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Endocannabinoids and immune regulation☆

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Abstract

Cannabinoid pharmacology has made important advances in recent years after the discovery of the cannabinoid receptors. These discoveries have added to our understanding of exogenous and endogenous cannabinoid signaling along with exploring the various pathways of their biosynthesis, molecular structure, inactivation, and anatomical distribution of their receptors throughout the body. The endocannabinoid system is involved in immunoregulation and neuroprotection. In this article, we have reviewed the possible mechanisms of the regulation of the immune response by endocannabinoids which include modulation of immune response in different cell types, effect on cytokine network, induction of apoptosis in immune cells and downregulation of innate and adaptive immune response. Studies from our laboratory have suggested that administration of endocannabinoids or use of inhibitors of enzymes that breakdown the endocannabinoids, leads to immunosuppression and recovery from immune-mediated injury to organs such as the liver. Thus, manipulation of endocannabinoids in vivo may constitute a novel treatment modality against inflammatory disorders.

Keywords

Endocannabinoids; Immune response

1. Endogenous cannabinoid system

The discovery of cannabinoid receptors occurring naturally throughout the vertebrate body and the availability of highly selective and potent canabimimetics led to the identification of a naturally occurring lipid signaling system termed the endocannabinoid system. Interestingly, the endocannabinoid system dates back very long in the evolution because it exists as an ancient plant signaling system regulating the plant immunity-related genes in response to infection and stress [1]. The stereoselective binding sites for endocannabinoid ligands have been found in invertebrate immunocytes and microglia showing that this system has been conserved throughout evolution from coelenterates to man and is therefore considered as a very successful evolutionary principle [2]. The endocannabinoid family comprises of a family of lipid transmitters serving as natural ligands for the cannabinoid receptors and the enzymes for biosynthesis and degradation of these ligands. The first endocannabinoid discovered was named as N-arachidonoylethanolamide (anandamide, AEA) from the Sanskrit “internal bliss” by Devane et al. [3]. AEA is an amide of arachidonic acid and ethanolamine. Subsequently, 3 years later, another ligand named 2-arachidonoylglycerol (2-AG) was discovered independently by Mechoulam et al. [4] and

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Sugiura et al. [5], which was found to be in much higher concentration in serum and brain than anandamide.

Mammalian tissues are known to express primarily two types of cannabinoid receptors which are G-protein coupled. These include CB1 receptors, cloned in Tom Bonner's laboratory in 1990 and CB2 receptors, cloned by Sean Munro in 1993. Both CB1 and CB2 receptors regulate the release of chemical messengers, where CB1 receptors are found mainly on neurons (and in some non-neuronal tissues like pituitary gland, immune cells and reproductive tissues), and CB2 receptors are found primarily on immune cells. Anandamide binds to the brain CB1 with high affinity and mimics the behavioral actions of exogenous cannabinoid, Δ^9-tetrahydrocannabinol (Δ^9-THC) when injected into rodents. 2-AG has similar affinities for both CB1 and CB2 receptors comparable to those of anandamide but it exhibits higher efficacy than anandamide. Other pharmacological targets for anandamide, other than cannabinoid receptors, have also been described. The most accepted receptor to be activated by anandamide and other synthetic analogs of endocannabinoids (methanamide, arachidonyl-2′-chloroethylamide) is TRPV1 (vanilloid VR1) receptor [6,7]. These findings have triggered an exponential growth in studies describing the endocannabinoid synthesis, their release [8], transport [9] and degradation [10], constituting the new ubiquitous "endogenous cannabinoid system".

The main pharmacological functions of the endocannabinoid system include neuromodulation, controlling motor functions, cognition, emotional responses, homeostasis and motivation. However, in the periphery, this system is an important modulator of autonomic nervous system, the immune system and microcirculation.

1.1. Biosynthesis of endocannabinoids

Endocannabinoids are not stored in vesicles or cells like neurotransmitters; rather their biosynthesis takes place on demand from lipid precursors present in the cytoplasmic membrane through enzyme activation in response to elevations of intracellular calcium. The immediate precursor to AEA is *N*-arachidonoyl phosphatidylethanolamine, which is formed from phosphatidylcholine and phosphatidylethanolamine and is converted to anandamide by the action of *N*-arachidonoyl phosphatidylethanolamine-phospholipase [11]. Synthesis of 2-AG depends on the conversion of 2-arachidonate-containing phosphoinositides to diacylglycerols (DAGs), which are then converted to 2-AG by the action of DAG lipase. Also, like anandamide, 2-AG is thought to be removed from its sites of action by cellular uptake and metabolized intracellularly [12]. Release of endocannabinoids is stimulated in a receptor-dependent manner by neurotransmitters. It is noteworthy that some effects of endogenously released anandamide and 2-AG may be enhanced through an "entourage effect" or 'ALIA' mechanism that relies on the corelease of other endogenous fatty acid derivatives as first described by Facci et al. [13]. These derivatives include palmitoylethanolamide (PEA) and oleamide, which can potentiate anandamide, and 2-linoleyl glycerol and 2-palmitoyl glycerol, which can potentiate 2-AG [6].

The endocannabinoids are removed from the site by cellular uptake processes such as simple diffusion, through membrane associated binding proteins or by a transmembrane carrier protein. Inside the tissue, their metabolism is catalyzed by fatty acid amide hydrolase (FAAH) [12,14]. FAAH is found postsynaptically mainly in soma and dendrites of principal neurons and is located on cytosolic surfaces of SER cisternae and mitochondria. In some tissues, however, endocannabinoids also undergo an oxidative catabolism through palmitoylethanolamide-preferring acid amidase (PAA) lipoxygenases, cyclooxygenase-2 and cytochrome P450. Thus, anandamide and 2-AG can be released from both neuronal and non-neuronal cells whenever the need arises, and they utilize analogous but distinct receptor-dependent pathways regulating the effects of primary messengers, such as

neurotransmitters and hormones. In certain disorders such as multiple sclerosis, cancer, intestinal disorders, cardiovascular disorders, pain, Parkinson's disease and excitotoxicity, the tissue concentration of endocannabinoids, cannabinoid receptor density and the cannabinoid receptor coupling efficiency increases resulting in the reduction of symptoms of these disorders. The endocannabinoid system has been shown to be involved in various physiological processes like lipogenesis, inflammation, food intake and nociception.

2. Endocannabinoid receptors in immune system

Endocannabinoids are believed to control immune functions and play a role in immune homeostasis. Immune cells express both CB1 and CB2 receptors, secrete endocannabinoids and have functional cannabinoid transport and breakdown mechanisms [15,16]. Human peripheral blood immune cells are reported to have different degrees of cannabinoid receptor expression with the following rank order: B cells > NK cells > monocytes > polymorphonuclear neutrophils > CD8 lymphocytes > CD4 lymphocytes [17]. The CB1 receptors are densely expressed in the central nervous system and mediate neurobehavioral effects. The expression levels of CB2 receptors in immune cells are 10–100 times greater than CB1 receptors. Moreover, CB2 receptor mRNA was also detected in the cortex of lymph nodes and the nodular corona of Peyer's patches [18].

3. Endocannabinoid biosynthesis and signaling in the immune system

There is significant biochemical evidence to suggest that biosynthesis, uptake and degradation of endocannabinoids occur in macrophages and leukocytes [15,16,19]. This finding supports the role of endocannabinoids as local modulators of immune and inflammatory reactions. It was observed that both RBL-2H3 basophil cells and J774 macrophages can biosynthesize AEA and PEA through hydrolysis of corresponding N-acylphosphatidylethanolamines. Both these cell lines could inactivate the two bioactive acylethanolamide by sequestering radiolabelled AEA and PEA from the culture medium. Furthermore, following uptake by basophils, AEA and PEA competed for the same inactivating enzyme which catalyzed their hydrolysis to ethanolamine. Maccarrone et al. demonstrated that LPS down-regulated FAAH expression and increased AEA levels in human peripheral lymphocytes [20,21]. The effect of this endotoxin on FAAH was not mediated by AEA-induced activation of cannabinoid receptors. In fact, the stimulatory action of LPS on AEA levels was considered to be due to the inhibition of FAAH as suggested by the observation that an increase in levels of AEA was also induced by an irreversible FAAH inhibitor. Varga et al. demonstrated that rat platelets and macrophages contained the endogenous cannabinoid, 2-AG, and in vitro exposure to LPS markedly increased 2-AG levels [22]. Thus, these results indicated that immune cells take part in regulating peripheral endocannabinoid system and endocannabinoid homeostasis. It has been shown that human mast cells also take up AEA followed by its hydrolysis by FAAH [21]. Thus, mast cells contribute towards regulation of peripheral endocannabinoid system thereby affecting endocannabinoid mediated effect in inflammation, vascular tone and other immune interactions [21].

The CB1 and CB2 receptors negatively regulate adenylyl cyclase activity through pertussis toxin-sensitive GTP-binding protein. Endocannabinoids have been well known to exhibit this property, which is considered to be an important mechanism of lymphocyte regulation [4,23]. As cAMP signaling cascade has a positive regulatory role in immune cell function, cannabinoid receptor stimulation could antagonize the early events in immune cell activation. Besides, CB1 and CB2 receptors also stimulate mitogen activated protein kinase (MAPK) activity. Endocannabinoids have also been shown to induce MAPK pathway, which is a CB2 receptor-mediated response [24]. Thus, it is now evident that cannabinoid

receptor stimulation in the immune system by the endocannabinoids triggers a complex regulation and modulation of cAMP pathways and the cannabinoid receptor stimulation of MAP kinase plays a key role in immune homeostasis and control. The importance of CB2 receptor activation in the immunomodulatory effects of endocannabinoids has been recognized and is supported by anti-inflammatory effects of CB2 receptor activation in many pathological conditions and disparate diseases ranging from inflammatory pain, myocardial infarction, stroke, hepatic I/R injury, gastrointestinal inflammatory disorders [25,26], liver inflammatory disorders [27] and atherosclerosis [28].

The effect of cannabinoids on immune functions appears to be transient which would allow the inhibitory effects to be overcome when the immune system needs to be activated during infections. This is supported by the downregulation of cannabinoid receptor expression when the immune cells are activated. Thus, the transient nature of cannabinoids on the immune system suggests that the side effects of the potential therapy may be minimal. Although the function of cannabinoid receptor on immune cells and the cross-talk between the immune system and endocannabinoids is yet to be fully defined, based on available data, we can hypothesize that the endocannabinoid signaling in lymphoid tissue may provide a tonic control of immune cell activation and therefore limit spontaneous activation of immune cell function. There have been a number of recent studies which have demonstrated that the endocannabinoids have both inhibitory effects and stimulatory impact on the immune system and may be actually important in homeostasis or control of the immune reactions.

4. Endocannabinoid system in the CNS immunity and inflammation

The inflammatory network of the CNS is an orchestrated collaboration of neuronal and non-neuronal cells, such as T-lymphocytes, oligodendrocytes, astrocytes and microglial cells. In recent years, there have been several in vitro, in vivo and clinical studies suggesting that endocannabinoid system plays an important role in the cellular network of communication in and between the nervous and immune system during neuronal damage and neuroinflammation. During inflammation in the CNS, there is a loss of balance and control, resulting in neuronal damage and neurological diseases [29]. Inflammatory processes are involved in many acute and chronic neurodegenerative autoimmune disorders. 2-AG in brain is produced in response to intracellular Ca^{2+} and stimulation of glutamate receptors, and is found at 200-fold higher concentrations in brain tissue. 2-AG is produced by microglial cells and astrocytes in response to stimulation of purinergic P2X7 receptors [30]. During injury, the ATP inside neuronal cells flood the extracellular space and thus stimulate purinergic receptors on microglia and astrocytes causing release of endocannabinoids on neuronal damage. This cellular endocannabinoid response is a restricted process involving short and local events affecting only few cells in vicinity.

Synthesis of AEA in the CNS is well regulated, and is released only on demand. Recently, it has been suggested that AEA is released by CNS tissue as a mechanism that controls and limits immune response in healthy and damaged brain [31]. It has also been reported that endocannabinoid-mediated neuroprotection is impaired during experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, as a consequence of high levels of IFN-gamma which disrupts the functionality of purinergic P2X7 receptors [31]. Mice lacking the CB1 receptor develop more severe neuronal damage following EAE induction, supporting a role for endocannabinoids in this inflammatory neurodegeneration [32]. Furthermore, pharmacological interventions aimed at enhancing the levels of the anandamide significantly attenuated the clinical and pathological features of EAE [33,34]. The homozygous 188–189 GG/GG polymorphism in CB2 receptor (substitution of glutamine at amino acid position 63 by arginine) has been shown to be associated with

autoimmune disorders [35]. Proliferative response in T-lymphocytes from CB2 188–189 GG/GG homozygotes was observed to be less susceptible to endocannabinoid-induced suppression. Dexanabinol (HU-211), a synthetic nonpsychotropic cannabinoid, significantly reduced the clinical symptoms of EAE and suppressed inflammation [36]. Arvanil, a synthetic capsaicin–anandamide hybrid has been shown to ameliorate EAE [37]. The neuroprotection provided by endocannabinoids has been considered to be a direct effect through CB1 receptors that are widely distributed in the mammalian brain in neocortex, hippocampus, basal ganglia, cerebellum and brainstem [38].

The existing literature is highly supportive of a beneficial immunomodulatory action mediated by endocannabinoid activation during both MS and EAE [39]. Virtually all immune cells involved in the pathophysiology of MS and EAE express functional cannabinoid receptors [40] and evidence exists that stimulation of these receptors mediates complex inhibitory actions in these cells, accounting, at least in part, for the protective effects of direct and indirect cannabinoid agonists in MS and animal models of this disease [33,34,41]. Recently, higher concentrations of AEA were reported in active lesions than in quiescent lesions in MS patients [31]. It has been observed that AEA and 2-AG, although sharing many pharmacological actions, have differential regulatory mechanisms, and that AEA but not 2-AG may be involved during pathological events [42]. Similar to this finding it was observed that AEA, but not 2-AG, was increased in the CSF of MS patients, indicating that the two endocannabinoids are differentially engaged during CNS inflammatory diseases [43].

5. Endocannabinoid and immune modulation

Endocannabinoids have important effects on immune functions. They modulate T- and B-lymphocytes proliferation and apoptosis, macrophage-mediated killing of sensitized cells, inflammatory cytokine production, immune cell activation by inflammatory stimuli, chemotaxis and inflammatory cell migration [44,45]. The immunosuppressive effect of endocannabinoids on immune cells is primarily considered to be mediated through CB2 receptors by the inhibition of the cAMP/protein kinaseA (PKA) pathway (by decreasing the expression of cAMP-responsive genes). Endocannabinoids also act at nuclear level, e.g. phosphorylation of IκB-α that enhances the transcription of several apoptotic genes regulated by NF-κB, by peroxisome proliferator-activated receptor gamma (PPAR-γ dependent inhibition of nuclear factor of activated T cells (NF-AT) and interference of cell cycle by activation of p21waf-1/cip-1 and induction of G1/S phase arrest [37].

6. Lymphocytes and haematopoietic cells

Schwarz et al. [46] demonstrated dose-dependent immunosuppressive effects of AEA on T and B human lymphocyte proliferation. AEA effect on DNA synthesis in T- and B-lymphocytes occurred very rapidly as the exposure of the cells during the final 4 h of culture was sufficient to achieve >40% inhibition. Low doses of AEA caused significant inhibition of lymphocyte proliferation and DNA fragmentation, inducing cell death by apoptosis. 2-AG has been shown to exhibit biological activity in mouse splenocytes by producing strong immunomodulatory activity on mitogen induced lymphocyte proliferation, mixed lymphocyte response, and on antibody forming cell responses to T-cell dependent and T-cell independent antigens [47]. However, this immunoenhancing effect of 2-AG is considered to be a part of rapid degradation of 2-AG into AA which could in turn serve as a precursor for prostaglandin E_2, which is a well characterized activator of adenyl cyclase, important for lymphocyte proliferation and antibody forming cell responses.

The antibody producing B cells have been shown to express high levels of CB2 receptors. Role of endocannabinoids has been implicated in migration of B cells along with induction

of some cytokines when present in low doses. Several in vivo and in vitro studies have demonstrated the inhibition of antibody formation by endocannabinoids and synthetic cannabinoids at micromolar concentrations [48,49]. This effect was shown to be mediated by cannabinoid receptor and cAMP-dependent signaling elements in vitro as pertussis toxin could block the suppressive effect on antibody formation in splenocyte culture [48]. In part, the effect of endocannabinoids on modulating the serum levels of immunoglobulin is also considered to be due to their effect on Th cell cytokine secretion/polarization rather than the direct effect on B cells themselves. The endocannabinoid, 2-AG, could stimulate the migration of splenocytes in a CB2-dependent manner [50]. In addition, CB2 receptor stimulation may be associated with B cell differentiation [51], suggesting the positive role of endocannabinoids in mobilizing B cells during immune functions. However, it is yet to be explored whether endocannabinoids exhibit a direct effect on B cells or affect indirectly through T cells and macrophages required for B cell activation. Valk et al. demonstrated that AEA stimulated proliferation of haematopoietic cell lines in synergy with other growth stimuli such as GM-CSF, G-CSF and IL-3 [52]. Derocq et al. also showed that AEA potentiated the growth of cytokine dependent haematopoietic cell lines [53]. However, this effect was not reversed by receptor antagonists, thereby providing evidence for CB1 and CB2 receptor-independent effects. Arvanil, a synthetic capsaicin–anandamide hybrid has been shown to downregulate activation of CD4+ T cells [37] and 2-methylarachidonyl-(2′-fluoroethyl)amide(F–Me–AEA), an analog of anandamide was found to inhibit forskolin-stimulated adenylate cyclase activity in splenocytes and thymocytes [54]. It has been suggested that the regulation of PPAR-γ activity is one of the mechanisms by which endocannabinoids influence T cell activity [55].

The effects of endocannabinoids on T cell cytokine production demonstrated that both TH1 and TH2 cytokines can be either inhibited or induced. AEA and PEA were tested on LPS and phytohemagglutin (PHA)-stimulated human peripheral blood mononuclear cells in vitro and on LPS-induced pulmonary inflammation in mice and results showed the downregulation of LPS-induced synthesis of TNF-α, IL-6, IL-8 and PHA-induced IL-4 synthesis at nanomolar concentrations [56]. IFN-γ is an important mediator in delayed-type hypersensitivity reactions. AEA also inhibited PHA-induced IFN-γ synthesis, however, PEA was ineffective thereby indicating the specific cell sensitivity to different molecular species of ethanolamides and pointing towards the importance of fatty acid ethanolamides as possible endogenous autocoids playing multiple down regulatory roles in the complex network of immune responses. 2-AG also caused human monocytes to produce decreased levels of cytokines and adhesion molecules, thereby exhibiting an immunosuppressive response. In phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore-stimulated splenocytes, 2-AG suppressed IFN-γ mRNA expression in a concentration-dependent manner, which was shown to be independent of CB2 receptor and mediated through inhibition of NF-AT nuclear translocation. Th1 type cytokines have been implicated in the pathogenesis of a number of autoimmune disorders such as multiple sclerosis, its animal model (EAE), rheumatoid arthritis and autoimmune hepatitis (AIH). Inhibition of Th1 cytokines and the shift to Th2 type response is considered to provide therapeutic benefit. Endocannabinoids have been shown to cause blockade of Th1 cytokines and increase the expression of Th2 cytokines [57,58], such as IL-4 and IL-10 that are important for humoral immunity and TGF-α which has immunosuppressive properties [59]. On the other hand, cytokines have been shown to influence directly the endocannabinoid system such as by regulation of degrading enzymes: IL-4 or IL-10 stimulated FAAH activity, whereas IL-12 and IFN-gamma reduced FAAH activity and protein expression of FAAH [20]. In activated Jurkat T cells, significant suppression of IL-2 by 2-AG was shown to be induced at concentrations as low as 1 μM, which is within an order of magnitude of the calculated endogenous 2-AG levels detected in human plasma. This suppression was mediated by a COX-2 metabolite of 2-AG [55].

Recently, our lab has reported that both exogenous and endogenous cannabinoids can attenuate concanavalin A (ConA)-induced acute hepatitis, a well established model for autoimmune hepatitis in which liver injury is T cell-mediated [60]. In our studies, exogenously administered anandamide in the hepatitis model led to a decrease in hepatic injury which correlated with decreased activities of liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT) levels, and other inflammatory cytokines. We also observed that 12 h after anandamide treatment, in ConA-injected mice, there was a significant decrease in inflammatory cytokines TNF-α, IL-1β, IL-6, IL-9, and IL-17 and in chemokines such as KC, eotaxin and monocyte chemoattractant protein-1. Blocking of CB1 or CB2 receptors reversed the anandamide-mediated suppression of hepatitis thereby indicating that anandamide was acting in a CB1- and CB2-dependent manner. Anandamide levels are elevated in the absence of FAAH activity [10].Mice which lacked FAAH enzyme (responsible for hydrolysis of anandamide) were found to be resistant to ConA-induced hepatitis and showed less severe liver tissue damage and decreased leukocyte infiltration [60]. Similarly, increased endogenous anandamide levels caused by administering mice with FAAH inhibitors MAFP or URB532, also caused a decrease in hepatic injury with significant decrease in AST levels upon ConA challenge. These findings are exciting and suggest the potential manipulation of endocannabinoids, including the use of FAAH inhibitors in the treatment of AIH.

7. Macrophages

Macrophages play an important role in both innate and adaptive immunity by mediating their effect through presenting antigen to T cells, phagocytosis of infectious agents and secreting acute phase proteins such as nitric oxide, TNF-α, IL-1 and IL-6. CB1 and CB2 receptors are widely expressed on monocytes/macrophages and microgial cells. AEA has been shown to inhibit macrophage-mediated killing of TNF-sensitive murine L929 fibroblasts [61]. AEA and PEA have also been shown to maximally inhibit LPS-induced expression of pro-inflammatory mediators such as nitric oxide production in murine RAW264.7 macrophages and microglia [62] and these effects were mediated by CB2 receptors. Whereas AEA diminished LPS-induced NO and interleukin-6 production, 2-AG inhibited IL-6 production but increased iNOS-dependent NO production [63]. It has been suggested that the discrepant results of 2-AG could be due to its bioactive metabolites, AA and PEG_2. 2-AG enhanced the adhesion of macrophage-like differentiated HL-60 cells to fibronectin and to vascular cell adhesion molecule-1 (VCAM-1) and adhesion of human monocytic leukemia U937 cells and peripheral blood monocytes. This enhanced adhesion has been suggested to be mediated by CB2 receptor, Gi–G_0-proteins, and phosphotidylinositol 3-kinase dependent pathways. Decreased accumulation of macrophage-like cells was observed at focal sites of infection in a murine model of Granulomatous Amebic Encephalitis and reduced macrophage chemotaxis by cannabinoids was observed in a murine model of atherosclerosis, suggesting their strong effect on macrophage migration which is mediated by CB2 receptor signaling. In a recent study, patients with coronary artery disease demonstrated the activation of the endocannabinoid system with elevated levels of blood endocannabinoids and increased expression of CB1 receptor in coronary atheroma, particularly in macrophages. CB1 receptor blockade exhibited anti-inflammatory effects on human macrophages, which might provide beneficial effects on atherogenesis [64]. Thus, these properties of endocannabinoids provide a rationale for their use as anti-inflammatory agents.

8. Mast cells

Mast cells are bone marrow derived, multifunctional immune cells populating connective and mucosal tissue as well as nervous system and are involved in inflammatory reactions.

Using mast cell lines it was shown that they express both CB1 and CB2 receptors. Both PEA and AEA bound to CB2 receptors but only PEA could down modulate mast cell activation in vitro and this effect was efficiently antagonized by AEA [13]. Endocannabinoids except PEA and PEA derivatives have also been shown to induce a non-lytic, energy and concentration-dependent histamine release in rat peritoneal mast cells [65]. However, this effect was shown to occur irrespective of the presence of cannabinoid receptors. The effect was partially reduced by pertussis toxin indicating that Gi- and G_0-protein were partly responsible for the effect. In addition, where synthetic ligands enhanced anti-IgE mediated histamine release, AEA and PEA treatment showed no effect. In contrast, it has been demonstrated that 2-AG mediated suppression of histamine release from guinea pig mast cells can be reversed by nitric oxide synthase inhibitor or a CB2 receptor antagonist [66]. While analyzing the effect of endocannabinoids on LPS-induced bronchopulmonary inflammation in mice, PEA was shown to diminish the level of TNF-α by 31.5% but with no effect on neutrophil recruitment. AEA, however, did not influence the inflammatory process but TNF-α level and neutrophil recruitment was decreased by 28% and 62%, respectively. Interestingly, AEA levels were found to be upregulated in biopsies from patients with colitis or in patients with diverticular diseases [67]. According to the notion that endocannabinoids are released on demand, such findings suggest their possible role in the restoration of tissue homeostasis in certain pathological conditions. In a recent study it was observed that CB1 and CB2 agonists prevented mast cell-dependent angiogenesis during granuloma formation, thus indicating that cannabinoids can control mast cell activation in a receptor-dependent manner [68].

Although PEA does not bind with high affinity to CB1 (K_i = 23.8 nm) or CB2 (K_i = 13.9 nm) receptors, it still maintains CB-like anti-inflammatory actions in several mast cell-mediated experimental models of inflammation. For example, PEA reduces edema in response to compound 48/80 [69] and carrageen [70]. Recently, a new drug containing PEA has been approved by Food and Drug administration for treatment of dermatitis. A recent pilot study aimed to assess the efficacy and safety of twice daily application of a topical emulsion containing 2% Adelmidrol, a PEA analog, in 20 pediatric patients suffering from atopic dermatitis showed 80% increase in symptom resolution via inhibition of nerve growth factor (NGF) release from mast cells [71].

9. Dendritic cells (DCs)

DCs play a major role as antigen-presenting cells and in the development of antigen-specific T cell responses. Human dendritic cells have been shown to express both CB1 and CB2 receptors. There have been several reports indicating that the endocannabinoid system plays a critical role in regulating DC growth and maturation [72]. The endogenous cannabinoid system is present in DCs and can be regulated by cell activation [72]. AEA, 2-AG and PEA were observed in lipid extracts from immature dendritic cells. Upon LPS activation, the quantity of 2-AG was increased in DCs. However, there was no increased expression of CB1, CB2 or FAAH observed. Furthermore, 2-AG has been suggested to act as a chemotactic molecule capable of recruiting DCs during innate immune response and in the presence of a TLR agonist, instructs a Th1-shifted adaptive response [73].

DCs are highly sensitive to cannabinoid-induced apoptosis which is considered as a cellular background of immunosuppressive property of endocannabinoids [74]. Studies from our laboratory demonstrated for the first time that both exogenous and endogenous cannabinoids induce apoptosis in DCs [74]. To investigate whether endogenous cannabinoids such as anandamide could induce apoptosis in DCs, various concentrations of anandamide were added to DCs. It was observed that concentrations of 20 μM caused marked apoptosis while lower concentrations were not effective. The lesser efficacy of anandamide as compared to

exogenous cannabinoids might be due to rapid hydrolysis of anandamide by endogenous enzymes such as FAAH. To prevent the activity of FAAH, various concentrations of a FAAH inhibitor were added to the culture in the presence or in the absence of anandamide and the induction of apoptosis was determined. These studies revealed that addition of a FAAH inhibitor could increase the induction of apoptosis by anandamide in DCs significantly. Furthermore, anandamide-induced apoptosis was mediated through cannabinoid receptors since addition of selective antagonists of CB1 (SR141716A) or CB2 (SR144528) to the cultures reversed the effects of anandamide.

10. Natural killer cells and neutrophils

Natural killer cells and neutrophils are involved in host defense against cancer and anti-microbial responses. In vitro studies on human NK cells demonstrated that cannabinoids can inhibit the constitutive expression of chemokines, IL-8, MIP1-α, MIP-1β, RANTES, phorbol ester stimulated TNF-α, GM-CSF and IFN-gamma. Studies have demonstrated that cannabinoids can suppress NK cell function such as cytolytic activity in rats, mice and humans, which is a cannabinoid receptor-dependent process [75–77].

The presence of cannabinoid receptors on neutrophils was first reported in 1993. Dose-dependent non-cytotoxic release of lysosomal enzymes from neutrophils has been shown to be induced by cannabinoid ligands. Recent studies have also shown that AEA failed to inhibit superoxide production in neutrophils although other synthetic cannabinoid treatment exhibited the inhibition property. This effect was shown to be independent of cannabinoid receptors [78].

10.1. Endocannabinoids and cancer

Recent applications of cannabinoids have included their potential use as anti-tumor agents [79,80] which relies on their ability to inhibit tumor angiogenesis [81,82] or directly induce apoptosis or cell cycle arrest in neoplastic cells [83–86]. Studies from our laboratory demonstrated that AEA can induce apoptosis in malignant immune cells [87]. Molt-4 human tumor cells when cultured for 4 h in the presence of various concentrations of anandamide (5, 10, 20, and 40 μM) were found to undergo significant levels of apoptosis as quantified using the TUNEL assay at concentrations of 20 μM or greater. Also, murine EL-4 tumor T cells were found to be more sensitive to AEA as much as exposure to 5 and 10 μM AEA was sufficient to trigger significant levels of apoptosis [87]. Role of the endocannabinoids as potential endogenous tumor growth inhibitors has been suggested in a study where it was observed that levels of both AEA and 2-AG were higher in precancerous polyps than in fully developed carcinoma in colon [88]. Recent in vivo studies proposed that selective targeting of CB2 receptor resulted in colorectal tumor growth inhibition via apoptosis which was mediated through the stimulation of ceramide [89]. In a xenograft model of thyroid cancer, substances that block endocannabinoid degradation increased levels of AEA and 2-AG in tissues and reduced tumor growth [90]. Various attempts have been made to inactivate cannabinoid degrading enzymes thus increasing the local concentration of endocannabinoids at the tumor cell surface leading to anti-tumor effects of CB-receptor signaling in various types of cancer including thyroid, brain and prostate cancers [90–93].

Although majority of the effects of cannabinoids are mediated through CB receptors, AEA has been shown to induce its effects on cancerous cells by interacting with TRPV1 receptor [94,95] or cholesterol rich lipid rafts [96]. Furthermore, it has been reported that signaling pathways are differentially regulated by cannabinoids in normal cells versus cancer cells. In malignancies, such as thyroid cancer, lymphoma, melanoma, pancreas and breast cancer, the levels of cannabinoid receptors are often higher in the tumors when compared to normal cells, resulting in increased sensitivity to cannabinoids in malignant cells [67,83,97–99].

Moreover, many animal studies have reported anti-proliferative and pro-apoptotic effects of cannabinoids on tumor cells but not on normal tissue [79,83,85,97]. Thus the role of the endocannabinoid system in cancer indicates that this system is involved in regulating many of the functions that are essential in cancer development.

11. Concluding remarks

The endocannabinoids exhibit complex regulatory effects on the immune system. As summarized in Table 1, endocannabinoids are involved in immune regulation by the suppression of cell activation, inhibition of pro-inflammatory cytokine production, NF-κB-dependent apoptosis and modulation of the functions of T helper subsets: Th1 and Th2. Endocannabnoids (AEA and 2-AG) and their congeners (PEA) can thus be considered as potent immunomodulators. The effect of endocannabinoids depends on cell type, their concentration and cellular environment. The effects of endocannabinoids on chemokines, adhesion molecules and expression of costimulatory molecules need further evaluation. To understand the pleotropic effects of endocannabinoids on immune system, future studies on differential cannabinoid receptor signaling using knockout mice and further identification of cannabinoid-like receptors needs to be undertaken in the coming years. It is also critical to know the production and regulation of endocannabinoids by the naïve and activated components of the innate and adaptive immune response. The image of endocannabinoid system now appears to be of a modulatory complex which affects the physiological functions in peripheral tissues and can thus be considered as a potential therapeutic target in the future. While several in vitro studies have delineated the immunomodulatory effects of endocannabinoids, their in-depth functions in vivo need to be addressed. Use of inhibitors of enzymes such as FAAH that breakdown the endocannabinoids, to modulate their levels and consequent regulation of immune response, can provide novel therapeutic modality against inflammatory and autoimmune diseases.

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Table 1

Effect of endocannabinoids on functions of different immune cells.

Immune cells	Functions affected	Receptor involved	Reference cited
T-lymphocytes	Proliferation; cell death by apoptosis; Th1/Th2 cytokine secretion, polarization; cell number	CB2	[46,47,55,56]
B-lymphocytes	Inhibition of antibody formation; Ig production; Ig isotype switching; proliferation; cell number	CB1 and CB2	[48,49,51]
Haematopoietic cell line	Cell growth	Non-CB1, CB2	[52,53]
Macrophages	Decreased Inflammatory mediators; antigen presentation; migration; phagocytosis; increased adhesion	CB2	[61–64]
Mast cells	Down modulate mast cell activation; decreased TNF-α; decreased mast cell-dependent angiogenesis	Non-CB1, CB2 CB1, CB2	[65] [66,68]
Dendritic cells	Growth and maturation; apoptosis; recruitment during innate immune response	CB1, CB2	[72–74]
Natural killer cells and neutrophils	Cytolytic activity; chemokines; cytokines	Non-CB1, CB2	[75–78]
Cancer cells	Cell cycle arrest; apoptosis; growth inhibitor	CB1, CB2 receptor, TRPV1, lipid rafts	[86–99]