Endocannabinoids and the Immune System in Health and Disease

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Abstract

Endocannabinoids are bioactive lipids that have the potential to signal through cannabinoid receptors to modulate the functional activities of a variety of immune cells. Their activation of these seven-transmembranal, G protein-

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coupled receptors sets in motion a series of signal transductional events that converge at the transcriptional level to regulate cell migration and the production of cytokines and chemokines. There is a large body of data that supports a functional relevance for 2-arachidonoylglycerol (2-AG) as acting through the cannabinoid receptor type 2 (CB2R) to inhibit migratory activities for a diverse array of immune cell types. However, unequivocal data that supports a functional linkage of anandamide (AEA) to a cannabinoid receptor in immune modulation remains to be obtained. Endocannabinoids, as typical bioactive lipids, have a short half-life and appear to act in an autocrine and paracrine fashion. Their immediate effective action on immune function may be at localized sites in the periphery and within the central nervous system. It is speculated that endocannabinoids play an important role in maintaining the overall "fine-tuning" of the immune homeostatic balance within the host.

Keywords

Anandamide • Antigen presentation • 2-Arachidonoylglycerol • Astrocyte • Basophil • Cannabinoid receptor • Chemokine • Cytokine • Dendritic cell • Endocannabinoid • Interferon • Interleukin • Lymphocyte • Macrophage • Mast cell • Microglia • Monocyte • Natural killer (NK) cell • Neutrophil • Nitric oxide

Abbreviations

2-AG	2-Arachidonoylglycerol
Abn-CBD	Abnormal cannabidiol
AEA	Anandamide
ALIA	Autacoid local inflammation antagonism
ApoE	Apolipoprotein E
Arg-1	Arginase 1
BBB	Blood–brain barrier
CB1R	Cannabinoid receptor type 1
CB2R	Cannabinoid receptor type 2
ConA	Concanavalin A
CNS	Central nervous system
COX	Cyclooxygenase
ECM	Extracellular matrix
FAAH	Fatty acid amide hydrolase
HIV	Human immunodeficiency virus
HSV-1	Herpes simplex virus type 1
HUVECs	Human umbilical vein endothelial cells
IL	Interleukin
IRAK1BP1	IL-1 receptor-associated kinase 1 binding protein
iNOS	Inducible nitric oxide synthase
IFN	Interferon

LC-APCI-MS	Liquid-chromatography-atmospheric pressure chemical ionization-mass spectrometry
L-NAME	L-NG-nitroarginine methyl ester
LPS	Lipopolysaccharide
LTB_4	Leukotriene B4
MAPK	Mitogen-activated protein kinase
mBSA	Methylated bovine serum albumin
MCP-1	Monocyte chemoattractant protein 1
mDCs	Myeloid dendritic cells
MHC	Major histocompatibility complex
MKP-1	Mitogen-activated protein kinase phosphatase 1
MS	Multiple sclerosis
NADA	*N*-Arachidonoyldopamine
NAGly	*N*-Arachidonoyl glycine
NK cell	Natural killer cell
NMDA	*N*-Methyl-D-aspartate
NO	Nitric oxide
PCR	Polymerase chain reaction
pDCs	Plasmacytoid dendritic cells
PGD2	Prostaglandin D2
PGE2	Prostaglandin E2
PMN	Polymorphonuclear
PPARγ	Peroxidase proliferator-activated receptor γ
PSGL1	P-selectin glycoprotein ligand 1
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
TAK1	TGF-β-activated kinase 1
Tat	Trans-activator of transcription
Tc	Cytotoxic T cells
TCR	T-cell receptor
Th cells	T helper cells
TLRs	Toll-like receptors
TMEV	Theiler's murine encephalomyelitis virus
TMEV-IDD	Theiler's murine encephalomyelitis virus-induced demyelinating disease
TNF	Tumor necrosis factor
TRAF6	TNF receptor-associated factor 6
T_{regs}	Regulatory T cells
VCAM	Vascular cell adhesion molecules

1 Introduction

The immune system in mammals contains a diverse array of cells. These cells function coordinately to drive their maturation, direct antimicrobial activities, and promote repair as well as damage of tissues (Table 1). Many of these activities are

Table 1 Cell markers and functional attributes associated with immune cells

Cell type	Key cell markers	Functional attributes
Astrocyte	CD44, GFAP, S100B Glast	Maintains brain homeostasis Defines brain micro-architecture Synaptic transmission
B lymphocyte (B cell)	CD19, CD20, CD21, CD40, MHC class II	Antigen presentation Antibody production
Basophil	CD13, CD107a, CD123, FcεRIα	Responds to parasitic infections Mediates allergic responses
Dendritic cell	CD11c, CD141, CD303	Antigen presentation
Eosinophil	CD23, CD88, FcεRIα	Responds to parasitic infections Mediates allergic responses
Macrophage	CD14, CD11b, CD68, CD86, MAC-1/MAC-3,	Phagocytosis Stimulation of lymphocytes and other immune cells
Mast cell	CD117, CD23 FcεRIα, CD203c	Mediates allergic responses
Microglia	CD11b, CD40, CD45, B7, ICAM-1	Brain homeostasis Scavenging Phagocytosis Antigen presentation
Monocyte	CD11c, CD14, CD45, CD68, CD163	Phagocytosis Macrophage precursor
Neutrophil	CD121a	Initial responder to acute inflammation
Natural killer (NK) cell	CD16, CD56	Destroys tumors and virus-infected cells
T lymphocyte (T cell)	CD2, CD3, CD4, CD8, TCR	Attacks infected or cancerous cells Regulates immune responses

CD cluster of designation, *GFAP* glial fibrillary acid protein, *ICAM* intercellular adhesion molecule, *MHC* major histocompatibility complex, *TCR* T-cell receptor

mediated through soluble factors, such as chemokines and cytokines (Table 2) that are released from immune cells and bind to cognate receptors and other cellular targets, setting in motion signaling cascades that culminate in the activation of select genes. This cross talk between different cell types occurs in a fashion that maintains overall homoeostatic balance of the immune system. Bioactive lipid molecules participate in the interplay of proinflammatory and anti-inflammatory factors. Included among these are endocannabinoids, principal among which are the amide and ester of the long chain polyunsaturated fatty acids anandamide (2-arachidonoylethanolamine, AEA) (Devane et al. 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al. 1995). To date, two receptors that meet strict pharmacological and biochemical characterization for designation as endocannabinoid receptors have been identified. The first of these, the cannabinoid receptor type 1 (CB1R), is found in the central nervous system (CNS) and testis and at lower levels in some immune cells (Matsuda et al. 1990 ; Herkenham et al. 1990; Galiegue et al. 1995; Gerard et al. 1991). The second of these, the cannabinoid receptor type 2 (CB2R), is localized primarily in immune cells. The CB2R is found at highest levels in B lymphocytes, followed in order by natural

Table 2 Select chemokines/cytokines and their functional attributes

Cytokine	Property	Cells that produce cytokines	Functional attributes
IL-1 RA	Proinflammatory	Monocytes, macrophages, neutrophils, hepatocytes	Natural antagonist to IL-1
IL-1α	Proinflammatory	Macrophages, microglia, neutrophils, endothelial cells	Activates other cells, chemotactic, inflammatory reaction
IL-1β	Proinflammatory	Leukocytes, endothelial cells, macrophages	Inflammatory reaction, cell proliferation, cell differentiation, apoptosis
IL-2	Proinflammatory	T cells	Development and maturation of T cells, stimulates or activates other cells
IL-4	Anti-inflammatory	T cells, basophils, mast cells	Stimulates B cells
IL-6	Anti-/ proinflammatory	T cells, macrophages, microglia	Fever, stimulates acute phase response
IL-8	Proinflammatory	Eosinophils, macrophages, microglia, endothelial cells	Activates other cells, chemotactic, inflammatory reaction; kills parasites and amplifies the inflammatory reaction.
IL-10	Anti-inflammatory	T cells, macrophages, microglia	Stimulates or activates other cells, activates other cells, chemotactic, inflammatory reactions
IL-12	Proinflammatory	Macrophages, dendritic cells, B cells	Activates NK cells, T-cell differentiation
IL-17A	Proinflammatory	T helper cells	Inflammatory reaction against pathogen
IL-23	Proinflammatory	Dendritic cells, macrophages	Proliferation of memory T cell, increased IFN-γ production
MIP-1α	Proinflammatory	Eosinophils, mast cells	Inflammatory reaction in allergic reactions, stimulates other cells, kills parasites
MIP-2	Proinflammatory	Monocytes, macrophages	Chemotactic for polymorphonuclear leukocytes and hematopoietic stem cells
Eotaxin (CCL11)	Proinflammatory	Eosinophils, monocytes, lymphocytes	Kills parasites and amplifies inflammatory reactions, chemotaxis of eosinophils
RANTES (CCL5)	Proinflammatory	Eosinophils, T cells	Kills parasites and amplifies inflammatory reaction
IFN-γ	Proinflammatory	T cells, NK cells	Stimulates or activates cells

(continued)

Table 2 (continued)

Cytokine	Property	Cells that produce cytokines	Functional attributes
KC	Proinflammatory	Macrophages, neutrophils, epithelial cells	Neutrophil chemotactic activity
MCP-5	Proinflammatory	Lymph node, thymus	Allergic reactions, immune response to pathogens
TNF-α	Proinflammatory	T cells, NK cells, mast cells, microglia	Stimulates or activates other cells, inflammatory reaction, chemotaxis

IL interleukin, *MIP* macrophage inflammatory protein, *RANTES* regulated upon activation normal T cell expressed and presumably secreted, *IFN* interferon, *KC* keratinocyte chemoattractant, *MCP* monocyte chemoattractant protein, *TNF* tumor necrosis factor

killer (NK) cells, monocytes/macrophages/microglia, and T lymphocytes (Galiegue et al. 1995; Schatz et al. 1997). The ordered distribution of these receptors suggests that certain immune cell subpopulations may be more responsive to endocannabinoids.

Endocannabinoids have been identified in immune cells such as monocytes/macrophages, basophils, lymphocytes, and dendritic cells (Matias et al. 2002). It has been suggested that 2-AG is the cognate functionally relevant endocannabinoid for the CB2R (Sugiura et al. 2000; Parolaro et al. 2002). Lee et al. (1995) reported that 2-AG suppressed the lymphoproliferation of splenocytes to bacterial lipopolysaccharide (LPS) and anti-CD3, an antibody that induces T-lymphocyte activation. However, this suppression occurred only at concentrations greater than 10 μM. Sugiura et al. (2000) also examined the effect of 2-AG on intracellular free Ca^{2+} concentrations in human HL-60 macrophage-like cells and found that this endocannabinoid induced a rapid transient increase in levels of intracellular free Ca^{2+}. The induced Ca^{2+} transient was blocked by a CB2R antagonist, consistent with the involvement of the CB2R in this response. In contrast, AEA was found to be a weak partial agonist for the CB2R. Based on these results, it was proposed that the CB2R was originally a 2-AG receptor and that 2-AG constituted the native cognate ligand.

On the other hand, Stefano et al. (2000) reported that 2-AG stimulated the release of nitric oxide (NO) from human immune and vascular tissues and invertebrate immunocytes, but that it did so through the activation of the CB1R. Berdyshev et al. (1997) reported that AEA diminished levels of the cytokines interleukin (IL)-6 (IL-6) and IL-8 from human monocytes, while Valk et al. (1997) indicated that AEA acted through the CB2R as a synergistic growth factor for hematopoietic cells. Derocq et al. (1998), using IL-3-dependent and IL-6-dependent mouse cell lines, proposed that AEA exerted a growth-promotion effect. However, both Berdyshev et al. (1997) and Derocq et al. (1998) concluded that the growth-promoting effect of AEA was cannabinoid receptor independent. Facci et al. (1995) reported that mast cells, bone marrow-derived cells found in mucosal and connective tissues and in the nervous system that play a role in tissue inflammation and neuroimmune interactions, expressed a peripheral cannabinoid receptor that was differentially sensitive to AEA. These cells reportedly expressed the CB2R that exerted negative

regulatory effects on mast cell activation. However, AEA did not down-modulate mast cell activation in vitro.

2 Effects of Endocannabinoids In Vitro

A variety of mammalian cell systems have been used as experimental models for documenting the in vitro effects of endocannabinoids on immune function. Early experiments involved the exogenous introduction of endocannabinoids to cultures of transformed immune cells or to immune cell subpopulations obtained from mice and humans. Such studies were complemented with those using mixed cell populations that putatively replicated more closely an in vivo condition that integrated cross talk between different immune cell types. The use of mixed cell populations allowed for the conducting of depletion and reconstitution studies in which selected immune cell subpopulations were removed from, or added to, the culture system in an attempt to identify the immune cell subpopulation targeted by a particular endocannabinoid. While early studies focused on the relevance of endocannabinoids in modulating the function of immune cells associated with the peripheral immune system, recent studies have centered on the effects of endocannabinoids on immune functionality within the central nervous system (CNS).

3 Immune Cells in the Peripheral Immune System

3.1 Basophils

Basophils are a type of polymorphonuclear (PMN) cell or "white" blood cell and make up less than 1 % of this blood cell type. When activated, basophils degranulate to release histamine, proteoglycans (e.g., heparin and chondroitin), and proteolytic enzymes (e.g., elastase and lysophospholipase). They also secrete lipid mediators such as leukotrienes and several cytokines such as IL-4. Basophils have receptors on their cell surface that bind IgE, an immunoglobulin involved in macroparasite defense and allergy. It is the bound IgE antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens. Vannacci et al. (2002) reported that 2-AG diminished the expression of CD63, a cell differentiation marker that is used to identify activated basophils. The inhibitory effect was found to be concentration dependent and reversed by the CB2R antagonist SR144528 and the NO synthase inhibitor L-NG-nitroarginine methyl ester (L-NAME). In guinea pig mast cells, the antigen-mediated release of histamine was found to be decreased by 2-AG in a dose-dependent fashion. The release of histamine was returned to control values by the CB2R antagonist SR144528. However, because L-NAME abrogated the inhibitory effects of 2-AG and reduced the immunological activation of both human basophils

and guinea pig mast cells, it was questioned whether the activation of cannabinoid receptors was linked directly to the modulation of CD63 expression.

3.2 Dendritic Cells

Dendritic cells are antigen-processing and antigen-presenting cells that are critical for the induction of primary immune responses, induction of immunological tolerance, and regulation of T-cell-mediated immune responses. Matias et al. (2002) reported that human dendritic cells harbored a constitutive endocannabinoid system. Using a liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) method, lipids extracted from immature dendritic cells were shown to contain 2-AG and AEA. The amounts of 2-AG were increased following cell maturation induced by LPS or by the major mite fecal allergen Der p 1. The investigators found that dendritic cells also expressed the CB1R, the CB2R, and fatty acid amide hydrolase (FAAH). Do et al. (2004) examined the effect of endocannabinoids on murine bone marrow-derived dendritic cells. Addition of AEA to dendritic cell cultures induced their apoptosis. The dendritic cells expressed the CB1R and CB2R. It was found that engagement of both receptors was necessary to trigger apoptosis. Chiurchiù et al. (2013) compared the level of cytokine production by myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) in healthy human subjects and multiple sclerosis (MS) patients, following in vitro stimulation of Toll-like receptors (TLRs)7/8 that play a key role in the innate immune system. They also evaluated the effect of AEA on these dendritic cell subsets and correlated cytokine levels with defects in the endocannabinoid system. It was found that mDCs obtained from MS patients produced higher levels of IL-12 and IL-6, whereas pDCs produced lower levels of interferon (IFN)-α compared to healthy subjects. AEA inhibited cytokine production from healthy mDCs and pDCs, as well as their ability to induce T-lymphocyte Th-1 and Th-17 lineages. Th1 cells produce IFN-γ, IL-2, and tumor necrosis factor (TNF)-β and mediate delayed type hypersensitivity (DTH) responses. Th-17 cells produce IL-17, IL-17F, and IL-22 and have been linked to inflammation and tissue injury in autoimmune disease. It was suggested that AEA had an immunomodulatory effect on mDCs and pDCs from MS patients, possibly as a reflection of an alteration of the expression of FAAH.

3.3 Eosinophils

Eosinophils comprise approximately 6 % of white blood cells (i.e., PMNs) in the bloodstream and multiply in response to parasitic infections or allergic reactions. Oka et al. (2004) reported that 2-AG induced the migration of human eosinophilic leukemia EoL-1 cells. The migration evoked by 2-AG was abolished in the presence of the CB2R antagonist SR144528 or by pretreatment of the cells with pertussis toxin, an exotoxin produced by the bacterium *Bordetella pertussis* that

prevents G_i signaling by G protein-coupled receptors. The inhibition of 2-AG-stimulated migration by the CB2R antagonist was consistent with involvement of the CB2R. It was suggested that 2-AG served as a chemoattractant for human peripheral blood eosinophils, but had no effect on neutrophils. It has also been demonstrated that 2-AG induces chemotaxis, a directed movement of cells toward a gradient of a chemical stimulus, of EoL-1 cells and human peripheral blood eosinophils in a CB2R-dependent manner (Kishimoto et al. 2006).

3.4 Lymphocytes

T lymphocytes play a central role in cell-mediated immunity and are distinguished from other lymphocytes, such as B lymphocytes and NK cells, by the presence of a T-cell receptor (TCR) on the cell surface. There are several subsets of T lymphocytes, each exhibiting a distinctive functional capability. These include T helper cells (T_h cells), CD4+ cells, cytotoxic T lymphocytes (T_c cells), CD8+ cells, memory T lymphocytes that persist long-term after an infection has resolved, regulatory T lymphocytes (T_{reg} cells), natural killer T (NKT)-lymphocytes, and gamma-delta (γδ) T cells. B lymphocytes function to produce antibodies against antigens, act as antigen-presenting cells, and develop into memory B cells following activation by antigen interaction. Schwarz et al. (1994) indicated that AEA caused an inhibition of mitogen-induced T- and B-lymphocyte proliferation. The effects of AEA on DNA synthesis in T and B lymphocytes occurred rapidly as exposure of the cells during the final 4 h of culture was sufficient to achieve greater than 40 % inhibition. Low concentrations of AEA that were shown to inhibit lymphocyte proliferation also caused DNA fragmentation. Lee et al. (1995) investigated the immunomodulatory effects of AEA and 2-AG in splenocytes obtained from $B_6C_3F_1$ mice. 2-AG produced a concentration-related inhibition of the mixed lymphocyte response that is used as a barometer of an individual's response to transplanted tissue or organ. It also inhibited the anti-CD3 monoclonal antibody-induced T-cell proliferation and LPS-induced B-cell proliferation. However, it had no inhibitory effect on phorbol-12-myristate-13-acetate/ionomycin-induced cell proliferation. Similarly, in vitro primary immunoglobulin M antibody-forming cell responses were found to be enhanced by 2-AG. Conversely, AEA exhibited no inhibitory effects on cell proliferative responses to stimulation by anti-CD3 monoclonal antibody, LPS, or phorbol-12-myristate-13-acetate/ionomycin treatment. AEA also showed no effect on the in vitro sheep erythrocyte antibody-forming cell response, while 2-AG exhibited no effect on basal adenylate cyclase activity in splenocytes. In addition, AEA showed negligible inhibitory effects on forskolin-stimulated adenylate cyclase activity and had no effect on basal adenylate cyclase activity in splenocytes. Coopman et al. (2007) found that 2-AG elicited the activation of downstream biochemical effectors based on assessment of phosphorylation of ERK1/2 MAP kinases in T lymphocytes. Since 2-AG inhibited CXCL12-induced chemotaxis, it was suggested that it played a modulatory role in activated T lymphocytes.

It has been demonstrated also that a cyclooxygenase (COX)-2 metabolite of 2-AG inhibits IL-2 secretion in activated T cells through peroxisome proliferator-activated receptor-γ (PPARγ) activation independent of the CB1R and CB2R. Rockwell et al. (2008) investigated the role of COX-catalyzed metabolism in the inhibition of IL-2 secretion by 2-AG. Pretreatment with nonselective and COX-2-selective inhibitors resulted in a complete abrogation of 2-AG-mediated suppression of IL-2 secretion. In contrast, pretreatment with COX-1-selective inhibitors had no effect upon 2-AG-mediated inhibition of IL-2 secretion. It was also demonstrated that while the potency of 2-AG was comparable between human Jurkat T cells and murine splenocytes, AEA was more potent in suppressing IL-2 production in Jurkat T cells compared to murine splenocytes. The COX-2 protein was detected in resting Jurkat T cells but not in resting mouse splenocytes. Furthermore, levels of COX-2 mRNA and protein were increased over basal levels 2h following the activation of Jurkat cells, whereas increases in COX-2 protein in murine splenocytes were not observed until 4h after cellular activation. The collective results suggested that the potency of AEA in the suppression of IL-2 secretion correlated with levels of COX-2 protein in T cells and that the 2-AG-mediated inhibition of IL-2 secretion was dependent upon COX-2-catalyzed metabolism.

Gasperi et al. (2014) found that 2-AG was able to initiate and complete the leukocyte adhesion cascade, by modulating the expression of selectins, cell adhesion molecules that are involved in lymphocyte homing and in chronic and acute inflammatory processes. A short exposure of primary human umbilical vein endothelial cells (HUVECs) to 2-AG was sufficient to prime them toward an activated state. Within 1 h of treatment, endothelial cells showed a time-dependent plasma membrane expression of P- and E-selectins, which both trigger the initial steps (i.e., capture and rolling) of leukocyte adhesion. The effect of 2-AG was mediated by the CB1R and CB2R and was long lasting, because endothelial cells incubated with 2-AG for 1 h released the proinflammatory cytokine TNF-α for up to 24 h. Consistently, TNF-α-containing medium was able to promote leukocyte recruitment. That is, human Jurkat T cells grown in conditioned medium derived from 2-AG-treated HUVECs showed enhanced L-selectin and P-selectin glycoprotein ligand-1 (PSGL1) expression, as well as increased efficiency of adhesion and transmigration. It was concluded that the in vitro data indicated that 2-AG, by acting on endothelial cells, could indirectly promote leukocyte recruitment.

3.5 Mast Cells

Mast cells are bone marrow-derived immune cells that are resident to several tissues in the body, including connective and mucosal tissues. These cells are involved in a variety of processes such as allergic inflammation, immune regulation, innate immunity, and parasite rejection (Metcalfe et al 1997). Mast cells (also known as mastocytes and labrocytes) contain granules rich in histamine and heparin. Facci et al. (1995) reported that mast cells contained the CB2R gene and expressed functional CB2R protein that was linked to negative regulatory effects on mast

cell activation. Although AEA bound to the CB2R, it was shown not to down-modulate mast cell activation in vitro. It was suggested that modulatory activities on mast cells supported the existence of an autacoid local inflammation antagonism (ALIA) mode of action. Lau and Chow (2003) reported that AEA, when used at concentrations higher than micromolar, induced the release of histamine. However, when mast cells were activated with anti-IgE, the histamine release that was induced was not affected by AEA. Furthermore, the histamine-releasing action of AEA on anti-IgE-induced histamine release was not reduced by the selective CB1R antagonist AM281 or the selective CB2R antagonist AM630. It was concluded that AEA, rather than suppressing mast cell activation, enhanced its activation. However, the high concentrations required, and the failure of cannabinoid receptor antagonists to reverse these effects, brought into question whether a functional linkage to a cannabinoid receptor existed. On the other hand, Vannacci et al. (2004) reported that the antigen-induced release of histamine from sensitized guinea pig mast cells was dose dependently reduced by 2-AG. The inhibitory action afforded by 2-AG was reversed by the selective CB2R antagonist SR144528 and unaffected by the selective CB1R antagonist AM251. The inhibitory action of 2-AG was reduced by the unselective NO synthase inhibitor L-NAME and reinstated by L-arginine, the physiological substrate. The inhibitory action of 2-AG also was reduced by the unselective cyclooxygenase (COX) inhibitor indomethacin and the selective COX-2 blocker rofecoxib. 2-AG also increased the production of nitrite from mast cells, an increase that was abrogated by the selective inducible NO synthase (iNOS) inhibitor L-NAME and N-(3-(aminomethyl)benzyl)acetamidine (1400W). 2-AG increased the generation of PEG_2 from mast cells, an increase that was abrogated by indomethacin and rofecoxib. In addition, mast cell challenge with antigen was accompanied by a net increase in intracellular calcium levels. 2-AG decreased the intracellular calcium level, an effect that was reversed by the CB2R antagonist SR144528 and the NO synthase inhibitor L-NAME. In unstimulated mast cells, 2-AG increased cGMP levels. This increase was abrogated by SR144528, L-NAME, indomethacin, and rofecoxib. The collective results suggested that 2-AG decreased mast cell activation in a manner that was linked functionally to the CB2R.

More recently, Sugawara et al. (2012) examined the effect of the activation of the CB1R on the biology of human skin mast cells in situ. The mast cell-rich connective tissue sheath of organ-cultured human scalp hair follicles was examined using quantitative immunohistomorphometry, ultrastructural analysis, and quantitative polymerase chain reaction (PCR). These assessments were conducted in concert with the use of CB1R agonists or antagonists, knockdown of CB1R expression, and the use of CB1R knockout mice. Mast cells within the connective tissue sheath of human hair follicles were shown to express functional CB1Rs. Their pharmacological blockade or gene silencing resulted in a stimulation of degranulation and the maturation of mast cells from resident progenitor cells in situ. This enhancement was shown to be, at least in part, stem cell factor dependent. CB1R agonists counteracted the mast cell-activating effects of classical mast cell secretagogues, substances that cause other substances to be secreted. A similar

outcome was obtained using CB1R knockout mice. It was suggested that locally synthesized endocannabinoids served to limit excessive activation and maturation of mast cells from resident progenitors through the mediation of a "tonic" CB1R stimulation.

3.6 Monocytes, Macrophages, and Macrophage-Like Cells

Monocytes constitute 2–10 % of leukocytes in humans and play multiple roles in immune function. These cells and their macrophage and dendritic cell progeny function in phagocytosis, antigen presentation, and cytokine production. Early studies indicated that endocannabinoids, principally AEA, inhibited the production of proinflammatory cytokines by macrophage-like cells. Cabral et al. (1995) reported that AEA inhibited mouse RAW264.7 macrophage-like cell killing of TNF-sensitive mouse L929 fibroblasts. Berdyshev et al. (1997) investigated the effects of AEA on the production of TNF-α, IL-4, IL-6, IL-8, IL-10, IFN-γ, p55, and p75 TNF-α soluble receptors by stimulated human peripheral blood mononuclear cells as well as [^{3}H]arachidonic acid release by non-stimulated and formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe, fMLP)-stimulated human monocytes. AEA was shown to diminish IL-6 and IL-8 production at low nanomolar concentrations but to inhibit the production of TNF-α, IFN-γ, IL-4, and p75 TNF-α soluble receptors at higher concentrations. The effect of AEA on IL-6 and IL-8 production disappeared when used at a higher concentration. AEA had no effect on IL-10 synthesis. The release of [^{3}H]arachidonate was stimulated only by high concentrations of AEA. Chang et al. (2001) compared the effects of AEA and 2-AG on LPS-induced NO, IL-6, and PGE_2 (prostaglandin E2) release from mouse J774 macrophage-like cells. AEA diminished LPS-induced NO and IL-6 production, while 2-AG inhibited the production of IL-6 but slightly increased inducible nitric oxide synthase (iNOS)-dependent NO production. AEA and 2-AG had no effect on LPS-induced PGE_2 production and COX-2 induction. It was proposed that the discrepant results of 2-AG on iNOS and COX-2 induction were due to its bioactive metabolites, arachidonic acid, and PGE_2, since incubation with these potentiated the induction of both iNOS and COX-2. The AEA metabolite, PGE_2-ethanolamide, had no effect on the production of the LPS-induced NO or IL-6. It was suggested that the results were consistent with a direct cannabinoid receptor activation leading to anti-inflammatory action through the inhibition of macrophage function. It was indicated, in addition, that 2-AG also served as a substrate for COX-catalyzing PGE_2 production, which in turn modulated the action of the CB2R. Ribeiro et al. (2010) investigated whether treatment in vivo with a low dose of AEA immediately prior to sensitization with ovalbumin that was injected at the base of the tail would have an immunosuppressive or immunostimulatory effect on the Th1 cell-mediated immune response in mice. It was reported that AEA, administered prior to sensitization, increased the Th1 response to ovalbumin in vivo and ex vivo. AEA increased the DTH response, splenocyte proliferation, and IFN-γ production in a co-culture of adherent and non-adherent splenocytes. Moreover,

administration of AEA prior to sensitization increased both the expression of dendritic cell co-stimulatory molecules (CD80/CD86) and IL-12/IL-23 (p40) production ex vivo. The direct effects of AEA in the IFN-γ/IL-4 balance of Concanavalin A (ConA)-stimulated splenocytes in vitro were also assessed. AEA at nanomolar concentrations increased the production of IFN-γ, but this production decreased at micromolar range. Thus, AEA induced both the increment of dendritic cell activation and IFN-γ production, considered likely factors involved in the increase of Th1 immune response.

Stefano et al. (2000) found that 2-AG caused human monocytes and immunocytes from the blue mussel, *Mytilus edulis*, to assume a round shape and become immobile, a transformation that was postulated to correlate with decreased production of cytokines and adhesion molecules associated with an immunosuppressive response. In addition, exposure of these cells to 2-AG resulted in NO release, which was blocked by the NO synthase inhibitor L-NAME and the CB1R antagonist SR141716A, but not by the CB2R antagonist SR144528. Similar results were obtained using cells related to the human vascular system. Treatment of human saphenous veins and atria with 2-AG stimulated basal NO release, which was antagonized by L-NAME and the CB1R antagonist. Kishimoto et al. (2003) examined the effects of 2-AG on the motility of human promyelocytic leukemia cells (HL-60 cells) that had been differentiated into macrophage-like cells. It was found that 2-AG induced the migration of these cells in a mode that was blocked by treatment with the CB2R antagonist SR144528 or pertussis toxin, consistent with a functional linkage to the CB2R and G_i/G_o proteins. In contrast, AEA did not induce migration by these cells. The 2-AG-induced migration was observed also for human monocytic leukemia U937 cells, human monocytic leukemic (THP-1) cells, and peripheral blood monocytes. Gokoh et al. (2005) reported that 2-AG enhanced the adhesion of HL-60 cells, differentiated into macrophage-like cells, to fibronectin and vascular cell adhesion molecule (VCAM)-1. The CB2R, G_i/G_o, intracellular free Ca^{2+}, and phosphatidylinositol 3-kinase were shown to be involved in 2-AG-induced augmented cell adhesion. 2-AG also enhanced the adhesion of U937 cells and peripheral blood monocytes. These results suggested that 2-AG played an essential role in inflammatory reactions and immune responses by inducing adhesion to extracellular matrix (ECM) proteins and adhesion molecules in several types of inflammatory cells and immune-competent cells. Gokoh et al. (2007) also examined the effect of 2-AG on the phagocytosis of opsonized zymosan by HL-60 cells that had been differentiated into macrophage-like cells. 2-AG augmented the phagocytosis of opsonized zymosan. Treatment of the HL-60 cells with the CB2R antagonist SR144528 or pertussis toxin abolished the effect of 2-AG, indicating that the CB2R and G_i/G_o were involved in the augmented phagocytosis. It was suggested that phosphatidylinositol 3-kinase and extracellular signal-regulated kinase were involved in this process since treatment of the HL-60 cells with wortmannin or PD98059 abrogated the 2-AG-augmented phagocytosis. More recently, Montecucco et al. (2009) assessed levels of endocannabinoids and related molecules during atherosclerosis development in mice. It was found that endocannabinoid-degrading enzymes were expressed by macrophages within

atherosclerotic lesions. In vitro, 2-AG induced monocyte migration, which corresponded to the levels observed in aortas. It was suggested that enhanced 2-AG levels in advanced atherosclerotic lesions triggered the inflammatory process by recruiting inflammatory cells and inducing ECM degradation through the CB2R. However, while this possibility was supported in vitro, in vivo experiments using the CB2R antagonist SR144528 failed to provide confirmation.

3.7 Natural Killer Cells

Natural killer (NK) cells are a type of cytotoxic lymphocyte that provides rapid responses to virally infected cells and tumor cells. NK cells are capable of recognizing stressed cells in the absence of antibodies and markers of the major histocompatibility complex (MHC). That is, they do not require activation in order to kill cells that are missing "self" markers of the MHC class 1. The functional role of NK cells is important since cells that are missing MHC 1 markers cannot be detected and destroyed by other immune cells, such as T cells. Kishimoto et al. (2005) examined the effects of 2-AG on the motility of human NK cells. It was found that 2-AG induced the migration of a line of natural killer leukemia cells (KHYG-1 cells) and human peripheral blood NK cells. The migration of NK cells induced by 2-AG was abolished by treating the cells with SR144528, a CB2R antagonist, suggesting that the CB2R was involved in the 2-AG-induced migration. In contrast, AEA did not induce migration of these cells.

3.8 Neutrophils

Neutrophils constitute approximately 60 % of PMNs and contain lysosomal enzymes in their cell granules that break down bacterial cells. In this capacity, they play a critical role in acute inflammation and provide a first line of defense against microbes. Chemotactic signals include IL-8, the streptococcal peptidase C5a, the bacterial protein derivative fMLP, and leukotriene B4. Kraft and Kress (2005) investigated the respiratory burst reaction of human whole-blood PMNs under the influence of cannabinoids using flow cytometry. In their natural whole-blood milieu, a CB2R-dependent stimulation of the PMN respiratory burst was found at nanomolar concentrations of methanandamide, a synthetic relatively stable derivative of AEA, whereas the short-living and rapidly hydrolyzed endogenous ligand AEA did not alter the burst reaction of whole-blood PMNs. However, the stimulatory cannabinoid effect was absent in isolated PMNs but could be transferred onto isolated polymorphonuclear leukocytes by adding the cell-free low-molecular mass plasma fraction (<5000 Da) of cannabinoid-incubated blood, consistent with an indirect mode of action that was dependent on humoral products or mediators. It was suggested that products of arachidonic acid metabolism acted as mediators of the cannabinoid-induced enhancement of the respiratory burst reaction of whole-blood PMNs. Kurihara et al. (2006) found that upon stimulation

with 2-AG, HL60 cells rapidly extended and retracted one or more pseudopods containing F-actin in different directions instead of developing a front/rear polarity typically exhibited by migrating leukocytes. Activity of Rho-GTPase RhoA decreased in response to CB2R stimulation, whereas that of Rac1, Rac2, and Cdc42 increased. Moreover, treatment of cells with the RhoA-dependent protein kinase (p160-ROCK) inhibitor Y27632 yielded a cytoskeletal organization similar to that observed for CB2R-stimulated cells. In human neutrophils, 2-AG did not induce motility or morphologic alterations. Pretreatment of neutrophils with 2-AG disrupted fMLP-induced front/rear polarization and migration and suppressed fMLP-induced RhoA activity. The 2-AG results were replicated using JWH015, a CB2R-selective agonist. The collective results suggested that the CB2R played a role in regulating excessive inflammatory responses by controlling RhoA activation that, in turn, resulted in a suppression of neutrophil migration.

On the other hand, there have been reports that receptors other than the CB2R, or pathways not involving this receptor, are involved in the modulation of neutrophil functional activities. McHugh et al. (2008) found that certain endogenous lipids, including AEA, inhibited human neutrophil migration at nanomolar concentrations in a biphasic manner. These investigators implicated a pharmacological target that was distinct from the CB1R and CB2R that was antagonized by the endogenous compound *N*-arachidonoyl l-serine. Balenga et al. (2011) suggested that GPR55 modulated CB2R-mediated responses. It was found that GPR55 was expressed in human blood neutrophils and that its activation augmented their migratory response toward 2-AG while concomitantly inhibiting neutrophil degranulation and reactive oxygen species (ROS) production. Chouinard et al. (2011) postulated that arachidonic acid released by 2-AG and AEA hydrolysis was metabolized into eicosanoids and that these metabolic products could, in turn, serve as mediators of some of the effects of endocannabinoids. It was concluded that while 2-AG activated human neutrophils, it did so indirectly as a result of 2-AG hydrolysis, de novo LTB_4 biosynthesis, and an autocrine activation loop that involved the LTB_4 receptor 1. In a subsequent series of studies, Chouinard et al. (2013) reported that 2-AG and arachidonic acid-stimulated neutrophils released a variety of antimicrobial effectors. Supernatants of neutrophils activated with nanomolar concentrations of 2-AG and arachidonic acid inhibited the infectivity of herpes simplex virus type 1 (HSV-1) and respiratory syncytial virus (RSV). In addition, these supernatants impaired the growth of *Escherichia coli* and *Staphylococcus aureus*. The impairment of bacterial growth correlated with the release of α-defensins, as well as a limited amount of the antimicrobial peptide LL-37 (cathelicidin). The effects of arachidonic acid and 2-AG were prevented by inhibiting LTB_4 biosynthesis or by blocking that of BLT_1. CB2R agonists or antagonists could neither mimic nor prevent the effects of 2-AG. It was suggested that the rapid conversion of 2-AG to arachidonic acid and their metabolism into LTB_4 (leukotriene B4) promoted 2-AG and arachidonic acid as multifunctional activators of neutrophils, exerting their effects primarily by activating the BLT_1 receptor.

4 Immune Cells in the Central Nervous System

The CNS harbors an endogenous "immune" system that includes astrocytes, microglial cells, and perivascular macrophages. Endocannabinoids have been linked to the modulation of these cells' functional capabilities and play a role in regeneration of damaged CNS tissue. For example, studies performed using the medicinal leech have provided insights into the regeneration of the CNS following mechanical trauma. Using an electrochemical NO-selective electrode to measure NO levels, Arafah et al. (2013) found that the time course of NO release in the injured leech CNS was partially under the control of AEA and 2-AG. The results showed that after injury concurrent with ATP production, purinergic receptor activation, NO production, microglial recruitment, and accumulation to the lesion site, an imbalance occurred in the endocannabinoid system.

4.1 Astrocytes

Astrocytes, or astroglia, are the most abundant cell type in the human CNS. These cells provide nutrients to nervous tissue, maintain extracellular ion balance, and play a role in the repair and scarring of the brain and spinal cord following traumatic injuries. They also elicit a plethora of proinflammatory cytokines upon secondary activation by inflammatory factors released from microglia. Molina-Holgado et al. (1997) showed that primary cultures of neonatal mouse cortical astrocytes stimulated with LPS (BALB/c mice strain) or Theiler's murine encephalomyelitis virus (TMEV) (SJL/J mice strain), used as an encephalomyelitis model for MS, released increased amounts of nitrites (NO_2-) and TNF-α. AEA blocked the release of NO_2- and TNF-α that was induced by LPS. In TMEV-stimulated astrocytes, AEA also suppressed the stimulatory effects of TMEV on both NO_2- and TNF-α. Molina-Holgado et al. (1998) also investigated whether AEA could modify IL-6 production by primary cultures of mouse brain cortical astrocytes infected with TMEV. Infection of a susceptible strain of mice resulted in virus persistence in the brain and chronic primary immune-mediated demyelination, which resembled MS. Astrocytes from susceptible (SJL/J) and resistant (BALB/c) strains of mice infected with TMEV released higher levels of IL-6. AEA caused an enhancement of the release of IL-6 by the TMEV-infected astrocytes. Treatment of TMEV-infected astrocytes with arachidonyl trifluoromethyl ketone, a potent inhibitor of the amidase that degrades AEA, potentiated the effects of AEA on IL-6 release. The selective CB1R antagonist SR141617A blocked the enhancing effects of AEA on IL-6 release by TMEV-infected astrocytes, suggesting a linkage to this cannabinoid receptor in the AEA-mediated enhancement of IL-6 release. Molina-Holgado et al. (2002) extended these studies regarding the role of the CB1R in mediating the actions of AEA. Coincubation of primary mouse astrocyte cultures with AEA resulted in an inhibition of LPS-induced release of NO. The inhibitory effect was abolished by the CB1R antagonist SR141716A. Furthermore, SR141716A alone increased NO release in response to LPS, suggesting that endocannabinoids

modified inflammatory responses. In addition, coincubation of astrocytes with the CB2R antagonist SR144528 abolished the inhibitory effects of AEA on LPS-induced NO release. Ortega-Gutiérrez et al. (2005) studied whether UCM707, a potent and selective AEA uptake inhibitor, was able to inhibit the production of proinflammatory mediators by LPS-stimulated astrocytes. UCM707 reduced NO release, iNOS expression, and the production of the proinflammatory cytokines TNF-α and IL-1β, while producing a slight increase in IL-6 levels.

4.2 Microglia

Microglia are resident macrophages in the brain and spinal cord. These cells exhibit many of the functional properties of macrophages in tissues at nonneuronal sites and upon stimulation release chemokines and proinflammatory cytokines. Kreutz et al. (2009) investigated whether the activation of the "abnormal cannabidiol" (abn-CBD) receptor, a receptor that is coupled to a G_i/G_o protein and is sensitive to abn-CBD (a synthetic isomer of CBD) that is inactive at the CB1R or CB2R, contributed to 2-AG-mediated neuroprotection. In their studies, they used an organotypic hippocampal slice culture system that included an excitotoxic lesion induced by *N*-methyl-D-aspartate (NMDA) that causes neuronal damage and accumulation of microglia within the granule cell layer. Application of abn-CBD or 2-AG to lesioned slice cultures resulted in a decrease in the number of microglia and neurons in the dentate gyrus. 2-AG was reported to exert a neuroprotective effect through the activation of abn-CBD-sensitive receptors on the microglia. It was indicated that 2-AG modulated migration and proliferation of microglia that then were rapidly activated following introduction of the brain lesion. Furthermore, these modulatory effects were attributed to the activation of CB2R and abn-CBD-sensitive receptors. McHugh et al. (2010) investigated the relationship between *N*-arachidonoyl glycine (NAGly), an endogenous metabolite of AEA that reportedly acts as an agonist at the G protein-coupled receptor GPR18. It was found that NAGly acted as an effective lipid recruiter of mouse BV-2 microglial-like cells and that it mimicked the effects of abn-CBD. NAGly exhibited marked potency for acting on GPR18 to elicit directed migration and proliferation. Based on these data, it was suggested that GPR18, also referred to as the *N*-arachidonoyl glycine receptor, was the "abn-CBD" receptor.

Navarrete et al. (2009) examined the role of AEA and *N*-arachidonoyldopamine (NADA) in the regulation of PGE2 synthesis in primary glial cells. It was demonstrated that NADA exerted a robust inhibition of PGE2 synthesis in LPS-stimulated cells, without modifying the expression or enzymatic activity of COX-2 and the production of PGD2 (prostaglandin D2). It was shown also that NADA prevented free radical formation in primary microglia. Thus, AEA and NADA exerted opposite effects on glial cells. The results suggested that NADA had the potential to serve as an antioxidative and anti-inflammatory agent acting through a mechanism that involved reduction in the synthesis of microsomal PGE in LPS-activated microglia.

Correa et al. (2009) investigated the effects of AEA on the inducible expression of IL-12p70 and IL-23 and their forming subunits in activated human and mouse microglia. They also studied the signaling pathways involved in the regulation of IL-12p70/IL-23 expression. It was reported that AEA inhibited the production of biologically active IL-12p70 and IL-23 and their subunits. Treatment of these activated cells with inhibitors of mitogen-activated protein kinases (MAPK) revealed that AEA acted through the ERK1/2 and JNK pathways to downregulate IL-12p70 and IL-23 and that these effects were mediated, at least in part, by the activation of the CB2R. These investigators (Correa et al. 2010) subsequently showed that AEA enhanced LPS/IFN-γ-induced IL-10 production in microglia by targeting the CB2R through the activation of ERK1/2 and JNK MAPKs. AEA also inhibited NF-κB activation by interfering with the phosphorylation of IκBα, an action that was postulated to account for the increase of IL-10 production. It was suggested that, by altering the cytokine network, AEA indirectly modified the type of immune responses within the CNS. Hernangomez et al. (2012) reported that AEA protected neurons from microglial-induced neurotoxicity through a CD200–CD200R interaction. CD200 is a membrane glycoprotein expressed in neurons that suppresses immune activity through its receptor CD200R that is located mainly in macrophages/microglia. It was indicated that the AEA-mediated increase of the expression of CD200R1 in LPS/IFN-γ-activated microglia was due to the activation of the CB2R. The neuroprotective effect of AEA disappeared when microglia were obtained from CD200R1$^{-/-}$ mice, lending support for a functional linkage to the CB2R. In the chronic phases of Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), used as a model of MS, the expression of CD200 and CD200R1 was reduced in the spinal cord. AEA-treated animals exhibited an upregulation in the expression of CD200 and CD200R1 to levels comparable to those found in sham animals. The AEA-treated animals also exhibited an increase in the expression of IL-10 and a reduction in that of IL-1β and IL-6. Because AEA upregulated the expression of CD200R1 in microglia, but failed to enhance CD200 in neurons, it was suggested that the AEA-induced upregulation of CD200 in the TMEV-IDD paradigm was mediated through the action of IL-10 as the level of this anti-inflammatory cytokine was shown to be increased in CD200-positive neurons.

5 Effects of Endocannabinoids In Vivo

Experimental models using animals such as guinea pigs and mice have been employed for nearly a century to document effects of various toxic and infectious agents on host resistance. These in vivo models have offered unique advantages for assessment of effects of drugs on infection and immunity due to their well-defined immune systems. Furthermore, the use of animal models has allowed for the definition of factors of host resistance that are targeted by drugs under stringently controlled conditions. As a result, acquisition of statistically significant data with minimal confounding variables has been possible, a condition that is difficult to

attain for human populations as a result of potential environmental toxic exposures and multiple drug use. The conducting of in vivo studies using endocannabinoids has been challenging, partially because these substances are readily degraded, necessitating that they be applied experimentally at relatively high doses. Furthermore, their intracellular fate, compartmentation, and processing within the host may be distinctive from that of exogenously introduced synthetic cannabinoids. Nevertheless, given these caveats, the results obtained with endocannabinoids through in vivo studies are in general agreement with those that have been derived from in vitro studies.

Maestroni (2004) suggested that 2-AG acted as a chemotactic substance that recruited dendritic cells or their precursors during the innate immune response. These cells, in the presence of a Toll-like receptor (TLR) agonist, display a T helper-1 (Th1)-shifted adaptive response. It was demonstrated that 2-AG injected intradermally in mice together with a soluble protein and a T helper-2 (Th2) priming TLR agonist during primary immunization shifted the memory response to the Th1 type. This effect was demonstrated by the enhanced hypersensitivity response and by the Th1 pattern of cytokines that were produced, a result that was abolished by the CB2R antagonist SR144528. It was postulated that 2-AG operated during the innate immune response by increasing the number of dendritic cells migrating to draining lymph nodes. Oka et al. (2005) investigated the pathophysiological roles of the CB2R and 2-AG in acute inflammation in the mouse ear that was induced by the topical application of 12-O-tetradecanoylphorbol-13-acetate. It was found following treatment that the amount of 2-AG was markedly augmented while that of AEA was not affected. The 12-O-tetradecanoylphorbol-13-acetate-induced ear swelling was blocked by the CB2R antagonist SR144528. In addition, the application of 2-AG to the mouse ear-evoked swelling was abolished by the CB2R antagonist SR144528. It was suggested that NO was involved in the ear swelling induced by 2-AG, implicating a role for neutrophils in this process, and that the CB2R and 2-AG played crucial stimulative roles during the course of the inflammatory reaction. Mimura et al. (2012) using the ear dermatitis model showed that the level of 2-AG increased upon serial 2,4-dinitrofluorobenzene challenges and was correlated with ear weight gain. The increased ear thickness in this allergy model was clearly suppressed in CB2R knockout mice, suggesting that generated endocannabinoids induced ear thickness through aberrant inflammatory responses and remodeling mediated through the CB2R. In addition, in an allergic bronchitis model induced by ovalbumin, the 2-AG level in bronchoalveolar lavage was increased and sustained during the elevation of inflammatory cell infiltration. DNA microarray analysis of human HL-60 cells revealed that the 2-AG ether, noladin ether, induced the expression of inflammatory chemokines/cytokines and cell growth factors. The data suggested that endocannabinoids that served as endogenous CB2R ligands that were upregulated upon disease progression in allergic models were involved in aberrant alterations of both inflammatory responses and tissue cell growth. Ribeiro et al. (2010) investigated whether treatment in vivo with a low dose of AEA immediately prior to in vivo sensitization with ovalbumin had an immunosuppressive versus an immunostimulatory effect on cell-

mediated immunity (i.e., Th1 response) in mice. Administration of AEA prior to sensitization resulted in an increase in the Th1 response to ovalbumin in vivo and ex vivo. AEA administration resulted in an increase in the DT response, splenocyte proliferation, and IFN-γ production in a co-culture of adherent and non-adherent splenocytes. Moreover, AEA administration prior to sensitization resulted in an increase in both the expression of dendritic cell co-stimulatory molecules (CD80/CD86) and IL-12/IL-23 (p40) production ex vivo. Direct effects of AEA in the IFN-γ/IL-4 balance of ConA-stimulated splenocytes in vitro also were assessed. AEA at nanomolar concentrations increased the production of IFN-γ. Thus, AEA induced both dendritic cell activation and IFN-γ production, key factors involved in the increase of the Th1 response. Jackson et al. (2014) observed that a single intraperitoneal administration of AEA caused rapid induction of myeloid-derived suppressor cells. This heterogeneous population of cells consisted of a mixture of granulocytic and monocytic subtypes and expressed arginase-1 (Arg-1), a binuclear manganese metalloenzyme that catalyzes the conversion of L-arginine into L-ornithine and urea, and iNOS. The myeloid-derived suppressor cells inhibited T-cell proliferation in vitro mediated through iNOS. In addition, mice were sensitized by a subcutaneous injection of methylated bovine serum albumin (mBSA). Twelve hours before mBSA rechallenge, mice were injected with myeloid-derived suppressor cells. Adoptive transfer of myeloid-derived suppressor cells led to suppression of methylated bovine serum albumin (mBSA)-induced DTH. Through the use of pharmacological inhibition, as well as genetic knockout mice, it was found that the induction of myeloid-derived suppressor cells by AEA was dependent on the CB1R. The induction of myeloid-derived suppressor cells by AEA was reduced in mast cell-deficient mice, while maintained in LPS-insensitive mice, showing that the induction of myeloid-derived suppressor cells by AEA was dependent, at least in part, on mast cells and independent of Toll-like receptor 4 (TLR4), a receptor that detects LPS from gram-negative bacteria and leads to the activation of the immune system. Chemokine analysis of AEA-treated wild-type mice showed an early spike of monocyte chemoattractant protein-1 (MCP-1). This spike was decreased in Kit (W/W-sh) mast cell-deficient mice, implicating a role of mast cells in the secretion of MCP-1 in response to AEA. Use of antibodies against MCP-1 or mice deficient in MCP-1 confirmed the linkage to MCP-1. It was concluded that endocannabinoids activated the CB1R on mast cells to induce MCP-1, which facilitated recruitment of monocytic myeloid-derived suppressor cells.

There have been a limited number of studies that have addressed the effects of endocannabinoids on peripheral, nonneuronal pathological processes. Sugamura et al. (2009) sought to determine whether the endocannabinoid system was involved in human atherosclerosis in which plaque builds up inside the arteries. They investigated whether pharmacological blockade of the CB1R could modulate proinflammatory activity in macrophages. Patients with coronary artery disease demonstrated the activation of the endocannabinoid system that was accompanied by elevated levels of blood endocannabinoids and increased expression of CB1R in coronary atheroma, an accumulation of fatty material within the inside lining of the arteries. It was indicated that blockade of the CB1R in macrophages with the

CB1R-selective antagonist rimonabant (SR141716A) led to anti-inflammatory effects on the part of these cells. Lenglet et al. (2013) assessed atherosclerosis in apolipoprotein E-deficient ($ApoE^{-/-}$) and $ApoE^{-/-}FAAH^{-/-}$ mice. These investigators observed enhanced recruitment of neutrophils, but not monocytes, to large arteries of $ApoE^{-/-}$ mice treated with the FAAH inhibitor URB597. Spleens of $ApoE^{-/-}FAAH^{-/-}$ mice had reduced CD4+FoxP3+regulatory T-cell content, and in vitro stimulation of splenocytes revealed significantly elevated IFN-γ and TNF-α production in the case of FAAH deficiency. It was concluded that increased AEA and related FAAH substrate levels were associated with the development of smaller atherosclerotic plaques with high neutrophil content that was accompanied by an increased proinflammatory immune response. Rettori et al. (2012) investigated the role of AEA in experimental periodontitis, an infectious disease leading to inflammation and destruction of tissue surrounding and supporting the tooth. In this disease, the progress of the inflammatory response depends on the host's immune system and risk factors such as stress. Experimental periodontitis was induced by a ligature around the first inferior molars and immobilization stress for 2 h twice daily for 7 days in a rat model. AEA was shown to diminish the inflammatory response in periodontitis even during a stressful situation. Donovan and Grundy (2012) examined cannabinoid modulation of sensory signaling from the gastrointestinal tract following an acute inflammatory response triggered by systemic administration of LPS. A segment of proximal jejunum was intubated to allow for measurement of intraluminal pressure in anesthetized rats. Afferent impulse traffic was recorded from a single isolated paravascular nerve bundle supplying the jejunal loop. It was found that the AEA transport inhibitor, VDM11, but not the FAAH inhibitor URB597, caused an increase in afferent activity. The VDM11 response was found to be linked to mediation by both the CB1R and CB2R. LPS evoked an increase in afferent activity that was reduced by the blockade of the CB1R and CB2R. It was concluded that endocannabinoids played a role in modulating afferent signaling and that, in this context, the endocannabinoid system represented a target for the treatment of visceral hypersensitivity. However, inhibition of the breakdown of endocannabinoids through the use of URB597 had no effect on baseline or LPS-induced afferent firing. These observations suggested that the uptake of endocannabinoids rather than their breakdown mediated by FAAH terminated their action in the gastrointestinal tract.

6 Effects of Endocannabinoids on Immune Function in the CNS

Since endocannabinoids have been shown to ablate immune functional activities, they have been proposed as candidate agents for treatment of a variety of pathological processes in the CNS that are characterized by a hyperimmune response. In this context, these substances afford several advantages. They have low cytotoxicity and readily cross the blood–brain barrier (BBB) due to their lipophilicity. Furthermore, as in the case of 2-AG, they target the CB2R that is expressed on selective

cell types that have immune function capability within the CNS. The selective targeting of the CB2R is important since the activation of the CB2R obviates untoward psychotropic effects that could be engendered if signal transduction were effected through the CB1R. Finally, due to the short half-life of endocannabinoids, their effects are postulated to be short-lived, thereby minimizing the potential for long-term perturbation of immune functional capability within the CNS. It is now apparent that resident immune cells within the CNS and eye harbor an endocannabinoid system (Suárez et al. 2010; Hu et al. 2010). Recently, Krishnan and Chatterjee (2014) suggested that endocannabinoids had potential as neurotherapeutic agents for select conditions linked to human immunodeficiency virus (HIV-1)-induced inflammation. They investigated whether the innate immune response in human retinal Muller glia could be modulated to combat inflammation since an increased inflammatory response can cause visual impairment during HIV infection in spite of successful antiretroviral therapy. AEA and 2-AG were used to alleviate cytotoxicity induced by the HIV-specified nonstructural transactivating protein Tat (trans-activator of transcription) and to rescue retinal cells. The neuroprotective effect of these endocannabinoids involved suppression of the production of proinflammatory cytokines and augmentation of that of anti-inflammatory cytokines. This altered expression was effected mainly through the activation of the MAPK pathway and regulated primarily by mitogen-activated protein kinase phosphatase-1 (MKP-1). Both endocannabinoids regulated cytokine production of the transcriptional level of the NF-κB complex, including that of IL-1 receptor-associated kinase 1 binding protein 1 (IRAK1BP1) and TGF-β-activated kinase 1/MAP3K7 binding protein 2 (TAB2), a signal transducer that acts as an adaptor molecule of TNF receptor-associated factor 6 (TRAF6) and TGF-β-activated kinase 1 (TAK1) and mediates the activation of TAK1 (a ubiquitin-dependent kinase of mitogen-activated protein kinase and IκB kinase). Nevertheless, there has been a paucity of studies that have addressed from an immunological perspective the effect of endocannabinoids on neuropathological processes in animal models and humans. The studies that have been conducted have focused on experimentally induced Alzheimer's disease (Piro et al. 2012), multiple sclerosis (Bittner et al. 2009; Rossi et al. 2011; Correa et al. 2011; Mestre et al. 2011; Lourbopoulos et al. 2011; Sanchez Lopez et al. 2014), Parkinson's disease (Fernandez-Suarez et al. 2014), and traumatic brain injury (Katz et al. 2014).

7 Summary and Conclusions

Lipid-derived messengers and their cognate receptors cooperate with other signaling molecules to modulate the functional activities of immune cells at nonneuronal and neuronal sites. Included among these bioactive lipids are endocannabinoids, endogenous cannabinoids that have the potential to signal through the seven-transmembranal, G protein-coupled CB1R and CB2R. Their ligation to, and activation of, these receptors sets in motion a series of signal transductional events that converge at the transcriptional level to regulate cell migration and the production of

cytokines and chemokines. There is a large body of data that supports a functional relevance for 2-AG as acting through the CB2R to inhibit migratory activities for a diverse array of immune cell types, and it has been suggested that 2-AG is the cognate functionally relevant endocannabinoid for the CB2R (Sugiura et al. 2000; Parolaro et al. 2002). AEA has been reported to inhibit immune functional activities, particularly the production of proinflammatory cytokines. However, unequivocal evidence that supports a functional linkage of AEA to a cannabinoid receptor in mediating these effects remains to be obtained. Endocannabinoids, typical of bioactive lipids, have a short half-life intracellularly and extracellularly and appear to act in an autocrine and paracrine fashion. Thus, it appears that their immediate effective action on immune function is at localized sites in the periphery and CNS. It is speculated that, in this context, endocannabinoids play an important role in maintaining the overall "fine-tuning" of the immune homeostatic balance within the host. Recognition that immune functional activities can be mediated through interaction of lipid ligands with specified receptors leading to the activation of signaling cascades should provide unique and novel insights into the development of therapeutics for the manipulation and ablation of untoward immunological events, including possibly those associated with infection with the HIV and other pathogens. Furthermore, definition of the functional relevance of lipid bio-effector molecules in the immune system could lead to the development of strategies for therapeutic intervention related to the use of illicit drugs that may interfere with endogenous immune homoeostatic and modulatory processes.

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