho-AEA intermediate that is dephosphorylated by phosphatases such as PTPN22 [51]; *3*) the generation of lyso-NAPE by PLA_2 activity followed by its hydrolysis by a lyso-PLD [52]; and *4*) the generation of a lyso-NAPE by PLA_2 activity and its hydrolysis into glycerophospho-*N*-arachidonyl-ethanolamine by the serine esterase ABHD4, followed by the activation of glycerophosphodiesterase 1 [53].

2-AG biosynthesis

Several pathways have been documented for the biosynthesis of 2-AG, and the generation of 1,2-diacylglycerol (DAG) is common to most of them. DAG can be produced by the hydrolysis of glyceryl-phosphatidylinositol by PLCs [21, 54] or by the combined action of PLD and phosphatidic acid phosphatase on glyceryl-phosphatidylcholine [55]. 2-AG is then obtained when AA-containing DAG species are hydrolyzed by DAG lipase-α or -β [56, 57]. 2-AG can also be obtained from the dephosphorylation of arachidonoyl-lysophosphatidic acid by a lysophosphatidic acid phosphatase [58] (Fig. 2).

ENDOCANNABINOID METABOLISM

Endocannabinoids released into the extracellular space are rapidly cleared by cellular uptake, hydrolysis, or both. Four distinct mechanisms by which endocannabinoids are transported from the extracellular space into the cytoplasm have been documented: *1*) facilitated transport by a carrier protein; *2*) gradient-driven passive diffusion coupled with intracellular metabolism by enzymes such as monoacylglycerol (MAG) lipase and FAAH; *3*) gradient-driven passive diffusion coupled with endocannabinoid sequestration by fatty acid-binding proteins; and *4*) caveolae-mediated transport. These mechanisms and recent data regarding endocannabinoid clearance from the extracellular space have been reviewed by Yates and Barker [59]. Following their uptake, 2-AG and AEA can be hydrolyzed by serine esterases into AA and glycerol/ethanolamine, or metabolized by a variety of enzymes involved in eicosanoid metabolism, including LOX, COX, and epoxygenases.

Hydrolysis of AEA

The membrane-associated FAAH-1 and -2 are the main enzymes responsible for the hydrolysis of AEA into AA and ethanolamine. Whereas FAAH-1 is expressed in rodents and humans, FAAH-2 is

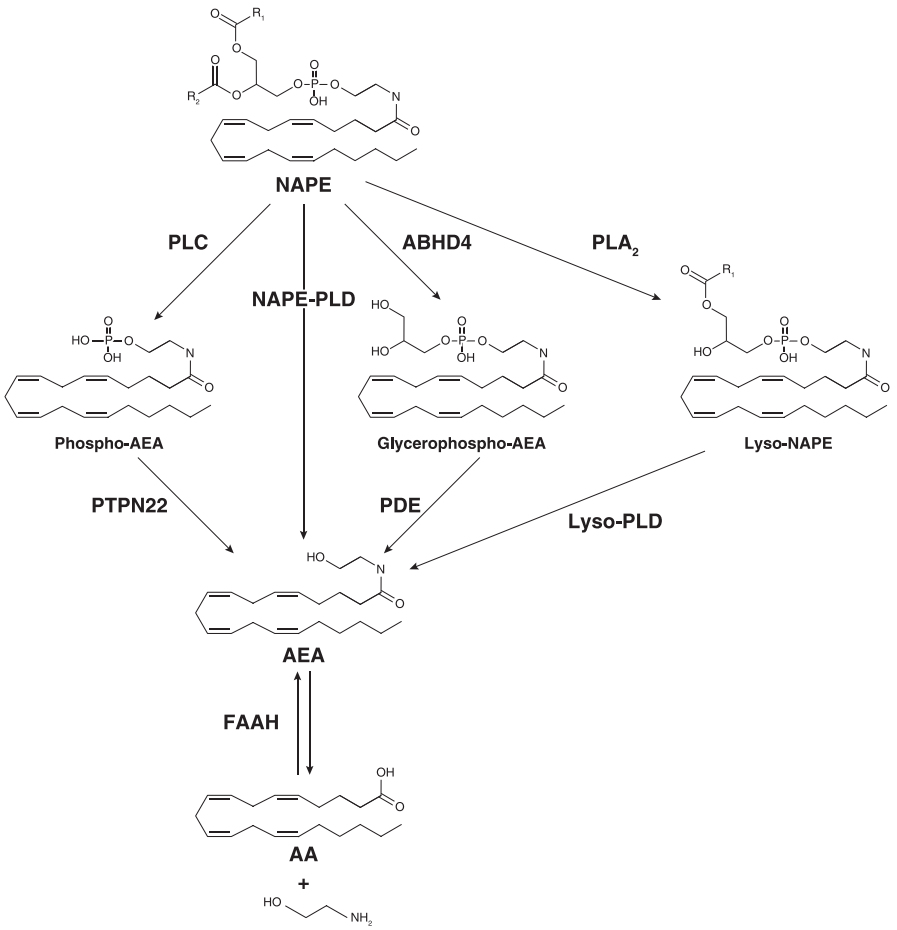

Figure 1. Biosynthetic pathways of AEA. AEA can be obtained by conjugation of ethanolamine and AA from the reverse reaction of FAAH. Otherwise, it is obtained from its precursor NAPE, which is produced from acylation of phosphatidyl-ethanolamines by *N*-acyl-transferases. NAPE can be metabolized into AEA by 4 pathways: *1*) hydrolysis of NAPE by NAPE-PLD; *2*) hydrolysis of NAPE by a PLC to generate a phospho-AEA, which is dephosphorylated by the phosphatase PTPN22; *3*) cleavage of NAPE by PLA_2 and the hydrolysis of the lyso-NAPE by a lyso-PLD; and *4*) the generation of a lyso-NAPE by PLA_2 activity and its hydrolysis by the serine esterase ABHD4, followed by a phosphodiesterase activity (glycerophosphodiesterase-1).

expressed in humans, but not in rodents. The tissue distribution profiles of FAAH-1 and -2 are slightly different: FAAH-1 is mainly found in the brain, kidney, liver, lung, prostate, testis, and small intestine; FAAH-2 is found mostly in the heart, kidney, liver, lung, prostate, and ovary. Notably, very little FAAH-1 or -2 has been detected by RT-PCR amplification of cDNA obtained from leukocytes [60–62]. The pharmacological or genomic deletion of FAAH-1 increases basal levels of AEA and abrogates the hydrolysis of exogenously added AEA [44, 63–65], emphasizing the key role of FAAH-1 and perhaps of FAAH-2 in AEA hydrolysis and establishing FAAH as a promising avenue for treating pain, anxiety, and possibly inflammatory disease. FAAH inhibition reduces nociception, anxiety, and depression, whereas other effects associated with cannabinoids, such as hypothermia and decreased coordination, are not observed [44, 66].

Hydrolysis of 2-AG

With the help of a biotinylated fluorophosphonate derivative, which covalently binds to and inactivates serine hydrolases, Blankman and colleagues [67] unraveled several enzymes capable of hydrolyzing 2-AG into AA and glycerol in the mouse brain. They found that, although MAG lipase accounts for 85% of 2-AG hydrolase activity, other enzymes such as α/β-hydrolase domain (ABHD)-6, ABHD12, and FAAH-1 are responsible for the remaining 15%. ABHD6 plays a key role in 2-AG hydrolysis (and CB_2 receptor activation) in the microglial cell line BV-2, which does not express MAG lipase. Moreover, the dual inhibition of FAAH and ABHD6 significantly increases 2-AG and AEA levels in neurons [68–70]. The importance of each 2-AG-hydrolyzing enzyme has yet to be determined in humans and may be different from that described in rodents. In that regard, other enzymes, such as carboxylesterases 1 and 2, are involved in 2-AG hydrolysis in cultured human THP-1 cells [71], also suggesting that the profiles of enzymes that hydrolyze 2-AG in the periphery and in the brain are different.

The effects of a pharmacological or a genetic disruption of MAG lipase underscores its crucial role in 2-AG hydrolysis in mice. Indeed, the administration of the MAG lipase inhibitor JZL184 results in a 10-fold increase in 2-AG levels while decreasing the levels of unesterified AA in the brain by ~50%, similar to that observed in MAG lipase-deficient animals [72, 73]. As opposed to FAAH disruption, the chronic inhibition of MAG lipase with JZL184 induces a loss of analgesic effects, development of physical dependence, impaired endocannabinoid-dependent synaptic plasticity, and CB_1 desensitization in the brain [73, 74].

OXYGENATION OF AEA AND 2-AG

The arachidonoyl moiety of AEA and 2-AG makes them susceptible to metabolism by eicosanoid biosynthetic enzymes,

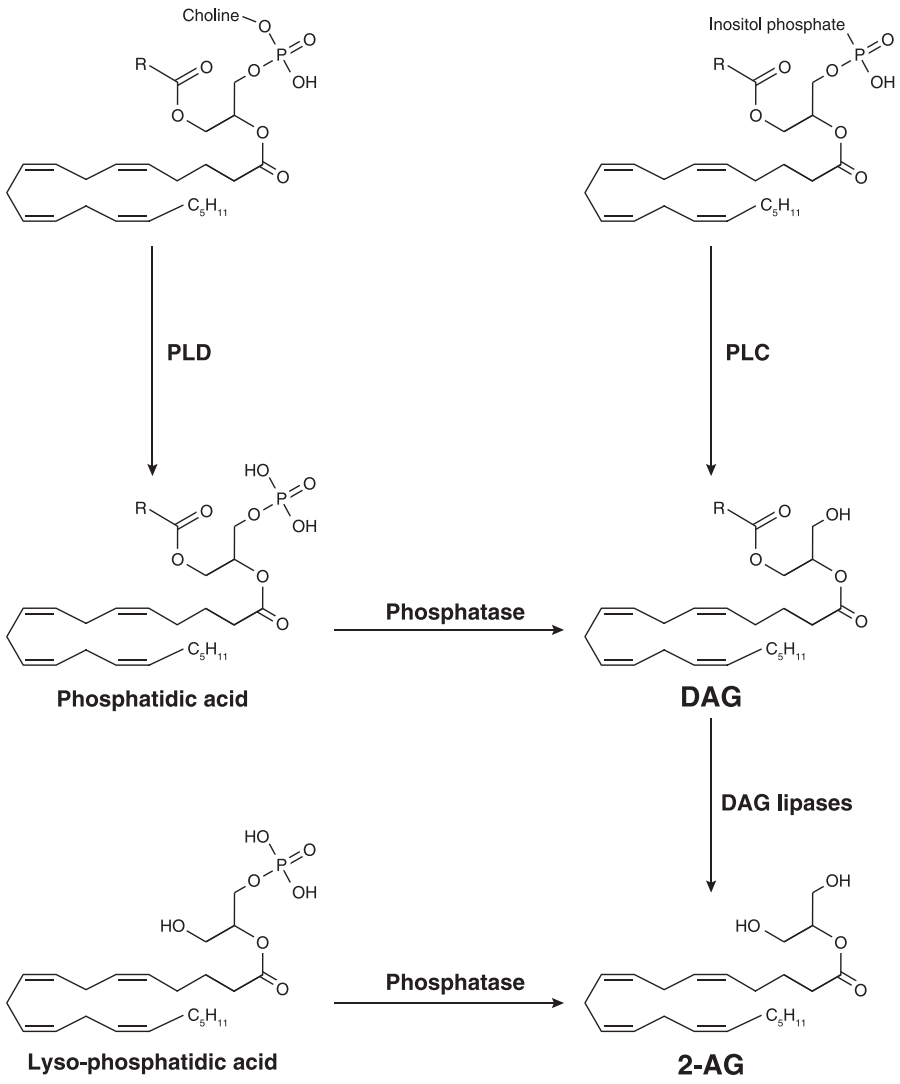

Figure 2. Biosynthetic pathways of 2-AG. 2-AG is obtained from DAG, which can be produced by the hydrolysis of glyceryl-phosphatidyl-inositol by PLC or by the combined action of PLD and phosphatidic acid phosphatase on glyceryl-phosphatidyl-choline. DAG-containing AA is then hydrolyzed by DAG lipase-α or -β to generate 2-AG. The latter can also be obtained from the dephosphorylation of arachidonoyl-lysophosphatidic acid by a lyso-phosphatidic acid phosphatase.

notably through the COX, LOX, and epoxygenase pathways. These are often seen as endocannabinoid inactivation pathways, considering that most of the obtained metabolites have limited or no affinity toward cannabinoid receptors. However, given the importance of eicosanoids in inflammation, endocannabinoid oxygenation should be seen as a unique process leading to the production of numerous bioactive lipids that will modulate the inflammatory response.

Cyclooxygenation

AEA was initially described as a good substrate for COX-2 by Yu and colleagues in 1997 [75]. They documented that AEA is metabolized into PGH_2-EA by human recombinant COX-2, but not COX-1, and into PGE_2-EA by a human foreskin fibroblast cell line that expresses COX-2. Of note, AEA metabolism by COX-2 is almost as efficient as that of AA. The biosynthesis of PGE_2-EA was later confirmed in RAW 264.7 cells [76]. Additional studies further demonstrated that COX-2 metabolism of AEA leads to the AEA-derived prostaglandins PGG_2-EA, PGH_2-EA, PGD_2-EA, PGE_2-EA, $PGF_{2\alpha}$-EA, and PGI_2-EA [77–79]. The first evidence that PG-EAs play important roles in the regulation of multiple pathways in vivo came from 2 studies: one showed that the pharmacological profile of $PGF_2\alpha$-EA is identical to that of the $PGF_2\alpha$ analog bimatoprost, currently used for the treatment of glaucoma [80], and the other demonstrated that PGD_2-EA, PGE_2-EA, and $PGF_2\alpha$-EA are produced in vivo in FAAH-deficient mice [81]. PG-EAs are poor activators of the CB_1 and CB_2 receptors [82] and are less potent in activating the PG receptors than PGs are [80, 83]. Although PG-EA can directly activate PPAR-γ [84] and prostaglandin E_2 (EP) receptors [85] (**Fig. 3**), several studies have highlighted the existence of a specific PG-EA receptor (reviewed in ref. 86).

2-AG can also be oxygenated by COX-2 as efficiently as by AA but, similar to AEA, the oxygenation of 2-AG by COX-1 is ineffective [87]. 2-AG can be metabolized into PGG_2-glycerol (G), PGH_2-, PGD_2-, PGE_2-, $PGF_{2\alpha}$-, PGI_2-, and HHT-G. Although thromboxane (TX) B_2-G could be detected, the conversion of 2-AG into TXA_2-G by TX synthase is minimal, compared to AA and PGH_2 [77]. Notably, PGE_2-G activates RAW 264.7 cells at picomolar concentrations, with a pharmacological profile distinct

TABLE 1. 2-AG and AEA biosynthesis by inflammatory cells

Cell type	Origin (cell line)	Endocannabinoid	References
Astrocytes	Mouse	AEA	[28]
		2-AG	[29]
Dendritic cells	Human (monocyte- derived)	2-AG	[30]
Lymphocytes	Human	AEA	[31]
Mast cells	Rat (RBL-2H3)	AEA	[32]
Microglia	Mouse	2-AG	[33]
	Rat (RTMGL1)	2-AG, AEA	[34]
Macrophages	Mouse (peritoneal)	2-AG	[35, 36]
	Mouse (J774)	AEA	[37, 38]
		2-AG	[38]
	Mouse (RAW 264.7)	AEA	[38, 39, 40, 41]
	Mouse (P388D1)	2-AG	[41, 42]
	Rat	2-AG, AEA	[38]
Platelets	Human	2-AG	[21, 42]
	Rat	2-AG	[39]

from that of PGE_2, with which it shares affinity for the EP_1 and EP_3 receptors [88]. This effect indicates that PG-G potently activates cells by interacting with PG-G-specific receptors .

Lipoxygenation

Soon after the identification of AEA and 2-AG as endocannabinoids, the metabolism of AEA by leukocyte types 12- and 15-LOX was documented [89, 90]. Three main LOX activities are observed in mammals. AA can be metabolized by 5-, 12-, and 15-LOX. Of note, AEA and 2-AG are good substrates for the leukocyte type 12- and 15-LOX enzymes, but are not oxidized by platelet type 12- or 5-LOX [89–92].

The oxidation of AEA by 12- and 15-LOX leads to the biosynthesis of 12(*S*)- and 15(*S*)-hydroperoxyeicosatetraenoyl-EA (12- and 15-HpETE-EA), respectively. They are then reduced to their hydroxyl derivative (e.g., 15-HETE-EA) [89, 90]. Even though 12-HETE-EA displays minimal binding to the CB_1 receptor [89, 93], these mediators seem to exert their biologic effects by activating vanilloid receptors [94–96]. 2-AG oxidation by 12- and 15-LOX results in the formation of 12- and 15-HETE-G [91, 92]. Very little is known regarding the biologic effects of 12- and 15-HETE-G and whether they activate cell surface receptors, but 15-HETE-G has been reported to be a PPARα agonist [92]. The biosynthesis of 12- and 15-HETE-EA has been observed in human platelets and granulocytes [93] incubated with AEA, whereas the biosynthesis of 12- and 15-HETE-G has been detected in human keratinocytes [91, 92].

Epoxygenation

2-AG and AEA are also substrates for P450 enzymes. The metabolism of AEA into 20-HETE-EA, 5,6-, 8,9-, 11,12- and

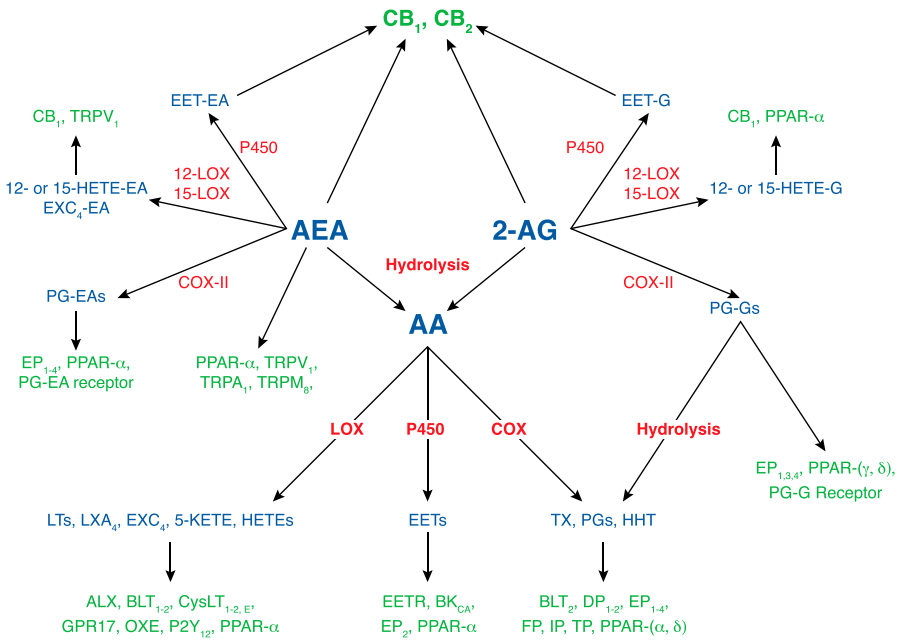

Figure 3. The complex metabolome of AEA and 2-AG is very likely involved in their ability to regulate inflammation. The endocannabinoids AEA and 2-AG can modulate inflammatory cell functions by activating the CB_1 and CB_2 receptors. However, their hydrolysis into AA or their metabolism by eicosanoid biosynthetic enzymes (red) results in a plethora of bioactive lipids that activate additional receptors (green). Thus, the final outcome of 2-AG and AEA as regulators of inflammation most likely results in the combination of effects involving cannabinoid, eicosanoid, and endocannabinoid metabolite receptors.

TABLE 2. Expression of CB receptors by human leukocytes

Leukocyte	Receptor	mRNA	Protein	References
Astrocytes	CB_1	+		[110]
	CB_2	−		[110]
Basophils	CB_1	+		[111]
	CB_2	+		[111]
B cells	CB_1	+		[112]
	CB_2	+	+ (FACS)	[102, 112, 113]
$CD4^+$	CB_1	−		[112]
	CB_2	+	+ (FACS)	[102, 112]
$CD8^+$	CB_1	−		[112]
	CB_2	+	+ (FACS)	[102, 112]
Dendritic cells	CB_1	+	+ (WB)	[30]
	CB_2	+	+ (WB)	[30]
Eosinophils	CB_1	+		[111]
	CB_2	+	+ (WB)	[104–106]
Mast cells	CB_1	+	+ (FACS), + (IHC)	114–116
	CB_2	+	+ (FACS)	[114]
Macrophages	CB_1	+	+ (FACS)	[117]
(monocyte-derived)	CB_2	+	+ (FACS)	[118]
Microglia	CB_1	−		[119]
	CB_2	+	+ (IHC)	[119]
Monocytes	CB_1	+	+ (IHC)	[102, 117]
	CB_2	+	+ (FACS)	[102, 113, 120]
Neutrophils	CB_1	+		[102]
	CB_2	+, −	+ (FACS), − (WB)	[102, 104–107]
NK cells	CB_1	+		[102]
	CB_2	+	+ (FACS)	[102, 112]
Platelets	CB_1		+ (WB), + (CM, WB)	[121]
	CB_2		+ (WB), − (CM, WB)	[122]
T lymphocytes	CB_1	+		[113, 123]
	CB_2	+	+ (FACS)	

CM, Confocal microscopy, IHC, immunohistochemistry; WB, Western blot; +, detected; −, not detected.

14,15-epoxyeicosatrienoic (EET)-EA has been observed in human liver, kidney, and brain [97, 98]. Similarly, 2-AG can be metabolized into 11,12-, and 14,15-EET-G [99], which promote the vasodilatation of rat mesenteric arteries [100]. Although no specific GPCR has been characterized for these P450-derived metabolites of AEA and 2-AG, they bind to CB_1 and CB_2 with the same affinity as AEA and 2-AG [99–101].

CANNABINOID RECEPTORS AND IMMUNE CELLS

After the cloning of CB_2 from HL-60 cells [13], numerous researchers investigated cannabinoid receptor expression by leukocytes and whether endocannabinoids and cannabinoids would modulate their functions. Leukocytes were initially shown to express the CB_2 receptor and, to a lesser extent, the CB_1 receptor [102, 103]. However, the level of expression of each CB receptor in immune cell subsets should be considered with caution, especially when only mRNA data are provided. Contamination by other cell types during isolation of a specific subset of leukocytes is frequent and difficult to avoid. Considering that mRNA can be overamplified very effectively by PCR, minor cell contamination can produce false positives. As an example, the removal of contaminating eosinophils in human neutrophil suspensions considerably diminishes the amplification of the CB_2 mRNA, thereby raising the question of whether neutrophils express that receptor [104–107]. Furthermore, initial results obtained with commercially available CB receptor antibodies can be misleading, given that they have shown many differences in expression patterns [108, 109]. **Table 2** summarizes most studies that have documented the presence or absence of CB receptors in human immune cells at the mRNA and protein levels.

ROLE OF THE CANNABINOID SYSTEM IN INFLAMMATION

Endocannabinoids levels fluctuate in many inflammatory conditions, notably brain injury [124], cerebral ischemia [125], hepatic ischemia-reperfusion injuries [126], Huntington disease [127], multiple sclerosis [128], rheumatoid

TABLE 3. Anti-inflammatory effects observed in CB receptor-deficient mice in vivo

Model	Intervention	Effects	References
TNBS-induced colitis	CB_1, CB_2 and $CB_{1/2}$ KO	↑ Colitis ↑ TNF-α and IL-1β	[141]
Sepsis caused by cecal ligation	CB_2 KO	↓ Sepsis-induced mortality ↓ IL-10, IL-6, and MIP-2	[142]
DNFB-induced contact hypersensitivity	CB_1, CB_2 and $CB_{1/2}$ KO	↑ Neutrophil recruitment ↑ Ear swelling	[133]
Hepatic IR injury	CB_2 KO	↑ Liver damage ↑ Neutrophil recruitment ↑ Inflammatory cytokines	[135]
Influenza infection	$CB_{1/2}$ KO	↑ APC function ↑ Pulmonary damage ↑ Inflammatory cell infiltration	[144]

DNBS, 2,4,4-trinitrobenzenesulphonic acid; DNFB, 2,4-dinitro-1-fluorobenzene; IR, ischemia reperfusion; KO, knockout; TNBS, 2,4,6-trinitrobenzene sulfonic acid

TABLE 4. Anti-inflammatory effects linked to enhanced AEA levels in vivo

Model	Species	Intervention	Effects	References
ConA-induced hepatitis	Mouse	AEA	↓ Liver damage ↓ Inflammatory cytokines	[134]
Collagen-induced arthritis	Mouse	FAAH KO FAAH-NS FAAH inhibitor URB597	↓ Severity of arthritis (pannus, inflammation, bone damage, cartilage damage)	[65]
Carrageenan-induced acute inflammation	Mouse	FAAH inhibitor URB937	↓ Paw edema ↓ Hyperalgesia ↓ Indomethacin-induced gastric lesions	[145]
Peripheral nerve injury	Mouse	FAAH inhibitor URB937	↓ Hyperalgesia and allodynia ↓ Indomethacin-induced gastric lesions	[145]
Antigen-induced arthritis	Mouse	FAAH inhibitor URB937	↓ Hyperalgesia	[145]
LPS-induced pulmonary inflammation	Mouse	AEA	↓ TNF-α ↓ Neutrophil recruitment	[137]
LPS-induced inflammatory pain	Mouse	FAAH KO	↓ Edema ↓ Hyperalgesia ↓ TNF-α and IL-1β	[140]
			↓ Allodynia	[146]
		FAAH inhibitors PF-3845, URB597 and OL-135	↓ Allodynia	[146]
DNBS-induced colon inflammation	Mouse	AEA reuptake inhibitor VDM11	↓ Colon inflammation ↓ MPO ↑ AEA	[132]
TMEV	Mouse	AEA reuptake inhibitor OMDM1-2	↓ Motor symptoms ↓ Microglial cell activation ↓ MHC class II	[143]
Osteoarthritis	Rat Guinea Pig	FAAH inhibitor URB597	↓ Nociception	[147]
Periodontitis	Rat	AEA	↓ TNF-α and IL-1β	[148]
Age-related long-term potentiation	Rat	FAAH inhibitor URB597	↓Age-related microglial activation ↓ Age-related increase in TNF-α and IL-1β	[149]
LPS-induced brain inflammation	Rat	FAAH inhibitor URB597	↓ TNF-α and IL-1β	[150]
Hypoxia-ischemia injury	Rat	AEA	↓ Apoptosis ↓ ROS production	[151]
Neuropathic pain (3 models)	Mouse Rat	FAAH inhibitor ST4070	↓ Nociception	[152]
Cyclophosphamide-induced cystitis	Mouse	FAAH KO	↓ NGF, COX-2, iNOS	[153]
Kaolin-carrageenan -induced osteoarthritis	Mouse	FAAH inhibitor URB597	↓ Leukocyte rolling and adhesion ↓ Inflammation-induced hyperemia	[154]
Traumatic brain injury	Mouse	FAAH inhibitor PF-3845	↓ COX-2 expression ↓ NOS-2 and ↑ Arg-1 in microglial cells ↓ Cortex lesion volume	[155]
mBSA-induced delayed-type hypersensitivity	Mouse	AEA	↓ Footpad swelling ↓ IL-17 and IFN-γ ↓ RORγT expression ↑ IL-10	[156]
TNBS-induced colitis	Mouse	FAAH inhibitor PF-3845	↓ Colon inflammation ↓ PGE_2	[157]
TMEV	Mouse	AEA	↓ IL-1β and IL-6 ↑ IL-10	[158]

FAAH-NS, FAAH expressed exclusively in nervous tissue; mBSA, methyl-bovine serum albumin; MPO, myeloperoxidase; NS, nervous system; TMEV, Theiler's encephalomyelitis virus; TNBS, 2,4,6-trinitrobenzene sulfonic acid

arthritis [129], atherosclerosis [130], sepsis [131], ulcerative colitis [132], contact dermatitis [133], and inflammatory pain [4]. In this regard, 3 main strategies have been used to define the immunomodulatory effects of endocannabinoids in vivo: *1*) increasing their levels by using 2-AG or AEA hydrolysis inhibitors; *2*) the administration of exogenous endocannabinoids or cannabinoids; and *3*) the genetic or pharmacological disruption of the CB receptors.

Anti-inflammatory effects of endocannabinoids and cannabinoids in vivo

Endocannabinoids have been shown to downregulate inflammation in numerous experimental models, such as experimental hepatitis [134], hepatic ischemia-reperfusion injury [135, 136], LPS-induced pulmonary inflammation [137], inflammatory pain [63, 138–140], trinitrobenzene sulfonic acid-induced colitis [132, 141], polymicrobial sepsis [142], contact hypersensitivity [133], and multiple sclerosis [143]. In these studies, activation of the cannabinoid system has been linked to decreased inflammatory cell recruitment and enhanced anti-inflammatory cytokine production. **Tables 3**, **4**, and **5** respectively summarize the anti-inflammatory effects in vivo of the CB receptors, AEA, and 2-AG, in vivo.

CB receptor agonists also downregulate the immune response in vivo [167–175], but can worsen *Legionella pneumophila* [176], herpes simplex virus-2 [177], and *Listeria monocytogenes* [178] infections and can increase tumor progression [179]. In these cases, the immunosuppressive effects induced by CB receptor activation on immune cells promote the proliferation of pathogens and cancer cells. High concentrations of CB receptor agonists can modulate inflammation independent of CB receptor activation. Peroxisome proliferator-activated receptors, which are known to modulate immune cell functions, are activated by high concentrations of the cannabinoid receptor agonists THC, WIN55212-2, CP 55940, and HU-210 [180-182]. **Table 6** summarizes the anti-inflammatory actions of the CB receptor agonists described to date.

Other cannabis constituents such as cannabidiol have anti-inflammatory properties, even though they do not activate CB receptors. Indeed, cannabidiol activates GPR55 [15] and inhibits the nucleoside transporter 1 [195]. The latter results in increased extracellular adenosine concentrations, which can putatively downregulate inflammatory cells by activating the adenosine A_{2A} receptor [196–198].

Anti-inflammatory effects of endocannabinoids and cannabinoids ex vivo

In agreement with the anti-inflammatory effects promoted by 2-AG and AEA in vivo, endocannabinoids can downregulate immune cell functions such as leukocyte migration [107, 199, 200], the production of reactive oxygen species [117, 201, 202], the release of proinflammatory cytokines [84, 117, 201, 203–205], and the increased release of the anti-inflammatory cytokine IL-10 [206]. However, some of the data are conflicting. For example, it has been published that AEA is a potent inhibitor of human

TABLE 5. Anti-inflammatory effects of enhanced 2-AG levels in vivo

Model	Species	Intervention	Effects	References
Acute experimental autoimmune encephalomyelitis	Mouse	2-AG	↑ Activation and ramification of microglia ↑ M2 macrophages	[159]
Acute inflammation	Mouse	MAGL inhibitor URB602	↓ Nociception ↓ Edema	[138]
Traumatic brain injury	Mouse	ABHD6 inhibitor WWL70	↓ Cortex lesion volume ↓ Neurodegeneration ↓ NOS-2 and COX-2	[160]
Formalin-induced inflammatory pain	Rat	MAGL inhibitor URB602	↓ Nociception	[139]
Hypoxia-ischemia injury	Rat	2-AG	↓ Apoptosis ↓ ROS production	[151]
LPS-induced expression of cytokines in frontal cortex and plasma	Rat	MAGL inhibitor JZL184	↓ TNF- α, IL-1β, IL-6, IL-10 ↓ AA levels	[161]
Alzheimer disease	Mouse	MAGL KO	↓ Gliosis and neuroinflammation ↓ TNF- α, IL-1β, IL-6, AA, PGE2, PGD2, TXB2 ↓ Amyloid plaques	[162]
Hepatic injury	Mouse	MAGL inhibitor JZL184 MAGL KO	↓ Cell death ↓ Neutrophil infiltration ↓ TNF-α and IL-1β	[163]
Carrageenan-induced paw edema	Mouse	MAGL inhibitor KML29	↓ Paw edema ↓ Mechanical allodynia	[164]
Sciatic nerve injury	Mouse	MAGL inhibitor KML29	↓ Mechanical and cold allodynia	[164]
Diclofenac-induced gastric hemorrhage	Mouse	MAGL inhibitor KML29	↓ Development of gastric hemorrhages	[164]
Chronic constriction injury	Mouse	JZL184 (MAGL) and diclofenac (COX)	↓ Mechanical and cold allodynia	[165]
LPS-induced lung injury	Mouse	MAGL inhibitor JZL184	↓ Leukocyte recruitment ↓ Adhesion molecule expression ↓ TNF-α, IL-6, MCP-1	[166]

JZL184, 4-nitrophenyl 4-(dibenzo[*d*][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate

TABLE 6. Anti-inflammatory effects of CB receptor agonists in vivo

Model	Species	Intervention	Effects	Receptor	References
TMEV	Mouse	WIN55-212	↓ ICAM-1 and VCAM-1 ↑ Motor coordination ↓ $CD4^+$ recruitment	PPARγ (GW9662)	[167]
Infection with *L. pneumophila*	Mouse	THC	↓ IFN-γ and IL-12 ↑ IL-4	CB_1 and CB_2 (KO)	[183]
Infection with influenza viruses	Mouse	THC	↓ Lymphocyte and monocyte recruitment ↑ Viral hemagglutinin	CB_1 and CB_2 (KO)	[184]
Experimental autoimmune uveoretinitis	Mouse	JWH-133	↓ Lymphocyte recruitment ↓ IFN-γ, TNF-α and IL-10	ND	[168]
Experimental autoimmune encephalomyelitis	Mouse	THC JWH-133	↓ Mononuclear cell recruitment ↓ IFN-γ and IL-2 ↓ T cell proliferation	CB_1 and CB_2 (KO)	[169]
Atherosclerosis $ApoE^{-/-}$ KO	Mouse	THC	↓ Atherosclerotic lesions ↓ Macrophage infiltration ↓ Leukocyte adhesion	CB_2 (SR144528)	[170]
		WIN55-212	↓ Atherosclerotic lesions ↓ Macrophage infiltration ↓ MCP-1, IL-6 and TNF-α	CB_2 (AM630)	[171]
Breast cancer cell injection	Mouse	THC	↓ Splenocyte proliferation ↑ Tumor mass ↑ Metastasis	CB_2 (SR144528)	[179]
Ovalbumin induced-asthma	Mouse	THC	↓ IL-2, IL-4 and, IgE	ND	[172]
Streptozotocin-induced diabetes	Mouse	THC	↓ Serum glucose ↑ Pancreatic insulin ↓ IFN-γ, TNF-α, IL-12	ND	[173]
Thioglycollate-induced peritoneal inflammation	Mouse	HU-210 WIN55-212	↓ Neutrophil recruitment ↓ MCP-1	CB_1 (SR141716)	[175]
Myocardial Ischemia-reperfusion injury	Mouse	WIN55-212	↓ MPO activity ↓ IL-1β and IL-8	CB_2 (AM630)	[174]
Allergen-induced airway inflammation	Mouse	THC	↓ Total cell count in bronchoalveolar lavage	ND	[185]
Ovalbumin-induced asthma	Guinea Pig	CP 55940	↓ MPO activity ↓ Mast cell degranulation ↓ TNF-α and PGD_2	CB_1 (AM-251) CB_2 (SR144528)	[186]
Amyloid -induced experimental Alzheimer's disease	Rat	WIN55-212	↓ Microglia cell activation ↑ Cognitive functions ↓ Toxic effects	CB_1 (SR141528)	[187]
CCI-induced allodynia	Rat	AM1241	↓ Allodynia	CB_2	[188]
Brain ischemia	Mouse	JWH-133	↓Microglia/macrophages ↓ Middle cerebral artery occlusion-induced gene expression of IL-6, TNF-α, MCP-1, MIP-1α, RANTES, iNOS	CB_2 (SR144528 and CB_2 KO)	[189]
Traumatic brain injury	Mouse	*O*-1966	↓Microglia/macrophage infiltration ↓ Blood-brain barrier disruption ↓ Neurodegeneration	CB_2	[190]
Periodontitis	Rat	Meth-AEA	↓ LPS-induced TNF-α and iNOS ↓ Alveolar bone loss	CB_1	[191]
Sepsis	Mouse	HU308	↓ Adherent leukocytes in submucosal venules	CB_2(AM630)	[192]
LPS-induced interstitial cystitis	Mouse	JWH015	↓ Leukocyte infiltration ↓ Myeloperoxidase activity ↓ IL-1α, IL-1β and TNF-α	CB_2	[193]
Stress-induced neuroinflammation	Mouse	JWH-133	↓ TNF-α and MCP-1 ↓ NF-κB, COX-2 and NOS-2 expression	CB_2	[194]

CCI, chronic constriction injury; ND, not determined

neutrophil migration [200], but some have reported it to be ineffective [104, 132, 207], and these discrepancies remain unexplained. Cannabinoid receptor activation by synthetic agonists mimics most of the anti-inflammatory effects of endocannabinoids, including inhibition of leukocyte migration [120, 199, 208–211] and of the release of proinflammatory mediators [143, 212–214]. Furthermore, some studies suggest that cannabinoid receptor activation induces a shift from the production of cell-mediated immunity (Th1) cytokines to humoral immunity (Th2) cytokines [143, 212, 213, 215]. **Tables 7** and **8** summarize the documented anti-inflammatory effects of endocannabinoids (2-AG and AEA) and cannabinoids on immune cells.

Proinflammatory effects of 2-AG, AEA, and cannabinoids in vivo

Although a large body of evidence supports endocannabinoids as anti-inflammatory mediators, several studies have reported a proinflammatory role of endocannabinoids in the development of inflammation in hypersensitivity [238, 239], nephropathy [240], cardiomyopathy [241], and experimental dermatitis [242, 243]. These proinflammatory effects of endocannabinoids have been associated with enhanced leukocyte recruitment, the production of reactive oxygen species and the release of proinflammatory cytokines (**Tables 9** and **10**). In agreement with the above-mentioned proinflammatory effects, endocannabinoids can also activate leukocyte functions and participate in the development of the inflammatory process. 2-AG participates in the recruitment of leukocytes [104, 106, 254, 259, 261, 264, 265] and the release of proinflammatory mediators. Endocannabinoids can also increase the release of proinflammatory cytokines and autacoids [130, 232, 257, 260, 266] and enhance phagocytosis, suggesting that they play a role in host defense [253, 265]. Of note, most of the proinflammatory effects attributed to endocannabinoids involve 2-AG, but not AEA (Table 10). In contrast to endocannabinoids, cannabinoids do not appear to stimulate leukocyte functions in experimental models of inflammation, and a limited number of studies have shown that cannabinoids

TABLE 7. Anti-inflammatory effects of 2-AG and AEA in vitro

Leukocyte	Origin	Intervention	Effects	Receptor	References
Astrocytes	Rat	AEA uptake inhibitor UCM707	↓ TNF-α, IL-1β and NO	CB_1 (SR141716) CB_2 (SR144528)	[201]
$CD4^+$	Human	AEA	↓ IFN-γ, TNF-α and IL-17	CB_2 (SR144528) (JWH-015)	[204]
$CD8^+$	Human	AEA	↓ IFN-γ and TNF-α	CB_2 (SR144528)	[204]
		AEA	↓ Migration induced by SDF-1	CB_2 (JWH-015)	[207]
Dendritic cells	Human	AEA	↓ R848-induced IL-6, IL-12 and IFN- α production	CB_2 (SR144528)	[216]
Macrophages	Mouse (RAW264.7)	AEA	↓ ROS, TNF-α and MCP-1	CB_1 (SR141716)	[117]
	Mouse (peritoneal)	2-AG	↓ TNF-α	ND	[217]
		AEA	↓ NO and IL-6	ND	[217]
	Mouse (peritoneal)	AEA	↓ Killing of TNF-sensitive cells	ND	[218]
Mast cells	Human	AEA	↓ Mast cell maturation and degranulation	CB_1 (KO)	[115]
Microglia	Mouse	AEA	↑ IL-10	CB_2 (SR144528)	[206]
	Mouse (microglia BV-2)	AEA	↓ NO	CB_2 (AM630)	[202]
		2-AG and AEA	↓ TNF-α release	ND	[219]
	Mouse	AEA	↓ IL-12p70 and IL-23 ↑ IL-10	CB_2 (SR144528)	[220]
Mononuclear cells	Human	AEA	↓ IL-8, TNF-α and IL-6	ND	[205]
Müller glia	Human	AEA and 2-AG	↓ Tat-induced retinal cell death	ND	[221]
			↓ TNF- α and IL-6 ↑ IL-10 and TGF-β		[222]
Neutrophils	Human	AEA	↓ Migration induced by fMLP and LTB_4	ND	[200]
		2-AG	↓ Migration induced by fMLP	CB_2 (SR144528)	[107]
Splenocytes	Human	2-AG and AEA	↓ IL-2	PPARγ	[203]
		AEA	↓ Plaque-forming cell assays	CB_2 (SR144528)	[223]
T cells	Human	AEA	↓ Cell proliferation	CB_2 (SR144528)	[204]
		AEA	↓ Cell proliferation ↑ Apoptosis	ND	[224]
		2-AG	↓ SDF-1-induced migration	CB_2 (JWH-015)	[199]
	Human (Jurkat)	2-AG	↓ IL-2	PPARγ	[225]
U87MG (malignant glioma)	Human	2-AG	↓ NF-κB activation ↓ Cell growth	CB_1 (AM281)	[226]
Platelets	Human	AEA	↓ Platelet aggregation	ND	[227]

ND, not determined; ROS, reactive oxygen species; SDF-1, stromal cell derived factor-1

TABLE 8. Anti-inflammatory effects of CB receptor agonists in vitro

Leukocyte	Origin	Intervention	Effects	Receptor	References
Astrocytes	Human	WIN55-212	↓ NO, TNF-α, IP-10, MCP-1 and RANTES	CB_1 (SR141716) CB_2 (SR144528)	[213]
$CD8^+$	Human	JWH-015	↓ Migration induced by SDF-1	CB_2 (AM630)	[208]
Dendritic cells	Mouse	THC	↑ NF-κB-dependant apoptosis	CB_1 (SR141716A) and CB_2 (SR144528)	[228]
Macrophages	Mouse (RAW264.7)	WIN55-212	↓ ROS	CB_2 (AM630)	[214]
	Mouse (RAW264.7)	WIN55-212	↓ NO production	CB_2 (SR144528)	[229]
	Mouse (peritoneal)	CP55, 940	↓ Migration induced by fMLP	CB_1 (SR141716)	[210]
		THC	↓ Migration induced by RANTES	CB_2 (SR144528)	[211]
			↓ Yeast phagocytosis	ND	[230]
		JWH-133	↓ IL-12p40 ↑ IL-10	CB_2 (SR144528)	[231]
	Mouse (J774)	THC	↓ NO, IL-6 and PGE_2	ND	[232]
	Mouse (Clone 63)	THC	↓ Activation of $CD4^+$	CB_2 (SR144528)	[233]
Mast cells	Rat (RBL-2H3)	WIN55-212 CP55, 940	↓ β-Hexosaminidase release	CB_2 (AM630)	[234]
Microglia	Mouse (microglia BV-2)	THC	↓ IL-1β, IFN-β and IL-6 ↓ Phosphorylation of STAT-1	ND	[212]
		CD-101	↓ LPS-induced production of NO, COX-2, TNF-α, IL-1β and IL-6	ND	[235]
Neutrophils	Human	JWH-133	↓ Migration induced by TNF-α	ND	[236]
	Mouse	JWH-133	↓ Migration induced by MIP-2α	CB_2 (KO)	[209]
Splenocytes	Mouse	JWH-015	↑ Apoptosis	ND	[237]
T cells	Human	JWH-133	↓ Migration induced by SDF-1	ND	[199]
		THC	↓ Cytokine proliferation switch to Th2	CB_2 (SR144528)	[215]
	Leukemia (Jurkat)	CP55, 940 WIN55-212 JWH-015	↓ Migration induced by SDF-1	CB_2 (AM630)	[208]
Thymocytes	Mouse	JWH-015	↑ Apoptosis	CB_2 (SR144528)	[237]

IP, interferon γ-induced protein; ND, not determined; SDF-1, stromal cell derived factor-1

stimulate immune cells [241, 243], suggesting that endocannabinoids, 2-AG in particular, modulate inflammation independent of CB receptor activation and through their numerous metabolites.

Involvement of endocannabinoid-derived metabolites in the regulation of inflammation

Given that both AEA and 2-AG are susceptible to metabolism by eicosanoid biosynthetic enzymes, the immunomodulatory role of

TABLE 9. Proinflammatory effects of endocannabinoids in vivo

Model	Species	Intervention	Effects	Receptor	References
Ovalbumin	Mouse	AEA	↑ Delayed-type hypersensitivity ↑ Cell proliferation ↑ IFN-γ	ND	[239]
Immunization	Mouse	2-AG and peptidoglycan keyhole limpet hemocyanin	↑ Delayed-type hypersensitivity ↓ IL-4	CB_2 (SR144528)	[238]
Cisplatin-induced nephropathy	Mouse	CB_1 (KO)	↓ Renal dysfunction ↓ Inflammatory response	CB_1	[240]
Doxorubicin-induced cardiomyopathy	Mouse	CB_1 (KO)	↓ Cell death ↓ Fibrosis ↓ Oxidative stress	CB_1	[241]
Oxazolone-induced contact dermatitis	Mouse	CB_2 (SR144528)	↓ Swelling ↓ MCP-1, MIP-1 and TNF-α ↓ Eosinophil recruitment	CB_2	[242]
TPA-induced ear inflammation	Mouse	CB_2 (SR144528)	↓ Swelling ↓ LTB_4 ↓ Eosinophil recruitment	CB_2	[243]
Endotoxin-induced uveitis	Rabbit	AEA	↑ Ocular inflammation	CB_1 (AM251)	[244]
Atherosclerosis ($ApoE^{-/-}$)	Mouse	FAAH KO	↑ Neutrophil recruitment in aortas ↑ IFN-γ and TNF-α	ND	[245]

ND, not determined; TPA, 12-*O*-tetradecanoylphorbol-13-acetate

endocannabinoids is very likely the combination of the effects of endocannabinoids and their metabolites. The hydrolysis of AEA and 2-AG results in increased AA levels, putatively promoting eicosanoid biosynthesis. However, FAAH and MAG lipase inhibitors not only increase the effects of endocannabinoids by preventing their hydrolysis, they also increase their likelihood of undergoing oxidation by COX-2, LOX, and p450 enzymes. A good example of this effect is the detection of PG-EA in FAAH knockout mice [81]. Moreover, proinflammatory mediators such as LPS reduce the expression of FAAH and promote COX-2 expression, which results in enhanced PG-EA and PG-G production [31, 87, 267].

COX-2 derivatives of AEA and 2-AG are the most studied endocannabinoid metabolites. Their biosynthesis has been observed in vivo in rat [268], and inflammatory mediators such as LPS and zymosan increase their production in vitro [35, 87] —not surprising considering that PG biosynthesis is a hallmark of inflammation. Among their biologic effects, PGD_2-EA and PGE_2-EA induce cell death in cancer cell lines [269, 270]. In addition, $PGF_{2\alpha}$-EA induces the contraction of the cat iris sphincter [70] and PGE_2-G induces Ca^{2+} mobilization, activation of PKC, and hyperalgesia and allodynia, and enhances glutamatergic synaptic transmission [88, 268, 271]. The reported biologic effects of COX-2-derived 2-AG and AEA metabolites are detailed in **Table 11**. Further studies are needed to identify the role of these mediators in inflammatory diseases.

Of note, 2-AG-derived COX-2 metabolites have a short half-life in vivo: 14 s in rat plasma and 10 min in human plasma [283]. PGE_2-G is hydrolyzed into PGE_2 and glycerol by several hydrolases, including MAG lipase, carboxylesterase 1, lysophospholipase 2, and palmitoyl protein thioesterase 1 [71, 284–286]. Thus, PG-Gs most likely act in the vicinity of their biosynthesis and then are rapidly cleared from the tissues. Moreover, some biologic effects attributed to PG-Gs may in fact be the consequence of their hydrolysis products. PGE_2-EA, in contrast, is not hydrolyzed but is slowly dehydrated into its isomer PGB_2-EA. It is far more stable in human and rat plasma, with a half-life greater than 5 h [283].

12- and 15-LOX also oxygenate endocannabinoids. In humans, 15-LOX is constitutively expressed in reticulocytes, eosinophils,

TABLE 10. Proinflammatory effects of endocannabinoids in vitro

Leukocyte	Origin	Intervention	Effects	Receptors	References
B cells	Human lymphoblast	2-AG	↑ Migration	CB_2 (SR144528)	[246]
	Mouse	2-AG	↑ Migration	CB_2 (SR144528)	[247, 248]
Dendritic cells	Human	2-AG	↑ Migration	CB_2 (SR144528)	[238]
Eosinophils	Human	2-AG	↑ Migration	CB_2 (SR144528)	[106] [249]
	Human	2-AG	↑ Migration and synthesis of LTC4 and EXC4	CB_2 (SR144528 and AM630)	[250]
Endothelial cells	HUVECs and Jurkat cells	2-AG	↑ P- and E-selectins on HUVECs ↑ PGSL1 on Jurkat cells	ND	[251]
Macrophages	Human	LPS	↓ IL-1β, IL-6, IL-8, TNF-α and MMP-9	CB_1 (SR141716A)	[130]
	Mouse (peritoneal)	CB_2 KO	↓ Apoptosis	ND	[252]
	Mouse (peritoneal)	2-AG	↑ Zymosan phagocytosis	CB_2 (SR144528)	[253]
	Human (HL-60 macrophages)	2-AG	↑ Actin polymerization ↑ Adhesion to fibronectin	CB_2 (SR144528)	[254]
		2-AG	↑ IL-8 and MCP-1 production	CB_2 (SR144528)	[255]
	Mouse (J774)	2-AG	↑ NO	ND	[256]
			↓ IL-6	ND	[232]
	Mouse (peritoneal)	DAG lipase β blockade	↓ PGE_2 ↓ PGD_2 ↓ LPS-induced TNF-α	ND	[36]
Mast cells	Rat (peritoneal)	AEA	↑ Histamine release	ND	[257]
	Human (RBL-2H3 cell line)	2-AG	↑ TNF-α ↑ β-Hexosaminidase	ND	[258]
Microglia	Mouse (microglial cell line BV-2)	2-AG	↑ Migration	CB_2 (SR144528)	[259]
Monocytes	Human	2-AG	↑ Adhesion to fibronectin	CB_2 (SR144528)	[256]
			↑ Migration	CB_2 (SR144528)	[254]
			↑ NO release	CB_1 (SR141716A)	[260]
Neutrophils	Human	2-AG	↑ LTB_4 biosynthesis ↑ MPO release	ND	[104]
NK cells	Human	2-AG	↑ Migration	CB_2 (SR144528)	[261]
Platelets	Human	2-AG	↑ Platelet aggregation	ND	[262] [263]

MMP, matrix metalloproteinase

TABLE 11. Effects of COX-2-derived metabolites of AEA and 2-AG on inflammation

Model	Effects	Metabolite	References
Aortic rings (rat)	AEA-induced vasorelaxation	COX-2 products Blocked by FAAH inhibitor URB597 Blocked by COX-2 inhibitor DFU Blocked by EP_4 antagonist GW627368X	[272]
Carcinoma (colorectal HT29)	AEA-induced cell death	COX-2 products Prevented by COX-2 inhibitor NS398 Mimicked by PGD_2-EA and PGE_2-EA	[269]
Carcinoma (cell line JWF2)	AEA-induced cell death	COX products Mimicked by PGD_2 and PGD_2-EA	[270]
Cat iris	Contraction of iris sphincter	$PGF_{2\alpha}$-EA>PGD_2-AEA ≈ PGE_2-EA	[70]
Hippocampus	Enhanced glutamatergic synaptic transmission Enhanced neuronal injury/death	PGE_2-G	[271]
HUVECs	Tissue factor release and PPAR-δ activation induced by 2-AG	PGI_2-G Prevented by COX-2 inhibitor NS398 Inhibited by PGI_2S siRNA	[273]
Lung (rabbit)	Pulmonary arterial pressure induced by 2-AG and AEA	COX-2 products Blocked by MAFP Blocked by COX-2 inhibitor nimesulide Blocked by EP_1 antagonist SC-19220	[274]
Monocytes (human and THP-1 cell line)	↓ LPS-induced TNF-α production	PGE_2-EA	[275]
Mouse macrophages (RAW 264.7)	Transcriptional activity of the IL-12p40 gene induced by AEA	COX-2 products Prevented by COX-2 inhibitor NS398 Mimicked by PGE_2-EA Blocked by EP_2 antagonist AH6809	[276]
	Ca^{2+} accumulation Activation of PKC	PGE_2-G	[88]
Mouse splenocytes	Inhibition of IL-2 release induced by AEA	COX-2 products Unaffected by FAAH inhibitor MAFP Blocked by COX-2 inhibitor NS398 Blocked by PPAR-γ antagonist T0070907	[84]
Platelets	Aggregation induced by 2-AG	COX products Prevented by COX inhibitor aspirin	[277]
Rat	Hyperalgesia and allodynia	PGE_2-G	[268]
Carrageenan-induced knee inflammation (rat)	↑ Nociception ↓ Paw withdrawal latency	$PGF_{2\alpha}$EA	[278]
Ex vivo human mucosal explant colitis	↓ Cytokine-evoked epithelial damage	PGE_2-EA $PGF_{2\alpha}$-EA	[279]
Renal medulla (mouse)	↓ Mean arterial pressure ↑ Renal blood flow	PGE_2-EA	[280]
Peritoneal macrophages (mouse)	↓ LPS-induced inflammation	PGD_2-G	[281]
T cells (Jurkat)	IL-2 release induced by 2-AG	COX-2 products Prevented by COX-2 inhibitor NS398	[203]
	↓ IL-2	15d-PGJ_2-G	[282]

and airway epithelial cells [287, 288]. Th2 cytokines such as IL-4/13 induce the expression of 15-LOX in monocytes, macrophages, and dendritic cells. 12-LOX is expressed in human platelets (reviewed in ref, 289). Although the biosynthesis of 12/15-HETE-AE and -G is observed ex vivo, their levels have never been reported in vivo. However, LOX inhibitors abrogate some of the effects of endocannabinoids, suggesting that the transformation by 12/15-LOX occurs and that these products have biologic activity. These effects are summarized in **Table 12**. The detection and modulation of 12/15-HETE-AE and -G biosynthesis in humans or animals could determine the biologic relevance of these metabolites. Although AEA and 2-AG are not oxidized by 5-LOX, the hydrolysis of AEA and 2-AG can lead to substantial levels of AA and potentially to significant eicosanoid biosynthesis. In this respect, our group recently documented a functional link between 2-AG and leukotriene B_4 (LTB_4) biosynthesis. We found that 2-AG activates neutrophil function, a process that requires its hydrolysis into AA, which is rapidly metabolized into LTB_4, which in turn activates human neutrophils through the leukotriene receptor BLT_1 [104]. Other researchers have also reported that eicosanoids mediate, at least in part, the effects of endocannabinoids [292, 296].

TABLE 12. Involvement of lipoxygenases in the effects of 2-AG and AEA

Model	Effects	Metabolite	References
Brain (mouse)	↑ NAPE-PLD activity ↓ AEA uptake ↓ DAG lipase activity	15-HETE-EA	[290]
Brain (rat)	Decreases ouabain-induced edema	12-HETE-EA	[291]
Bronchus (guinea pig)	Contraction induced by AEA	LO products Blocked by LO inhibitor 5,8,11-ETI	[95]
Carcinoma (PC-3)	2-AG-induced cell invasion	12-LO products Mimicked by AA and 12(S)-HETE Blocked by 2-AG hydrolysis inhibitor OTFP Blocked by 12-LOX inhibitor baicalein	[292]
Distal colon (guinea pig)	2-AG-induced contraction of longitudinal muscle	LOX products Blocked by LOX inhibitor NDGA	[293]
Neuroblastoma (human)	FAAH inhibition induced by AEA and 2-AG	LOX products Mimicked by AA Blocked by 5-LOX inhibitor MK886	[294]
Neutrophil (human)	2-AG -induced migration, kinase activation, Ca^{2+} mobilization, and antimicrobial peptide release	LTB_4 Mimicked by AA Blocked by 2-AG hydrolysis inhibitor MAFP Blocked by FLAP antagonist MK-0591 Blocked by LTB_4 receptor antagonist CP 105,696	[64, 105]
Vagus nerve (Guinea Pig)	AEA-induced depolarization	LOX products Blocked by LOX inhibitor 5,8,11-ETI	[96]
Sciatic nerve injury (Rat)	FAAH inhibition reduces nociception	LOX products Blocked by 12/15-LOX inhibitor baicalein	[295]

CONCLUDING REMARKS

A growing body of evidence supports the immunomodulatory roles of 2-AG and AEA. First, CB receptors are expressed by human leukocytes. Second, mice in which CB receptors are deficient display an altered, usually more severe, inflammatory phenotype. Third, mice in which endocannabinoid levels are increased, either by a genetic or a pharmacological blockade, also display an altered inflammatory phenotype. When 2-AG and AEA were identified as CB receptor ligands, the story was relatively simple: they modulated cell functions by activating CB receptors. However, the large metabolome derived from 2-AG and AEA, which includes eicosanoids, significantly increases the complexity of research into the role of endocannabinoids in inflammation.

It is important to pursue research in this field, keeping in mind that perhaps 1 or more 2-AG or AEA metabolites participate to inflammation in a coordinated and timely fashion and that additional receptors, aside from CB_1 and CB_2, are involved (Fig. 3). This possibility is important, given that eicosanoid biosynthetic enzyme expression is constantly changing during the course of an inflammatory response. In this regard, 2-AG can be the source of proinflammatory leukotrienes in human neutrophils [104] and prostaglandins in murine macrophages [297], which are both usually involved in the acute phase of inflammation. Further, the 2-AG metabolite PGD_2-G has been shown to promote the resolution of inflammation in the lungs of mice [281], indicating that biosynthesis of the latter may occur during the resolution phase and may participate in the anti-inflammatory effects of COX-2 previously described [298].

Finally, the blockade of AEA and 2-AG hydrolysis not only increases endocannabinoid levels, but also their availability to undergo oxidation by COX and LOX enzymes. Therefore, endocannabinoid hydrolysis inhibitors may have a more complex impact than the intended increase in cannabinoid receptor signaling. This possibility should be taken into consideration in future studies, especially when assessing the potential of AEA and 2-AG hydrolysis inhibitors in the treatment of inflammatory diseases. It is also important to establish in humans which enzymes hydrolyze 2-AG and AEA, as well as their expression profiles, to develop pharmacological tools that can limit inflammation and possibly promote its resolution.

AUTHORSHIP

C.T., F.C., J.S.L., and N.F. designed the study and wrote the manuscript.

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DISCLOSURES

The authors declare no competing financial interests.

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KEY WORDS:
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