THE FASEB JOURNAL • REVIEW • www.fasebj.org

Cannabinoids, inflammation, and fibrosis

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ABSTRACT: Cannabinoids apparently act on inflammation through mechanisms different from those of agents such as nonsteroidal anti-inflammatory drugs (NSAIDs). As a class, the cannabinoids are generally free from the adverse effects associated with NSAIDs. Their clinical development thus provides a new approach to treatment of diseases characterized by acute and chronic inflammation and fibrosis. A concise survey of the anti-inflammatory actions of the phytocannabinoids Δ^9-tetrahydrocannabinol (THC), cannabidiol, cannabichromene, and cannabinol is presented. Mention is also made of the noncannabinoid plant components and pyrolysis products, followed by a discussion of 3 synthetic preparations—Cesamet (nabilone; Meda Pharmaceuticals, Somerset, NJ, USA), Marinol (dronabinol; THC; AbbVie, Inc., North Chicago, IL, USA), and Sativex (*Cannabis* extract; GW Pharmaceuticals, Cambridge United Kingdom)—that have anti-inflammatory effects. A fourth synthetic cannabinoid, ajulemic acid (AJA; CT-3; Resunab; Corbus Pharmaceuticals, Norwood, MA, USA), is discussed in greater detail because it represents the most recent advance in this area and is currently undergoing 3 phase 2 clinical trials by Corbus Pharmaceuticals. The endogenous cannabinoids, including the closely related lipoamino acids, are then discussed. The review concludes with a presentation of a possible mechanism for the anti-inflammatory and antifibrotic actions of these substances. Thus, several cannabinoids may be considered candidates for development as anti-inflammatory and antifibrotic agents. Of special interest is their possible use for treatment of chronic inflammation, a major unmet medical need.—Zurier, R. B., Burstein, S. H. Cannabinoids, inflammation, and fibrosis. FASEB J. 30, 3682–3689 (2016). www.fasebj.org

KEY WORDS: endocannabinoids · specialized proresolving mediators · anti-inflammatory · antifibrotic

Preparations derived from *Cannabis* have been the source of medical therapies since the earliest records on pharmacobotany (1). Many beneficial effects of *Cannabis* on the human body, including those on "rheumatism" were noted 4000 yr ago in a work reported by Hui-Lin Li called *Pen-tsao* (2). The term cannabinoid usually refers to compounds that activate the G-protein-coupled cannabinoid receptors 1 and 2 (CB1 and -2). CB1 receptors, located mainly on neurons in the hippocampus and basal ganglia, mediate the psychoactive actions of cannabinoids (3). CB2 receptors are present mainly on tissue and circulating cells of the immune system (4). However, many *Cannabis* components that do not activate either receptor are sometimes called cannabinoids. Given that the *Cannabis* plant contains more than 60 cannabinoids and 200–250 noncannabinoid constituents, it follows that the therapeutic benefits of marijuana are related to some combination of these compounds. We review the current knowledge of the mechanisms whereby phytocannabinoids, noncannabinoid plant components, and their pyrolysis products aid in the control of inflammation and fibrosis. We also address the development of synthetic cannabinoids as treatment for patients with diseases characterized by chronic inflammation and subsequent fibrosis. The ability of some cannabinoids to facilitate the resolution of inflammation by stimulating the action of several specialized proresolving mediators (SPMs), an important emerging concept, is also discussed. The roles of endogenous cannabinoids (endocannabinoids) and the closely related lipoamino acids in control of inflammation are also discussed.

ABBREVIATIONS: 2-AG, 2-arachidonoylglycerol; AJA, ajulemic acid; ARCI-M, Addiction Research Center Inventory-Marijuana; CB1/2, cannabinoid receptor 1/2; CBD, cannabidiol; COX, cyclooxygenase; DMH, dimethylheptyl; FAAH, fatty acid aminohydrolase; FDA, U.S. Food and Drug Administration; LINGly, *N*-linoleoyl glycine; LX, liposin; Mcl-1, myeloid cell leukemia 1; MOA, mechanism of action; NAGly, *N*-arachidonoyl glycine; NLINgly, NSAID, nonsteroidal anti-inflammatory drug; PBM, peripheral blood monocyte; PG, prostaglandin; PPAR-γ, peroxisome proliferator-activated receptor-γ; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SPM, specializing proresolving mediators; THC, Δ^9-tetrahydrocannabinol

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doi: 10.1096/fj.201600646R

CANNABIS CONSTITUENTS

Experiments with Δ^9 -tetrahydrocannabinol (THC; **Fig.1**), the main psychoactive cannabinoid in the plant, have been helpful in understanding the anti-inflammatory actions of the nonpsychoactive cannabinoids (5, 6). Although there is rich documentation of the anti-inflammatory actions of several of the nonpsychoactive constituents, that literature

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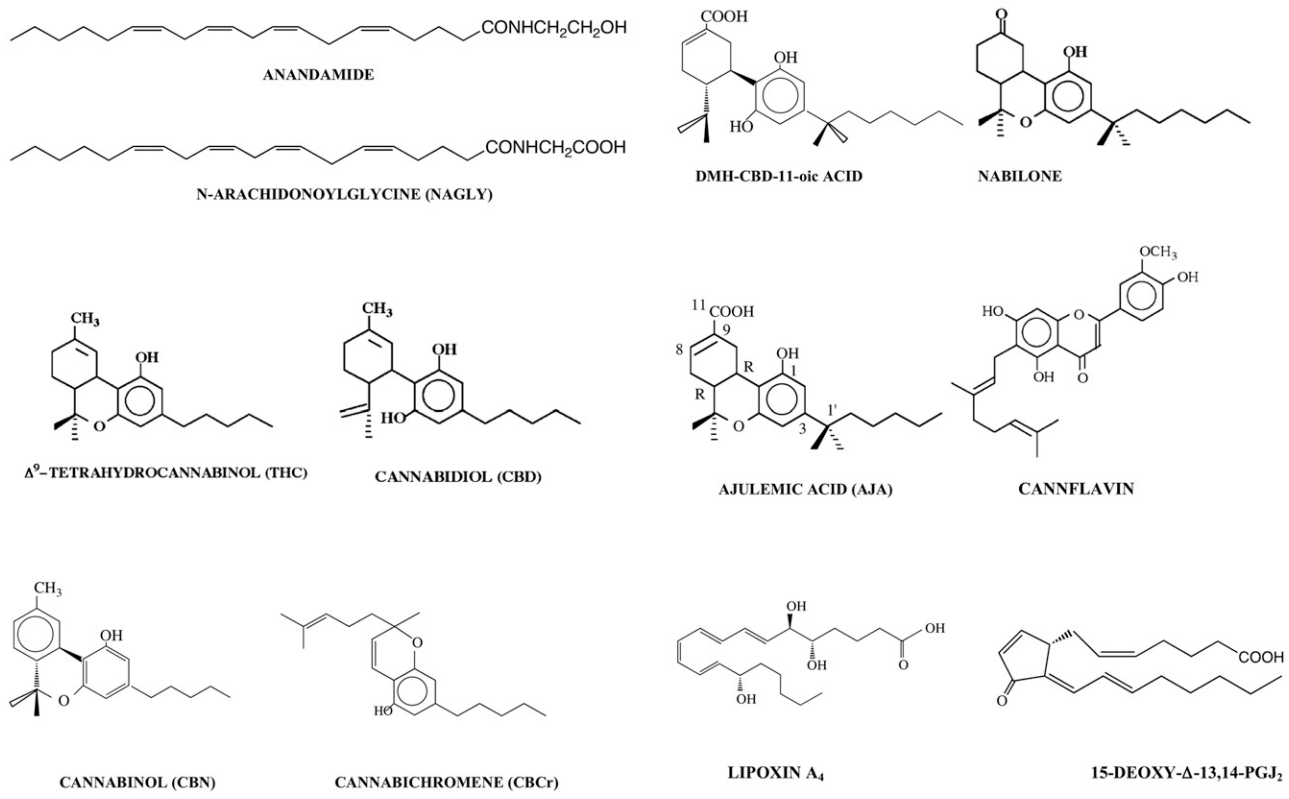

Figure 1. Structures of compounds discussed in this review.

has been crowded out by discussions about psychoactivity and legalization of marijuana. For example, the noncannabinoid, prenylated flavone cannflavin is 30 times more potent as an inhibitor of cyclooxygenase (COX) than the time-honored anti-inflammatory drug aspirin. In addition, Sofia *et al.* (7, 8) demonstrated the anti-inflammatory actions of a crude extract of THC and of the nonpsychoactive *Cannabis* constituents cannabidiol (CBD) and cannabinol in a carrageenan-induced paw edema model of acute inflammation in rats. They showed in the same model that THC is 80 times more potent than aspirin and twice as potent as hydrocortisone and that the nonpsychoactive constituent cannabichromene also suppresses the induced inflammation (9–11). Moreover, CBD reduced acute inflammation in a murine model of collagen-induced arthritis (12). The precise mechanisms whereby CBD reduces inflammation are not clear. CBD does reduce production of the proinflammatory cytokine TNF-α and induces reduction of fatty acid amino-hydrolase (FAAH) activity, thereby increasing production of anandamide, an anti-inflammatory endocannabinoid. Volatile oil components of *Cannabis sativa* suppress COX1 activity (13, 14), and pyrolysis products of CBD exhibit activity in COX-1-suppression assays (15). Several of the most abundant cannabinoid and noncannabinoid constituents of the plant are not psychoactive (16). Thus, it is clear that cannabinoid and noncannabinoid constituents of *Cannabis* are potential nonpsychoactive anti-inflammatory agents.

As the most abundant nonpsychoactive cannabinoid in *Cannabis*, CBD has been studied extensively for its anti-inflammatory properties. As noted, it is active in a murine model of collagen-induced arthritis. In addition, CBD reduces carrageenan-induced paw edema in rats (17) and intestinal inflammation in mice (18). CBD also counters psychoactivity, sedation, and tachycardia induced by THC (19).

SYNTHETIC CANNABINOIDS

Synthetic cannabinoids are being developed in an effort to separate psychoactivity from their analgesic and anti-inflammatory actions. The dimethylheptyl-11-oic-acid analog of CBD (DMH-CBD-11-oic acid; Fig. 1) reduces joint inflammation and tissue injury (cartilage degradation and bone erosion) in collagen-induced arthritis in mice (20). Hydrogenation of DMH-CBD-11-oic acid yields 4 distinct epimers (21). Hydrogenation at different double bonds leads to compounds with different bioactivities, none of which depend on CB1 activation. Thus, several potential therapeutic agents devoid of psychotropic activity may eventually be derived from this one phytocannabinoid. Three cannabinoids—Cesamet (nabilone; Meda Pharmaceuticals, Somerset, NJ, USA), Marinol (dronabinol; THC; AbbVie, Inc., North Chicago, IL, USA), and Sativex (*Cannabis* extract; GW Pharmaceuticals, Cambridge, United Kingdom)—which activate both the CB1 and -2 receptors have been approved by the U.S. Food and Drug Administration (FDA) for clinical use.

Nabilone (Fig.1), a dimethylheptyl analog of THC, is approved in many countries, including the United States,

for treatment of the severe nausea and vomiting associated with chemotherapy. It is also used for the management of neuropathic pain and pain associated with cancer and fibromyalgia (22). It is used most commonly as an adjunctive therapy and as such results in small but significant reductions in pain.

Dronabinol, THC, was approved by the FDA in 1985 for treatment of nausea and vomiting in patients receiving cancer chemotherapy who failed to respond to conventional antiemetics. It is administered in capsule form. Dronabinol has also been used as an appetite stimulant for patients with wasting diseases such as cancer and HIV/AIDS (23).

THC with CBD (Sativex) is approved in 24 countries for the treatment of muscle spasticity associated with multiple sclerosis. Sativex was granted fast track designation by the FDA in 2014. Administered as a mint-flavored oral spray, it is now in phase 3 clinical trials in the United States for treatment of cancer-related pain. Similar to nabilone and dronabinol, Sativex treatment has the same potential adverse effects as marijuana (24). In a double-blind placebo-controlled 5 wk study of 58 patients with rheumatoid arthritis (RA) who received Sativex by oral spray (25), pain at rest, pain on movement, sleep quality, and clinical responses (disease activity score 28) were improved significantly by Sativex.

Ajulemic acid (AJA; CT-3; Resunab; Corbus Pharmaceuticals, Norwood, MA, USA) is a synthetic cannabinoid derived from a modification of THC-11-oic acid, the major metabolite of THC. Extension of the pentyl side chain from 5 to 7 carbons, addition of 2 methyl groups to increase receptor affinity, and a carboxylic acid at the 9 position to reduce blood–brain barrier penetration, results in the formation of AJA (1′,1′-dimethylheptyl-THC-11-oic acid; Fig. 1 (26). AJA, administered by mouth, is 50–100 times more potent than THC as an analgesic (27). The recently developed preparation of AJA has 12 times greater affinity for CB2 than for CB1, which renders it nonpsychoactive at therapeutic doses (28). The anti-inflammatory and antifibrotic actions of AJA have been demonstrated in several *in vitro* systems and in animal models. Anti-inflammatory effects were first demonstrated in arachidonic acid–induced rodent paw edema (26). In an adjuvant-induced arthritis model, rats treated with 0.1 mg/kg AJA 3×/wk for 5 wk did not display evidence of active synovitis or cartilage or bone damage, whereas control animals had cartilage degradation and bone erosion that resembled RA (29). In other experiments, rats treated with AJA at up to 30 mg/kg/d for 5 d, did not show signs of physical dependence on the drug (30).

In studies designed to explore mechanisms of AJA action, it was found that addition of AJA to human peripheral blood and synovial fluid monocytes *in vitro* reduces production of the proinflammatory, bone-degrading cytokine IL-1β (31). It is of interest that AJA did not reduce production of TNFα in these studies, given the finding that clinical trials of TNFα inhibitors in patients with systemic lupus erythematosus (SLE) have been limited by toxicity and increases in disease activity (32, 33). We have observed (unpublished data) that oral administration of single doses of 3–10 mg AJA to healthy volunteers reduced pro-IL-1β gene expression in and secretion of IL-1β from LPS-stimulated peripheral blood monocytes (PBMs) (**Table 1**; Zurier RB, Rossetti RG, Burstein SH, *et al.*, unpublished data). In contrast, AJA had no appreciable effect on TNFα mRNA levels or TNFα secretion. Maximum serum concentration of AJA reached 0.15 μM at 5 h after a dose of 10 mg AJA (Corbus Pharmaceuticals, unpublished data).

No marijuana-like CNS effects were noted in the volunteers, as assessed by the Addiction Research Center Inventory-Marijuana (ARCI-M; developed in conjunction with the Addiction Research Center of the National Institute on Drug Abuse (National Institutes of Health, Bethesda, MD, USA) scale (34) (**Table 2**; Corbus Pharmaceuticals, unpublished data). Overall, the ARCI-M scores for treated subjects were not different from baseline scores or from placebo-treated subjects. The largest mean number of items positively responded to by subjects given AJA was 0.5 and occurred when the inventory was given at the second hour after administration. The largest mean number of positive responses among placebo-treated subjects was 0.375 and occurred before administration. The mean response rate of placebo-treated subjects at h 2 was 0:25. These numbers are relatively small compared to those from experienced marijuana smokers. Subjects smoking marijuana report mean scores of 5.2 and, under placebo or presmoking conditions, 0.7–1.0. The relatively few positive responses obtained in this study were not dose related. These results suggest that AJA is not psychoactive at the doses tested.

Addition of AJA (3–30 μM) to human monocyte-derived macrophages reduces steady-state levels of IL-6 mRNA and the subsequent secretion of IL-6 from LPS-stimulated cells (35). IL-6 is a multifunctional cytokine that contributes to inflammation and tissue injury in several diseases. It has been identified in kidney biopsy tissue from patients with SLE with active glomerulonephritis (36). Skin biopsies from patients with SLE have exhibited increased expression of IL-6 in active sites (37), and plasma levels of IL-6 have correlated with lupus arthritis (38). In addition, higher levels of IL-6 in synovial fluid increase the risk of joint destruction in patients with RA (39). Because activation of osteoclasts is central to the pathogenesis of bone erosion in patients with RA, the influence of AJA on osteoclast differentiation and survival was investigated (40).

TABLE 1. *IL-1β secretion from stimulated PBMs from healthy volunteers administered AJA orally in capsules*

Dose (mg)	Reduction of IL-1β secretion 5 h after AJA (%)
3	14.6
6	35.6
10	47.4

Results are presented as percentage reduction in 3 subjects. PBMs were isolated from peripheral blood of healthy male volunteers by Ficoll-Hypaque separation. Rested cells were stimulated or not with 10 ng/ml LPS 18 h for IL-1β secretion (cytokine ELISA) in supernatants. Oral administration of safflower oil (AJA control) did not influence IL-1β secretion from stimulated PBMs. IL-1β secretion from a 49-yr-old man before and 5 and 24 h after oral administration of safflower oil in placebo capsules was 390–410 pg/ml.

TABLE 2. *ARCI-M scores for AJA given orally in capsules*

Dose (mg)	*n*	Hour 0	1	2	4	8	12	24	Overall mean
1	6	0.5	0.167	0.0	0.0	0.0	0.0	0.0	0.095
3	6	0.0	1.25	1.67	1.25	0.167	0.0	0.0	0.619
6	6	0.5	0.167	0.33	0.0	0.5	0.167	0.167	0.262
10	6	0.33	0.0	0.0	0.0	0.0	0.0	0.0	0.047
All active	24	0.33	0.42	0.5	0.33	0.167	0.04	0.04	0.262
All placebo	8	0.375	0.125	0.25	0.25	0	0.0	0.0	0.143

This was a single-center, phase 1, double-blind, randomized, placebo-controlled study to assess 4 single increasing oral doses of AJA (1, 3, 6, and 10 mg) in 4 groups of 8 healthy male adult subjects. Thirty-two eligible consenting subjects were randomized to 1 of the 4 groups and within each group of 8 subjects, 2 were randomized to receive placebo, and 6 subjects were randomized to receive AJA. Plasma concentrations of AJA were determined by liquid chromatography/tandem mass spectrometry, and pharmacokinetic parameters were calculated by noncompartmental methods. Monitoring for the occurrence of adverse events, changes in physical examination, vital signs (blood pressure, pulse rate, respiration), electrocardiograms, rating scales (mood scale and ARCI-M) and clinical laboratory tests (biochemistry, hematology, urinalysis) were performed before and after administration of the study drug to assess safety, tolerability, and psychoactivity. The Addiction Research Center Inventory-Marijuana (ARCI-M) scale was used to identify possible marijuana-like effects of AJA. This 12-item questionnaire was developed to represent the perceptions and feelings experienced by subjects when smoking marijuana.

Addition of AJA to cell cultures suppressed development of multinucleated osteoclasts (osteoclastogenesis), and prevented further osteoclast formation in cultures in which osteoclastogenesis had already begun. Addition of AJA to fibroblastlike synovial cells also reduces production of matrix metalloproteinases, enzymes that facilitate cartilage and bone destruction (41).

In addition to its capacity to bind to CB2, AJA binds to and activates peroxisome proliferator–activated receptor (PPAR)-γ (42). PPARγ receptors are members of a family of nuclear receptors that modify the transcription of target genes in response to a variety of signaling proteins. They are expressed on immune cells, such as monocytes and macrophages and regulate inflammatory responses through inhibitory effects on expression of proinflammatory cytokines and eicosanoids (43). AJA binds directly to a second site in the PPARγ receptor that is separate from that utilized by other partial agonists, such as the thiazolidinediones (44). Thus, the problems of weight gain, fluid retention, and heart failure caused by the thiazolidinediones, have not been seen in animals or humans given AJA. Activation of PPARγ by AJA suppresses IL-8 promoter activity. IL-8 is a chemoattractant cytokine with specificity for the neutrophil, the major cell involved in acute inflammation. Thus, suppression of neutrophil migration and reduction of enzyme release from neutrophil granules limits acute inflammation and tissue injury. The loss of PPARγ in fibrotic tissues results in enhanced signaling by TGFβ, a major fibrogenic cytokine, and compounds that activate PPARγ reduce fibrosis in a murine model of scleroderma (45). AJA exhibits antifibrotic effects in murine models of systemic sclerosis (46) and reduces collagen synthesis in dermal fibroblasts of patients with scleroderma (47).

The discovery of SPMs (48) has broadened our understanding of how inflammation is controlled: not simply by passive cessation of proinflammatory mediators, but also by an increase in programmed cell death (apoptosis) of immune-inflammatory cells and by activation of stop signals that lead to resolution of inflammation. It is the lack of resolution of inflammation that is in large part responsible for the signs and symptoms of diseases characterized by chronic inflammation, tissue injury, and fibrosis. Novel actions of AJA include its capacity to induce apoptosis in human T lymphocytes (49) and to increase production of 2 proresolving eicosanoids—prostaglandin (PG)J_2 and LXA_4 (Fig. 1)—that facilitate the resolution of inflammation (50, 51).

In a phase 2 proof-of-principal trial, 21 patients with neuropathic pain received twice daily doses of 20 mg AJA in a double-blind, placebo-controlled manner for 7 d (52). No clinically significant adverse events were noted. A significant reduction in neuropathic pain was noted in 30% of patients.

ENDOGENOUS CANNABINOIDS

Endocannabinoids are groups of naturally occurring members of the eicosanoid superfamily that can activate cannabinoid receptors and are derivatives of long-chain fatty acids, primarily arachidonic acid. They are produced rapidly from lipid precursors, are released from neurons by neurotransmitters or from immune cells by inflammatory agents, and can subsequently activate cannabinoid receptors on the same or on adjacent cells. Some are metabolized rapidly by the serine hydrolase FAAH to release the free fatty acid.

Anandamide (Fig. 1), the amide conjugate of arachidonic acid and ethanolamine, is one of the most important of the endocannabinoids and is well named. *Ananda*, from the Sanskrit word for bliss, alludes to the capacity of anandamide to increase motivation and pleasure (53). Other endocannabinoids include 2-arachidonylglycerol (2-AG), and virodhamine. Enzymes known to hydrolyze endocannabinoids include FAAH, monoglyceride lipase, and *N*-acetylethanolamine. Endocannabinoids

act to regulate inflammation and immune responses (54). Anandamide reduces mitogen-induced T- and B-lymphocyte proliferation, probably because of increased apoptosis (55). Anandamide concentrations are increased in cerebrospinal fluid and in circulating lymphocytes of patients with multiple sclerosis (56), perhaps as an attempt at regulation of the neuroinflammation characteristic of the disease. In a murine model of colitis, CB1-knockout ($CB1^{-/-}$) mice exhibited far more inflammation than animals with intact CB1 receptors (57). CB1 and -2 are up-regulated on gingival fibroblasts of patients with periodontitis. Anandamide reduces production of *Porphyromonas gingivalis* LPS-induced IL-6, IL-8, and monocyte chemoattractant protein-1 by these cells. Anandamide also blocks LPS-triggered activation of NFκB, a protein complex that controls transcription of DNA, cytokine production, and cell survival (58). Anandamide also suppresses TNFα-induced NFκB activation by direct inhibition of I-κB kinase, the enzyme responsible for NFκB activation (59). Of interest is the observation that the inhibitory activity was independent of CB1 and -2 activation. Another endocannabinoid, 2-AG, appears to inhibit COX-2 *via* the CB1 receptor and cause down-regulation of the MAPK/NFκB signaling systems (60). Thus, further investigation and a better understanding of the regulation of endocannabinoid production and metabolism may lead to new therapy for diseases characterized by chronic inflammation and fibrosis.

LIPOAMINO ACIDS

An endogenous subfamily of eicosanoids, the lipoamino acids, are structurally and metabolically related to the endocannabinoids and also exhibit analgesic, anti-inflammatory, and proinflammatory resolving properties (61). The best-studied member of this family is *N*-arachidonoyl glycine (NAgly), which is similar in structure to anandamide (Fig. 1). Indeed, oxidation of the hydroxyl group of anandamide leads to NAgly, and NAgly inhibits the FAAH-mediated metabolism of anandamide with moderate potency. There is evidence to suggest that rather than acting through the CB1 or -2 receptors, NAgly binds and activates an orphan G- protein-coupled receptor GPR18 (62). In addition, GPR18, expressed on human leukocytes, binds directly to resolvin D2 (RvD2), an immunoresolvent synthesized during the resolution phase of inflammation. In studies with mice, GPR18, bound to RvD2, stimulated macrophage phagocytosis of bacteria (*Escherichia coli* and *Staphylococcus*) and apoptosis of PMNs, thereby enhancing clearance of bacteria, limiting PMN infiltration, accelerating resolution, and reducing tissue injury. These protective actions were substantially reduced in GPR18-deficient mice (63).

It has long been thought that acute inflammation, a primitive, protective response, simply resolves—or not—on its own, spontaneously. It is now clear that just as mediators of inflammation initiate and sustain the inflammatory response, so also do lipid mediators, including LXs, resolvins, protectins, and maresins, together called SPMs, facilitate an active process of resolution of inflammation, a series of events that prevent chronic inflammation, tissue injury, and fibrosis, and promote a return of tissue to physiologic homeostasis (64). As exemplified by the animal study cited above, a deficiency of SPM impairs resolution of inflammation, just as an abundance of inflammation mediators increases the intensity of inflammation. As noted, select cannabinoids, such as AJA and NAgly, stimulate particular SPMs. In an effort to identify the precise proresolving actions of SPMs, a set of quantitative resolution indices designed to determine the active components (inflammatory and resolving) of a particular resolution process were introduced (65). In addition, the impact of a known therapeutic agent on the resolution process can be determined. For example, down-regulation of the intracellular protein myeloid cell leukemia 1 (Mcl-1) induces apoptosis of human PMNs but does not impair their phagocytosis by macrophages, a series of actions crucial to resolution. In a murine model of bacteria-induced (*E. coli*) lung inflammation, down-regulation of inflammatory cell Mcl-1 accelerated resolution time, maintained appropriate lung function, and enhanced bacterial clearance (66). These results may in future be applied directly to treatment of patients with cystic fibrosis who experience ongoing pulmonary inflammation, even though their lung bacterial infections are cleared by antibiotics.

Just as different prostaglandins derive from different fatty acids (proinflammatory PGE_2 from arachidonic acid, anti-inflammatory PGE_1 from linoleic acid) so, too, do lipoamino acids derive from different fatty acids. *N*-linoleoyl glycine (LINgly), for example, at doses as low as 0.3 mg/kg, reduced leukocyte migration into an area of inflammation in a murine model of peritonitis (67). In addition, LINgly treatment increases production by cells in the peritoneum of the proresolving eicosanoid 15-deoxy-$\Delta^{13,14}$-PGJ_2 (Fig. 1). A small group of *N*-linoleoyl analogs have been studied for their ability to stimulate PGJ_2 production in mouse macrophage RAW cells. The D-alanine derivative was the most active, whereas the D-phenylalanine showed almost no response. A high degree of stereo specificity was observed when comparing the D- and L-alanine isomers, the latter being the less active, a finding that suggests the response is receptor mediated.

McHugh *et al.* (68–70) found that recruitment of BV-2 microglia by NAgly results in anti-inflammatory actions in the brain. They reported that NAgly potently acts on GPR18 to produce directed migration, cell proliferation, and perhaps other MAPK-dependent actions. These results advance our understanding of the lipid-based signaling mechanisms used in the CNS to actively recruit microglia to sites of injury. The NAgly-GPR18 pathway offers a novel approach to development of therapeutic agents to elicit a population of regenerative microglia, or alternatively, to prevent the accumulation of misdirected, proinflammatory microglia that contribute to and intensify neurodegenerative disease. These effects on microglia may also apply to inflammation in the periphery. The concept of an inflammatory reflex (71), a reflex circuit that maintains immunologic homeostasis mediated by the vagus nerve, is pertinent to a discussion of CNS regulation of inflammation. The CNS receives input from

TABLE 3. *Summary of the targets and anti-inflammatory actions of substances discussed in this review*

Substance	Target proteins	Response
Anandamide	CB1/CB2	T- and B-lymphocyte proliferation reduced
NAGLY	GPR-18	Recruitment of microglia stimulates PGJ_2
THC	CB1/CB2	Antiedema activity; adjuvant induced arthritis
CBD	PPARγ FAAH	Reduces collagen-induced arthritis; increases anandamide
CBN	-	Reduces collagen-induced arthritis
CBCr	-	Reduces collagen-induced arthritis
Dimethylheptylcannabidiol-11-oic acid	-	Reduces collagen-induced arthritis
Nabilone	CB1/CB2	Chronic pain management and antiemetic
AJA	CB2/ PPARγ	Stimulates PGJ_2 and LXA_4
Cannflavin	COX-1/COX-2	Inhibits PGE_2 synthesis
$LPXA_4$	ALXR	Inhibits NF-κB
PGJ_2	PPARγ	Inhibits NF-κB

the peripheral immune system *via* inflammatory cytokines and chemokines that inform resident microglia and neurons, which in turn act to reduce further production of the cytokines. The result is that, for example, patients with RA who receive anti-TNFα treatment develop changes in brain activity before resolution of inflammation (redness, swelling, heat, and pain) in the affected joints and before reduction in C-reactive protein, a circulating marker of inflammation. It appears that the nervous system is hardwired to monitor the presence of cytokines and molecular products of invaders. It may well be that the lipoamino acids promote resolution of inflammation through the inflammatory reflex. Thus, the lipoamino acids, including the cannabinoids and endocannabinoids, contain a multitude of compounds to investigate as potential new, effective, and safe treatments for diseases characterized by chronic inflammation, tissue injury, and fibrosis.

CONCLUSIONS

From the reports presented in this review, it may be concluded that several cannabinoids can be considered candidates for development as anti-inflammatory agents (**Table 3**). These compounds are generally free from the adverse effects associated with drugs now in clinical use. In addition, cannabinoids apparently act on inflammation through mechanisms that are different from those of other agents such as NSAIDs. A putative mechanism of action (MOA) of cannabinoids on inflammation is shown in **Fig. 2**, in which 2 well-studied examples, AJA and NAgly, are illustrated. The initial event is the binding to and activation of CB2 (for AJA) and GPR18 (for NAgly) at low doses in cells that are part of the immune system. In both cases an increase in release of free arachidonic acid leads to the increased production and release of proresolving eicosanoids such as PGJ_2 and LXA_4. Ultimately, this process results in an increase in the rate of resolution of chronic inflammation. These released eicosanoids may also act locally on fibroblast-like cells to reduce TGFβ production and signaling, resulting in turn, in a decrease in collagen synthesis and subsequent fibrosis. At high doses, AJA can activate PPARγ, which may also result in a reduction of fibrosis. As is true of all MOAs, this one will probably be modified as more data are reported. Regardless of the MOA, it appears likely that some of the cannabinoids

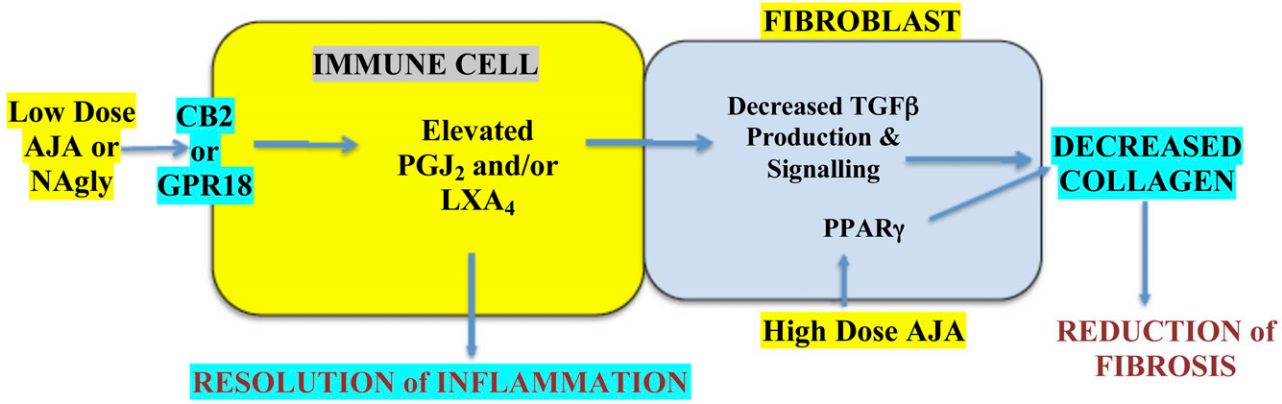

Figure 2. A proposed mechanism for the anti-inflammatory and antifibrotic actions of selected cannabinoids is presented. Two examples are shown; the synthetic cannabinoid AJA and the endocannabinoid NAgly. The former activates the CB2 receptor, and the latter activates the orphan receptor GPR-18. In cells of the immune system, this results in increased levels of the proresolving eicosanoids PGJ_2 and LXA_4. Ultimately, this process produces an increase in the rate of resolution of chronic inflammation. A second outcome is the action on fibroblast cells, resulting in decreased collagen production and reduced fibrosis.

will be developed into safe and effective anti-inflammatory drugs. FJ

ACKNOWLEDGMENTS

The authors thank Corbus Pharmaceuticals, Inc. for providing unpublished findings and Grant Kaufman for help in preparing Fig. 1. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

R. B. Zurier and S. H. Burstein conceived of the content of the article and wrote the manuscript.

REFERENCES

1. Russo, E. B. (2007) History of cannabis and its preparations in saga, science, and sobriquet. *Chem. Biodivers.* **4**, 1614–1648
2. Li, H.-L. (1975) The origin and use of cannabis in Eastern Asia: their linguistic cultural implications. In *Cannabis and Culture* (Rubin, V., ed.), Mouton, The Hague, The Netherlands
3. Mackie, K. (2005) Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb. Exp. Pharmacol.*, **168**, 299-325
4. Castaneda, J. T., Harui, A., Kiertscher, S. M., Roth, J. D., and Roth, M. D. (2013) Differential expression of intracellular and extracellular CB(2) cannabinoid receptor protein by human peripheral blood leukocytes. *J. Neuroimmune Pharmacol.* **8**, 323–332
5. Klein, T. W., and Newton, C. A. (2007) Therapeutic potential of cannabinoid-based drugs. *Adv. Exp. Med. Biol.* **601**, 395–413
6. Fimiani, C., Liberty, T., Aquirre, A. J., Amin, I., Ali, N., and Stefano, G. B. (1999) Opiate, cannabinoid, and eicosanoid signaling converges on common intracellular pathways nitric oxide coupling. *Prostaglandins Other Lipid Mediat.* **57**, 23–34
7. Sofia, R. D., Knobloch, L. C., and Vassar, H. B. (1973) The anti-edema activity of various naturally occurring cannabinoids. *Res. Commun. Chem. Pathol. Pharmacol.* **6**, 909–918
8. Sofia, R. D., Nalepa, S. D., Vassar, H. B., and Knobloch, L. C. (1974) Comparative anti-phlogistic activity of delta 9-tetrahydrocannabinol, hydrocortisone and aspirin in various rat paw edema models. *Life Sci.* **15**, 251–260
9. Wirth, P. W., Watson, E. S., ElSohly, M., Turner, C. E., and Murphy, J. C. (1980) Anti-inflammatory properties of cannabichromene. *Life Sci.* **26**, 1991–1995
10. Wirth, P. W., Watson, E. S., ElSohly, M. A., Seidel, R., Murphy, J. C., and Turner, C. E. (1980) Anti-inflammatory activity of cannabichromene homologs. *J. Pharm. Sci.* **69**, 1359–1360
11. Turner, C. E., and Elsohly, M. A. (1981) Biological activity of cannabichromene, its homologs and isomers. *J. Clin. Pharmacol.* **21**(8-9, Suppl)283S–291S
12. Malfait, A. M., Gallily, R., Sumariwalla, P. F., Malik, A. S., Andreakos, E., Mechoulam, R., and Feldmann, M. (2000) The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA* **97**, 9561–9566
13. Burstein, S., Varanelli, C., and Slade, L. T. (1975) Prostaglandins and cannabis-III: inhibition of biosynthesis by essential oil components of marihuana. *Biochem. Pharmacol.* **24**, 1053–1054
14. Burstein, S., Taylor, P., El-Feraly, F. S., and Turner, C. (1976) Prostaglandins and cannabias-V: identification of p-vinylphenol as a potent inhibitor of prostaglandin synthesis. *Biochem. Pharmacol.* **25**, 2003–2004
15. Spronck, H. J., Luteijn, J. M., Salemink, C. A., and Nugteren, D. H. (1978) Inhibition of prostaglandin biosynthesis by derivatives of olivetol formed under pyrolysis of cannabidiol. *Biochem. Pharmacol.* **27**, 607–608
16. Razdan, R. K. (1986) Structure-activity relationships in cannabinoids. *Pharmacol. Rev.* **38**, 75–149
17. Costa, B., Giagnoni, G., Franke, C., Trovato, A. E., and Colleoni, M. (2004) Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. *Br. J. Pharmacol.* **143**, 247–250
18. Rajesh, M., Mukhopadhyay, P., Bátkai, S., Haskó, G., Liaudet, L., Drel, V. R., Obrosova, I. G., and Pacher, P. (2007) Cannabidiol attenuates high glucose-induced endothelial cell inflammatory response and barrier disruption. *Am. J. Physiol. Heart Circ. Physiol.* **293**, H610–H619
19. Russo, E., and Guy, G. W. (2006) A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med. Hypotheses* **66**, 234–246
20. Sumariwalla, P. F., Gallily, R., Tchilibon, S., Fride, E., Mechoulam, R., and Feldmann, M. (2004) A novel synthetic, nonpsychoactive cannabinoid acid (HU-320) with antiinflammatory properties in murine collagen-induced arthritis. *Arthritis Rheum.* **50**, 985–998
21. Ben-Shabat, S., Hanus, L. O., Katzavian, G., and Gallily, R. (2006) New cannabidiol derivatives: synthesis, binding to cannabinoid receptor, and evaluation of their antiinflammatory activity. *J. Med. Chem.* **49**, 1113–1117
22. Tsang, C. C., and Giudice, M. G. (2016) Nabilone for the management of pain. *Pharmacotherapy* **36**, 273–286
23. Badowski, M. E., and Perez, S. E. (2016) Clinical utility of dronabinol in the treatment of weight loss associated with HIV and AIDS. HIV AIDS (Auckl.) **8**, 37–45
24. Russo, M., Naro, A., Leo, A., Sessa, E., D'Aleo, G., Bramanti, P., and Calabrò, R. S. (2016) Evaluating Sativex® in neuropathic pain management: a clinical and neurophysiological assessment in multiple sclerosis. *Pain Med.* **17**, 1145–1154
25. Blake, D. R., Robson, P., Ho, M., Jubb, R. W., and McCabe, C. S. (2006) Preliminary assessment of the efficacy, tolerability and safety of a cannabis-based medicine (Sativex) in the treatment of pain caused by rheumatoid arthritis. *Rheumatology (Oxford)* **45**, 50–52
26. Burstein, S. H., Audette, C. A., Breuer, A., Devane, W. A., Colodner, S., Doyle, S. A., and Mechoulam, R. (1992) Synthetic nonpsychotropic cannabinoids with potent antiinflammatory, analgesic, and leukocyte antiadhesion activities. *J. Med. Chem.* **35**, 3135–3141
27. Burstein, S. (2005) Ajulemic acid (IP-751): synthesis, proof of principle, toxicity studies, and clinical trials. *AAPS J.* **7**, E143–E148
28. Tepper, M. A., Zurier, R. B., and Burstein, S. H. (2014) Ultrapure ajulemic acid has improved CB2 selectivity with reduced CB1 activity. *Bioorg. Med. Chem.* **22**, 3245–3251
29. Zurier, R. B., Rossetti, R. G., Lane, J. H., Goldberg, J. M., Hunter, S. A., and Burstein, S. H. (1998) Dimethylheptyl-THC-11 oic acid: a nonpsychoactive antiinflammatory agent with a cannabinoid template structure. *Arthritis Rheum.* **41**, 163–170
30. Dajani, E. Z., Larsen, K. R., Taylor, J., Dajani, N. E., Shahwan, T. G., Neeleman, S. D., Taylor, M. S., Dayton, M. T., and Mir, G. N. (1999) 1′,1′-Dimethylheptyl-delta-8-tetrahydrocannabinol-11-oic acid: a novel, orally effective cannabinoid with analgesic and anti-inflammatory properties. *J. Pharmacol. Exp. Ther.* **291**, 31–38
31. Zurier, R. B., Rossetti, R. G., Burstein, S. H., and Bidinger, B. (2003) Suppression of human monocyte interleukin-1beta production by ajulemic acid, a nonpsychoactive cannabinoid. *Biochem. Pharmacol.* **65**, 649–655
32. Aringer, M., Houssiau, F., Gordon, C., Graninger, W. B., Voll, R. E., Rath, E., Steiner, G., and Smolen, J. S. (2009) Adverse events and efficacy of TNF-alpha blockade with infliximab in patients with systemic lupus erythematosus: long-term follow-up of 13 patients. *Rheumatology (Oxford)* **48**, 1451–1454
33. Williams, E. L., Gadola, S., and Edwards, C. J. (2009) Anti-TNF-induced lupus. *Rheumatology (Oxford)* **48**, 716–720
34. Haertzen, C. A. (1965) Addiction Research Center Inventory (ARCI): development of a general drug estimation scale. *J. Nerv. Ment. Dis.* **141**, 300–307
35. Parker, J., Atez, F., Rossetti, R. G., Skulas, A., Patel, R., and Zurier, R. B. (2008) Suppression of human macrophage interleukin-6 by a nonpsychoactive cannabinoid acid. *Rheumatol. Int.* **28**, 631–635
36. Aringer, M., and Smolen, J. S. (2005) Cytokine expression in lupus kidneys. *Lupus* **14**, 13–18
37. Mikita, N., Ikeda, T., Ishiguro, M., and Furukawa, F. (2011) Recent advances in cytokines in cutaneous and systemic lupus erythematosus. *J. Dermatol.* **38**, 839–849
38. Ball, E. M., Gibson, D. S., Bell, A. L., and Rooney, M. R. (2014) Plasma IL-6 levels correlate with clinical and ultrasound measures of arthritis in patients with systemic lupus erythematosus. *Lupus* **23**, 46–56
39. Kotake, S., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T., and Kashiwazaki, S. (1996) Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. *J. Bone Min. Res.* **11**, 88–95

40. George, K. L., Saltman, L. H., Stein, G. S., Lian, J. B., and Zurier, R. B. (2008) Ajulemic acid, a nonpsychoactive cannabinoid acid, suppresses osteoclastogenesis in mononuclear precursor cells and induces apoptosis in mature osteoclast-like cells. *J. Cell. Physiol.* **214**, 714–720
41. Johnson, D. R., Stebulis, J. A., Rossetti, R. G., Burstein, S. H., and Zurier, R. B. (2007) Suppression of fibroblast metalloproteinases by ajulemic acid, a nonpsychoactive cannabinoid acid. *J. Cell. Biochem.* **100**, 184–190
42. Liu, J., Li, H., Burstein, S. H., Zurier, R. B., and Chen, J. D. (2003) Activation and binding of peroxisome proliferator-activated receptor gamma by synthetic cannabinoid ajulemic acid. *Mol. Pharmacol.* **63**, 983–992
43. O'Sullivan, S. E., and Kendall, D. A. (2010) Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease. *Immunobiology* **215**, 611–616
44. Ambrosio, A. L., Dias, S. M., Polikarpov, I., Zurier, R. B., Burstein, S. H., and Garratt, R. C. (2007) Ajulemic acid, a synthetic nonpsychoactive cannabinoid acid, bound to the ligand binding domain of the human peroxisome proliferator-activated receptor gamma. *J. Biol. Chem.* **282**, 18625–18633
45. Wu, M., Melichian, D. S., Chang, E., Warner-Blankenship, M., Ghosh, A. K., and Varga, J. (2009) Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. *Am. J. Pathol.* **174**, 519–533
46. Lucattelli, M., Fineschi, S., Selvi, E., Garcia Gonzalez, E., Bartalesi, B., De Cunto, G., Lorenzini, S., Galeazzi, M., and Lungarella, G. (2016) Ajulemic acid exerts potent anti-fibrotic effect during the fibrogenic phase of bleomycin lung. *Respir. Res.* **17**, 49
47. Gonzalez, E. G., Selvi, E., Balistreri, E., Akhmetshina, A., Palumbo, K., Lorenzini, S., Lazzerini, P. E., Montilli, C., Capecchi, P. L., Lucattelli, M., Baldi, C., Gianchecchi, E., Galeazzi, M., Pasini, F. L., and Distler, J. H. (2012) Synthetic cannabinoid ajulemic acid exerts potent antifibrotic effects in experimental models of systemic sclerosis. *Ann. Rheum. Dis.* **71**, 1545–1551
48. Buckley, C. D., Gilroy, D. W., and Serhan, C. N. (2014) Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity* **40**, 315–327
49. Bidinger, B., Torres, R., Rossetti, R. G., Brown, L., Beltre, R., Burstein, S., Lian, J. B., Stein, G. S., and Zurier, R. B. (2003) Ajulemic acid, a nonpsychoactive cannabinoid acid, induces apoptosis in human T lymphocytes. *Clin. Immunol.* **108**, 95–102
50. Zurier, R. B., Sun, Y. P., George, K. L., Stebulis, J. A., Rossetti, R. G., Skulas, A., Judge, E., and Serhan, C. N. (2009) Ajulemic acid, a synthetic cannabinoid, increases formation of the endogenous proresolving and anti-inflammatory eicosanoid, lipoxin A4. *FASEB J.* **23**, 1503–1509
51. Stebulis, J. A., Johnson, D. R., Rossetti, R. G., Burstein, S. H., and Zurier, R. B. (2008) Ajulemic acid, a synthetic cannabinoid acid, induces an antiinflammatory profile of eicosanoids in human synovial cells. *Life Sci.* **83**, 666–670
52. Karst, M., Salim, K., Burstein, S., Conrad, I., Hoy, L., and Schneider, U. (2003) Analgesic effect of the synthetic cannabinoid CT-3 on chronic neuropathic pain: a randomized controlled trial. *JAMA* **290**, 1757–1762
53. Monteleone, A. M., Di Marzo, V., Aveta, T., Piscitelli, F., Dalle Grave, R., Scognamiglio, P., El Ghoch, M., Calugi, S., Monteleone, P., and Maj, M. (2015) Deranged endocannabinoid responses to hedonic eating in underweight and recently weight-restored patients with anorexia nervosa. *Am. J. Clin. Nutr.* **101**, 262–269
54. Skaper, S. D., and Di Marzo, V. (2012) Endocannabinoids in nervous system health and disease: the big picture in a nutshell. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **367**, 3193–3200
55. Schwarz, H., Blanco, F. J., and Lotz, M. (1994) Anadamide, an endogenous cannabinoid receptor agonist inhibits lymphocyte proliferation and induces apoptosis. *J. Neuroimmunol.* **55**, 107–115
56. Centonze, D., Bari, M., Rossi, S., Prosperetti, C., Furlan, R., Fezza, F., De Chiara, V., Battistini, L., Bernardi, G., Bernardini, S., Martino, G., and Maccarrone, M. (2007) The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain* **130**, 2543–2553
57. Massa, F., Marsicano, G., Hermann, H., Cannich, A., Monory, K., Cravatt, B. F., Ferri, G. L., Sibaev, A., Storr, M., and Lutz, B. (2004) The endogenous cannabinoid system protects against colonic inflammation. *J. Clin. Invest.* **113**, 1202–1209
58. Nakajima, Y., Furuichi, Y., Biswas, K. K., Hashiguchi, T., Kawahara, K., Yamaji, K., Uchimura, T., Izumi, Y., and Maruyama, I. (2006) Endocannabinoid, anandamide in gingival tissue regulates the periodontal inflammation through NF-kappaB pathway inhibition. *FEBS Lett.* **580**, 613–619
59. Sancho, R., Calzado, M. A., Di Marzo, V., Appendino, G., and Muñoz, E. (2003) Anandamide inhibits nuclear factor-kappaB activation through a cannabinoid receptor-independent pathway. *Mol. Pharmacol.* **63**, 429–438
60. Zhang, J., and Chen, C. (2008) Endocannabinoid 2-arachidonoylglycerol protects neurons by limiting COX-2 elevation. *J. Biol. Chem.* **283**, 22601–22611
61. Burstein, S. H. (2014) The cannabinoid acids, analogs and endogenous counterparts. *Bioorg. Med. Chem.* **22**, 2830–2843
62. Burstein, S. H., McQuain, C. A., Ross, A. H., Salmonsen, R. A., and Zurier, R. E. (2011) Resolution of inflammation by N-arachidonoylglycine. *J. Cell. Biochem.* **112**, 3227–3233
63. Chiang, N., Dalli, J., Colas, R. A., and Serhan, C. N. (2015) Identification of resolvin D2 receptor mediating resolution of infections and organ protection. *J. Exp. Med.* **212**, 1203–1217
64. Serhan, C. N. (2014) Pro-resolving lipid mediators are leads for resolution physiology. *Nature* **510**, 92–101
65. Schwab, J. M., Chiang, N., Arita, M., and Serhan, C. N. (2007) Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* **447**, 869–874
66. Lucas, C. D., Dorward, D. A., Tait, M. A., Fox, S., Marwick, J. A., Allen, K. C., Robb, C. T., Hirani, N., Haslett, C., Duffin, R., and Rossi, A. G. (2014) Downregulation of Mcl-1 has anti-inflammatory pro-resolution effects and enhances bacterial clearance from the lung. *Mucosal Immunol.* **7**, 857–868
67. Burstein, S., McQuain, C., Salmonsen, R., and Seicol, B. (2012) N-Amino acid linoleoyl conjugates: anti-inflammatory activities. *Bioorg. Med. Chem. Lett.* **22**, 872–875
68. McHugh, D., Hu, S. S., Rimmerman, N., Juknat, A., Vogel, Z., Walker, J. M., and Bradshaw, H. B. (2010) N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. *BMC Neurosci.* **11**, 44
69. McHugh, D., Wager-Miller, J., Page, J., and Bradshaw, H. B. (2012) siRNA knockdown of GPR18 receptors in BV-2 microglia attenuates N-arachidonoyl glycine-induced cell migration. *J. Mol. Signal.* **7**, 10
70. McHugh, D., Page, J., Dunn, E., and Bradshaw, H. B. (2012) Δ(9)-Tetrahydrocannabinol and N-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. *Br. J. Pharmacol.* **165**, 2414–2424
71. Diamond, B., and Tracey, K. J. (2011) Mapping the immunological homunculus. *Proc. Natl. Acad. Sci. USA* **108**, 3461–3462

Received for publication June 4, 2016.
Accepted for publication July 11, 2016.