MINIREVIEW

The Cannabinoid System and Cytokine Network (44546)

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Abstract. **Many advances have been made in the last few years concerning our understanding of the receptors and ligands composing the cannabinoid system. Likewise, the science surrounding cytokine biology has advanced enabling us to measure these proteins more precisely as well as understand and interpret the meaning of changes in their levels. Scientists wishing to study the health consequences of smoking marijuana as well as understand the possible role of endogenous cannabimimetic ligands in immune regulation have continued to study the influence of these substances on the regulation and development of the cytokine network. Research has shown that two major cannabinoid receptor subtypes exist and that subtype 1 (CB1) is expressed primarily in the brain whereas subtype 2 (CB2) is expressed primarily in the periphery. A variety of ligands for these receptors based on the cannabinoid structure have been synthesized and studied as well as low affinity compounds, noncannabinoid ligands, and endogenous ligands derived from fatty acid eicosanoids. Highly selective receptor antagonists have also been introduced and studied. Synthetic, low affinity ligands such as (+)-HU-211 and DMH-11C have been shown to cause anti-inflammatory effects possibly through inhibiting the production and action of TNF-α and other acute phase cytokines. In addition, suppression of TNF and other cytokines such as GM-CSF, IL-6, IFNγ, and IL-12 has also been seen following exposure to high affinity and psychoactive ligands such as marijuana and THC. However, some of these ligands have also been shown to increase rather than decrease interleukins such as IL-1, IL-4, IL-10, and IL-6, cytokines such as TNF-α, and chemokines such as IL-8, MIP-1, and RANTES. The endogenous ligand, anandamide, has been shown in culture to either suppress the proliferation response to prolactin or enhance the response to cytokines such as IL-3 and IL-6. This eicosanoid has also been shown to increase the production of interleukins and other cytokines. Cannabinoid receptors have been shown to be involved in some but not all of these effects. It is clear that psychoactive and nonpsychoactive compounds have demonstrated effects *in vivo* and *in vitro* on the production and function of a variety of cytokines. Depending upon the model system, these effects are often conflicting, and the involvement of cannabinoid receptors is unclear. However, enough evidence exists to suggest that the cannabinoid system significantly impacts the functioning of the cytokine network, and this association may provide clues to the mechanisms of certain immune diseases and form the basis for new immunotherapies.** [P.S.E.B.M. 2000, Vol 225:1–8]

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Since the last review in 1995 (1), many new findings have added to our understanding of the biology of the cannabinoid system and the interfacing of this system with the cytokine network. Previously we summarized existing studies demonstrating that Δ^9-tetrahydrocannabinol (THC) modulates the production by rodent cells of a handful of cytokines including IFNs, TNF-α, IL-1, and IL-2. Since that report, a wider array of cannabimimetic agents

has been tested for cytokine effects ranging from marijuana to cannabinoid analogs such as HU-211, and in addition, the diversity of cytokines affected by the drugs has increased significantly to include not only the interleukins but also the chemokines. The biological consequences of these drug-induced cytokine changes have also increased in scope from a few isolated immune tests and infection paradigms to models involving cancer, hematopoietic colony formation, inflammation, autoimmune disease, catalepsy, and brain injury. Knowledge about the cannabinoid system has also increased, providing a better understanding of its organization and function. In the following, we will review recent information on the cannabinoid system of ligands and receptors and then summarize recent studies involving cytokine modulation by cannabimimetic agents and related compounds. Several recent reviews in this area have also appeared (2–6).

Cannabinoid Receptors

By the late 1980s, there was good pharmacological evidence that marijuana cannabinoids and derived analogs caused cellular changes by interacting with specific receptors (6). The first of these receptors (CB1) was serendipitously discovered and cloned in 1990 from a rat brain cDNA library (7). The DNA sequence suggested a protein in the family of seven transmembrane (7TM; Fig. 1), G protein-coupled receptors that includes more than 2000 receptors for a variety of neurotransmitters, hormones, and peptides including the chemokine, IL-8 (8–10). In addition to rat CB1, the mouse gene has also been cloned (11) and encodes a product containing 473 amino acids with an extracellular amino end, intracellular carboxy tail, and putative G protein binding sites (Fig. 1). Ligands appear to bind to CB1 in transmembrane alpha helices 2, 3, 4, and 5 (12–14). CB1 is highly expressed in the brain hippocampal formation, basal ganglia, and molecular layer of the cerebellum (15) probably accounting for the psychoactive effects of cannabinoids. Expression of CB1 outside of the brain has been reported in testis and in cells of the immune system (16, 17); however, the function in these tissues is unclear.

A second cannabinoid receptor (CB2) was cloned in 1993 from a human cell line, HL-60, cDNA library (18). This gene encoded a protein of only 360 amino acids and only 44% identity to the rat CB1 receptor. However, CB2 had the structure of a 7TM, G protein-coupled receptor and displayed high affinity for cannabinoid ligands when expressed in COS cells (18). The mouse and rat genes have also been cloned and encode proteins of 347 (mouse) and 361 (rat) amino acids with only 82% (mouse) or 81% (rat) identity to the human CB2 (Fig. 1) (19, 20). CB2 is referred to as the peripheral cannabinoid receptor because it is expressed in abundance outside of the brain and especially in immune organs such as spleen (2, 18).

Several groups have developed and reported on CB1 knockout mice (21, 22). These mice are hyporesponsive in a number of behavioral and physiological tests but were not tested for immune or cytokine activity. Furthermore, although the mice generally appeared to be healthy and free of immune defects, one of the groups reported that the knockout mice had a significantly higher mortality rate of unknown etiology (22). These mice, as well as CB2 knockouts, will be useful in determining the role of cannabinoid receptors in normal physiological responses including immune responses and also determining which cannabinoid effects are receptor mediated.

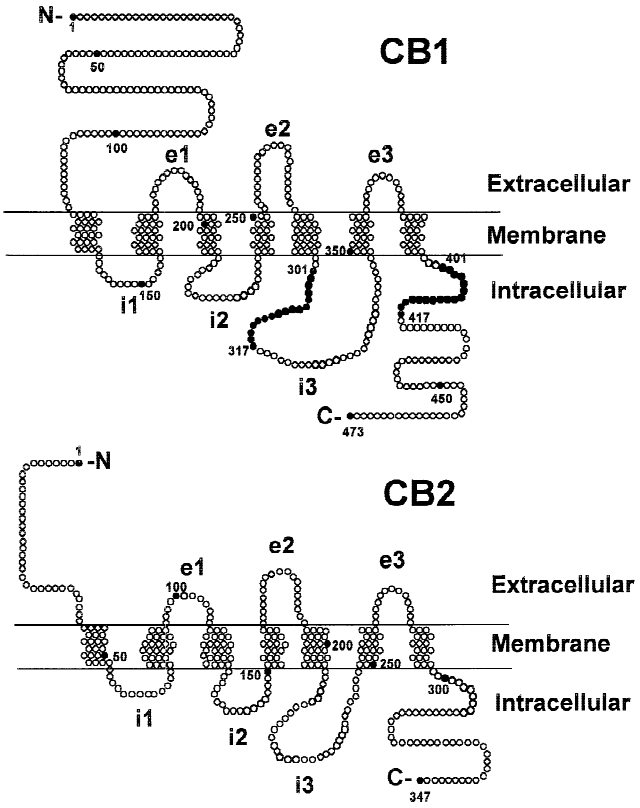

Figure 1. Mouse cannabinoid receptors of subtype 1 (CB1) and subtype 2 (CB2). Each receptor has an extracellular domain containing the amino (N) terminus as well as seven transmembrane regions, three extracellular (e) loops, and three intracellular (i) loops. In CB1, an intracellular tail containing the carboxy (C) terminus also contains putative G protein binding sites (dark circles). Binding sites are also believed to be in the third intracellular loop (58).

Cannabinoid Ligands and Cannabimimetic Agents

THC was the first cannabinoid ligand to be structurally defined and synthesized (Fig. 2) (23). Analogs of THC were studied over the years leading to the description of high affinity, nonclassical cannabinoids such as (−)-CP55 940 (24) and synthetic cannabinoids such as (−)-HU-210 (25). Interestingly, (+) enantiomers such as (+)-HU-211 have little cannabimimetic activity and receptor affinity (26) although as discussed below, they can still modulate cytokines. In addition to cannabinoid compounds, other types of drugs such as WIN55,212 (an aminoalkylindole) bind to

CB1 and CB2 inducing cannabimimetic activity (27). These classical and nonclassical cannabinoids and related structures have contributed to our understanding of the distribution and function of CB1 and CB2. However, a major advance in the understanding of the cannabinoid system came with the discovery of an endogenous ligand for cannabinoid receptors. This substance is not a cannabinoid but rather an eicosanoid. It was isolated from swine brain and was identified as arachidonylethanolamide (Fig. 3) and named anandamide (28). Anandamide is produced in brain and peripheral tissues such as spleen (29) that would position it in areas where receptors are present thus facilitating a role in endogenous cannabimimetic activity in these various areas of the body. It is also produced by cells of the immune system such as macrophages (30) and other leukocytes (31). In addition to anandamide, other endogenous fatty acids have been identified with cannabimimetic activity. One of these, isolated from canine gut, was shown to be a glycerol derivative of arachidonic acid termed 2-arachidonyl glycerol (Fig. 3) (32). This substance binds to cannabinoid receptors and possesses cannabimimetic activity with a potency comparable to anandamide but less than THC (32). Another ligand found in the periphery is palmitoylethanolamide (PEA) (Fig. 3). This is an N-acylamide like anandamide and is known to be generated in inflammatory conditions (33) and believed to downregulate inflammation. Also, PEA has been shown to inhibit mast cell function by binding to CB2 receptors (34, 35) leading some to speculate

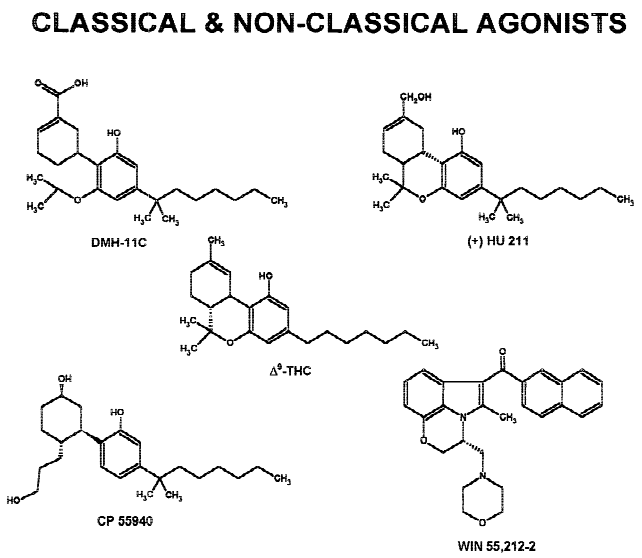

Figure 2. The classical cannabinoid Δ^9-THC (Δ^9-tetrahydrocannabinol) is depicted along with synthetic derivatives such as DMH-11C (1′,1′ dimethylheptyl-Δ^8-tetrahydrocannabinol-11 oic acid) and HU-211 ([+]11-hydroxy-Δ^8-tetrahydrocannabinol-1,1-dimethylheptyl). The nonclassical cannabinoid, CP55940 ([1α,2α-(*R*) 5α]-(−)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol), is also depicted along with the aminoalkylindole WIN55212-2 (R-(+)-(2,3-dihydro-5-methyl-3-[{4-morphonolinyl}-methyl]pyrol[1,2,3-de]-1,4-benzoxazin-6-yl)(1-napthalenyl) methanone monomethanesulfonate).

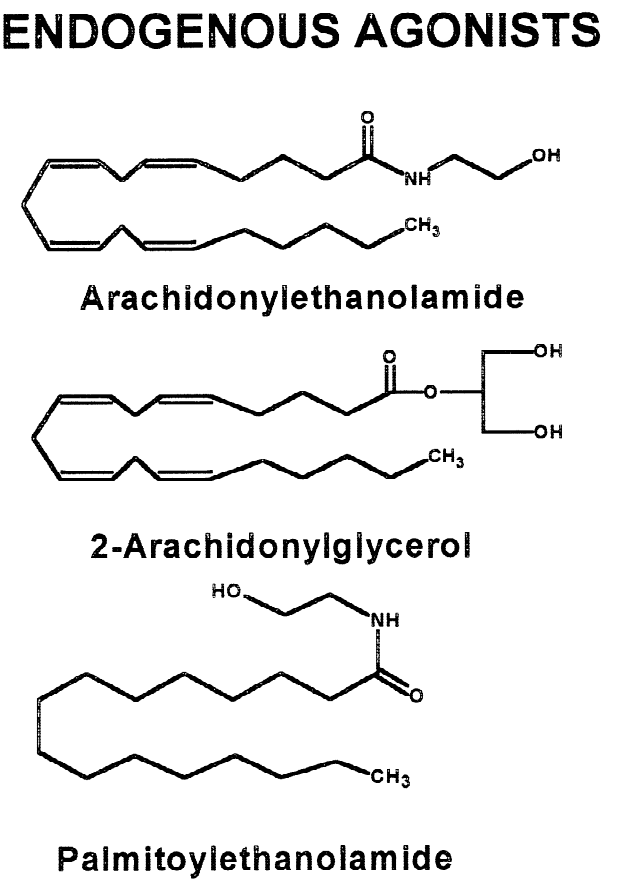

Figure 3. The endogenous eicosanoid agonists are depicted. Arachidonylethanolamide is anandamide.

that PEA is the major endogenous ligand for the peripheral cannabinoid system (35, 36).

Receptor Antagonists

In addition to cannabimimetic agonists, receptor antagonists have also been described. The first of these was the orally active antagonist with high affinity for CB1 termed SR141716A (Fig. 4). This compound was shown in rat brain membrane preparations (CB1 rich) to inhibit the binding of various receptor agonists with a K_i in the n*M* range while binding in splenocyte membranes (CB2 rich) was inhibited in only the μM range (37). In addition, when fed orally to mice, the SR compound inhibited a battery of agonist-induced effects (e.g., antinociception) at relatively low doses. Other studies suggested that SR141716A binds to CB1 in the 4th and 5th transmembrane regions of the protein (12).

An antagonist for CB2 has also been described and termed SR144528 (Fig. 4). This compound inhibited agonist binding to rat splenocyte membranes with a K_i in the n*M* range while inhibiting binding to brain membranes in the μM range (38). Furthermore, competitive binding studies with CHO cells expressing either CB2 or CB1 showed the preferential interaction of the antagonist with CB2 express-

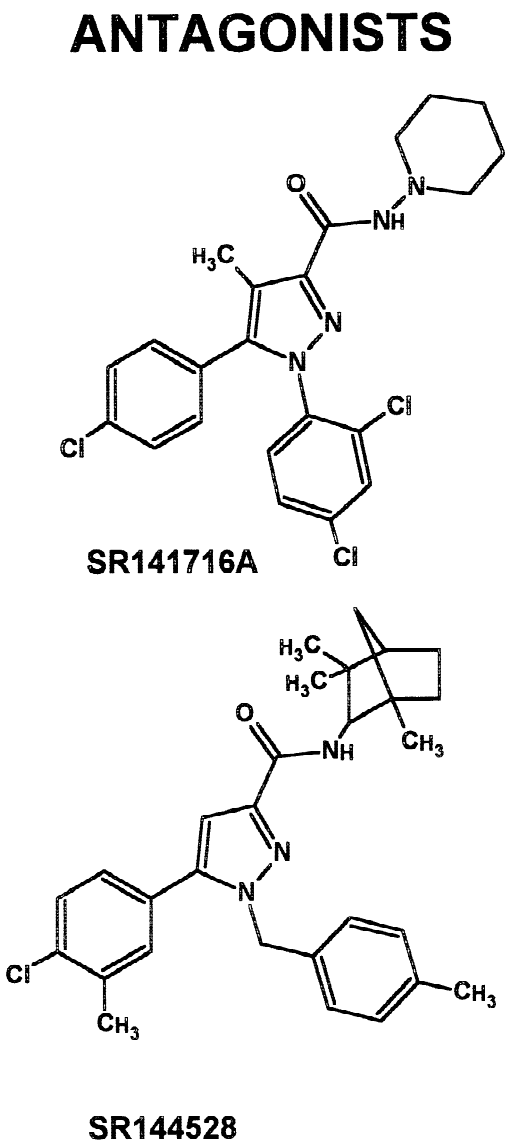

Figure 4. The cannabinoid receptor antagonists are SR141716A, specific for CB1, and SR144528, specific for CB2.

ing cells. In addition to these SR compounds based on the pyrazole nucleus, others have been reported more recently based upon other chemical structures (39).

Besides inhibiting the binding and function of cannabimimetic agents, the SR compounds act as inverse agonists in cell models displaying constitutive CB1 or CB2 activity (40). Thus, in certain cell types, treatment with SR compounds alone may cause changes in biological function by suppressing the constitutive activity of CBRs as well as the activity of other G protein-coupled receptors (40).

Anti-TNF-α and Anti-Inflammatory Properties of Synthetic Cannabinoids

The derivative of THC, (+)-HU-211, does not bind to CBRs nor exert cannabimimetic effects but instead appears to act as an antagonist of the NMDA (N-methyl-D-aspartate) receptor. Because these receptors have a role in brain injury, the effect of HU-211 treatment on neuroprotection was investigated in several models of brain injury (Fig. 5) (41, 42). Rats were injected intracisternally with a virulent strain of *Streptococcus pneumonia* and either untreated, treated with antibiotics only, or treated with antibiotics plus HU-211. The cannabinoid-treated group faired better than the antibiotic only-treated group with less mortality and less evidence of blood-brain barrier damage (41). Although not tested directly, this improved outcome could have been due to the attenuation of the acute phase cytokine response in the animals that is known to account partially for the pathology in this model. However, in another rat model of brain injury, HU-211 was shown to suppress brain levels of TNF-α directly as well as reduce mortality and improve clinical outcomes (42). The mechanism of the drug effect was not shown, but it was speculated that HU-211 was working through the NMDA receptor to inhibit cytokine production.

Another synthetic cannabinoid, dimethylheptyl-11 oic acid (DMH-11C), is a derivative of THC-11 oic acid and has been shown to be orally active in ameliorating symptoms in acute and chronic inflammation models (Fig. 5) (43). For acute inflammation, mice were injected with IL-1β and TNF-α into subcutaneous air pouches and the leukocyte inflammatory response measured with or without DMH-11C treatment. Under these conditions, drug treatment suppressed the leukocyte influx in response to IL-1β and TNF-α injection. The drug effect on chronic inflammation was also tested using the adjuvant arthritis model in rats. Again, DMH-11C treatment attenuated the joint swelling that developed over time in the animals. Little was presented as to the mechanisms of the drug effects other than to show evidence suggesting that DMH-11C inhibited cyclooxygenase 2, an enzyme known to be involved the pathophysiology of inflammation.

Marijuana Smoking Modulates Pulmonary Cytokines

To our knowledge, only one study has reported the effect of human marijuana smoking on cytokine production. In this study, pulmonary alveolar macrophages were removed from four subject groups and studied in tissue culture (Fig. 6) (44). The four groups were nonsmokers, or

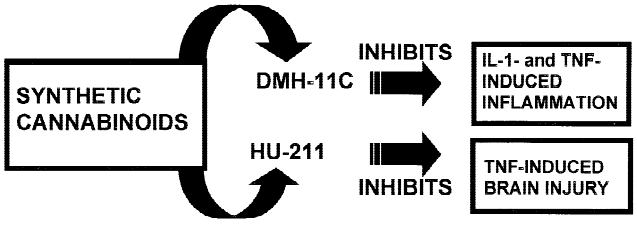

Figure 5. Cannabinoid analogs such as DMH-11C and HU-211 have been reported to inhibit inflammation and brain injury. Acute and chronic inflammation induced in mice responded to oral administration of DMH-11C. Closed head injury symptoms in rats and accompanying TNF-α production was inhibited by HU-211.

smokers of either cigarettes, marijuana, or cocaine. Macrophages were isolated and tested for various immune activities including the production of TNF-α, GM-CSF, IL-6, and TGFβ in response to LPS. Interestingly, the authors found that marijuana smoking alone resulted in a decrease in the macrophage production of TNF-α, IL-6, and GM-CSF. Cells from smokers of cigarettes or cocaine were fully competent to produce these cytokines. On the other hand, TGFβ production was not affected by marijuana or tobacco smoking but was decreased in cells from cocaine smokers. Although mechanisms of these drug effects were not presented, it was speculated that the suppression by marijuana smoking of inflammatory cytokines and the coincident neutral effect on anti-inflammatory cytokines such as TGFβ might lead to an imbalance in host defenses conducive to the spread of pulmonary infections and tumors (44).

THC Modulates Cytokines

Our previous review discussed the findings that THC treatment either decreased or increased the production of IFNs, TNF-α, or IL-1β as well as modulated the production of IL-2 and IL-2 receptor proteins (1). We also reported that THC injection into mice suppressed the production of the Th1 cytokine, IFNγ, lowering resistance to infection (45). More recent evidence shows that THC injection suppresses Th1 activity by decreasing IL-12 production and IL-12 receptor function (Fig. 6) (46), and that the drug is functioning through both CB1 and CB2 receptors. It is not clear at this time if the receptors involved are in the brain, periphery, or both; nor is it clear how the receptors are linked to Th immunity. Possibly, CB1-mediated central effects are acting through the hypothalamo-pituitary-adrenal axis thus affecting Th activity as a consequence of corticosterone mobilization (47). On the other hand, CB2 receptors expressed on immune cell subpopulations might be involved in modulating the cytokine environment controlling the relative maturation of one type of Th cell over another. Additional studies are needed to examine these and other possibilities.

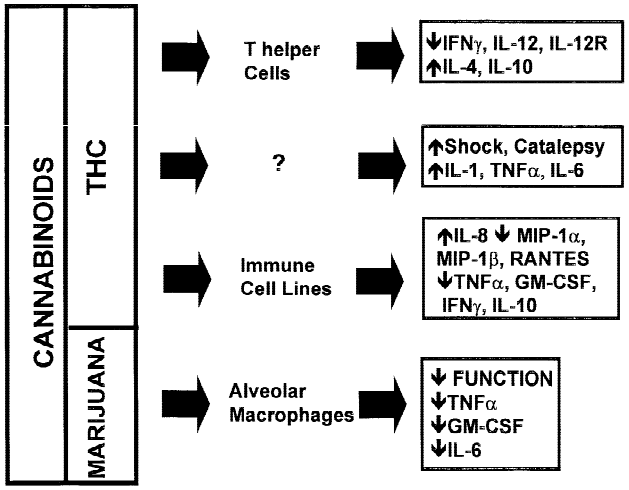

Figure 6. Marijuana smoking and cannabinoids such as Δ^9-THC modulate the cytokine responses of various immune cells. Mice injected with THC were affected such that T helper 1 cytokines such as IFNγ and IL-12 as well as IL-12 receptor (R) were suppressed (↓) whereas T helper 2 cytokines such as IL-4 and IL-10 were increased (↑). Also, THC injection into mice increased catalepsy and shock along with serum IL-1, TNF-α and IL-6 by cells of unknown origin (?). Human cell lines, representing major immune subpopulations, were modulated in culture by THC treatment in terms of cytokine and chemokine production capability. Human lung alveolar macrophages, taken from marijuana smokers, were deficient in functions such as phagocytosis and killing of bacteria and suppressed in the production of TNF-α, GM-CSF, and IL-6.

Several, seemingly nonrelated, other conditions in rodents induced by THC injection have been linked to the mobilization of cytokines. These conditions are infection-induced shock and THC-induced catalepsy (Fig. 6). THC injection into mice 24 hr after an infection with *Legionella pneumophila* causes shock symptoms and death within hours (48). The drug injection was linked to an increase in blood of acute phase cytokines such as TNF-α and IL-6 in the affected animals. This hypermobilization was shown to be pathogenic because neutralization with injections of anticytokine antibodies protected the mice from shock and death (48). Similar results were obtained in a model of THC-induced catalepsy. Here, IL-1β and TNF-α injections were able to augment the cataleptic effect of low-dose THC whereas antibodies to these cytokines attenuated the high-dose THC cataleptic effect (49). Studies such as these show that THC injection is capable of either inducing or augmenting the production of acute phase cytokines in animals suggesting that cannabimimetic activity may be linked to the regulation of these potent substances. It is possible that the observations on these proinflammatory effects of THC are related to those reported above concerning the apparent anti-inflammatory effects of nonpsychoactive cannabinoids.

Proinflammatory and anti-inflammatory effects of THC have been demonstrated in the same study. Human cell lines representing subpopulations ranging from T cells to eosinophils were incubated with THC and constitutive production of cytokines and chemokines measured by ELISA (50). Generally speaking, anti-inflammatory effects were observed in the various cell types with TNF-α, GM-CSF, and IFNγ all decreasing from drug treatment (Fig. 6). However, proinflammatory effects were also observed in that chemokines, especially IL-8, were increased following THC treatment, and the anti-inflammatory cytokine, IL-10, was decreased. From these studies, it is apparent that different immune cell subpopulations have varying thresholds of responsiveness to THC and other cannabimimetics, some of which is probably CBR mediated and some not. Additional studies are needed to analyze cytokine responses of single subpopulations passing through various stages of differentiation and maturation. Also, these studies must define the extent of CBR expression and function in relation to cannabinoid responsiveness and cytokine production.

Anandamide Modulates Responses Cytokines

The past few years have seen an increase in the number of studies examining the effects of the endogenous canna-

bimimetics on the cytokine biology of various cells systems. One such study reported that anandamide (as well as other CBR ligands) inhibited the proliferation of the human breast cancer cell lines, MCF-7 and EFM-19, through a mechanism involving prolactin (Fig. 7) (51). The antiproliferative effect was not due to enhanced apoptosis but rather a reduction in the number of cells entering the S phase of the cell cycle. Other experiments showed that the antiproliferative effect was CB1 receptor mediated, and the mitogenic effect of prolactin on these cells was inhibited by anandamide. This latter effect was of importance in that anandamide was shown to suppress the expression of prolactin receptor components, and this suppression of receptor function accounted for the antiproliferative effect.

As opposed to inhibition of proliferation, anandamide treatment has also been reported to increase cytokine-induced proliferation. Culturing of normal, mouse bone marrow cells in the presence of IL-3 and anandamide produced more hematopoietic colonies than culturing with IL-3 only (Fig. 7) (52). Furthermore, the myeloid cell line, 32Dcl3, proliferated to a greater extent in the presence of anandamide, and the growth factor effect was observed when anandamide was co-cultured with factors other than IL-3, such as GM-CSF, G-CSF, and erythropoietin. Paradoxically, the anandamide effect was not shared by other cannabimimetic agents, but the effect was dependent upon the expression of CB2. The authors concluded that anandamide is a synergistic growth stimulator for hematopoietic cells; however, the molecular link between CB2 signaling and growth factor receptor signaling was unclear (52). In this regard, the CB2 gene has been described as a proto-oncogene and appears to be the target of murine leukemia virus insertion and subsequent tumor formation (53). Possibly this property links it in some way to enhanced growth factor-induced proliferation.

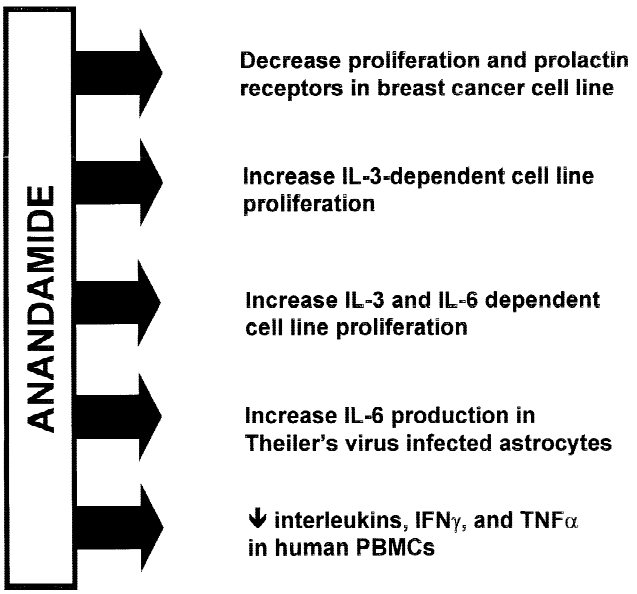

Figure 7. Anandamide exerts in culture a variety of cellular effects associated with cytokine biology. It modulates cellular responses to prolactin, IL-3, and IL-6 and also modulates the production of different cytokines such as IL-6 and IFNγ.

Other investigators have reported a CBR-independent growth-promoting effect of anandamide in hematopoietic cell cultures in the presence of either IL-6 or IL-3 (Fig. 7) (54). In these studies, the lymphoid cell line, B9, and the myeloid line, FDC-P1, were shown to express mRNA for both CB1 and CB2 and were stimulated to enhanced proliferation in the presence of cytokines and a variety of eicosanoids including anandamide, methanandamide, and palmitoylethanolamide. However, linkage of these effects to CBR ligation was not observed in that the high affinity CBR ligand, CP55940, was without effect, and the enhancement of proliferation was not blocked by receptor inhibitors such as pertussis toxin and the two SR antagonists. In addition to proliferation, anandamide was also shown to stimulate MAP kinase activity and like proliferation this effect was not block by the SR compounds. From these results, and because MAP kinase was also stimulated by arachidonic acid, the authors concluded that anandamide was activating biological processes in a nonreceptor-mediated way. This is not surprising for fatty acid compounds that can readily penetrate the cell membrane, and in fact this has been suggested in other studies (55). Whatever the mechanisms, endogenous cannabimimetics may participate in the growth factor-induced maturation and differentiation of hematopoietic cells, thus playing a role in the growth and development of immune function.

Anandamide Modulates Cytokine Production

Anandamide, in addition to modulating cellular responsiveness to various cytokines, has also been reported to increase the production of cytokines under varying conditions. For example, murine brain cortical astrocyte cultures infected with Theiler's murine encephalomyelitis virus produced more IL-6 in the presence of anandamide (Fig. 7) (56). This effect was blocked by the CB1 antagonist, SR141716A, suggesting that these receptors were involved in the effect. Studies in human peripheral blood mononuclear cells examining a wide variety of cytokines demonstrated that anandamide as well as palmitoylethanolamide and THC either increased or decreased cytokine release depending upon drug concentration (57). For example, IL-6 and IL-8 release were diminished by low doses of anandamide whereas TNF-α, IFNγ, and IL-4 were inhibited at higher drug concentrations. These studies underscore the potential wide-ranging role of cannabimimetics in immunomodulation through effects on cytokine production.

Conclusions

The number of publications dealing with the relationship between the cannabinoid system and the cytokine network has increased dramatically in the past few years. These studies have more precisely defined the complex of receptors and ligands that make up the cannabinoid system, and, armed with this information, investigators have increasingly

asked important questions concerning the influence of this system on the regulation and development of the cytokine network. It appears that CB2, to a greater extent than CB1, is involved in immune cell and cytokine biology. Furthermore, a variety of psychoactive and nonactive cannabinoids as well as a variety of cannabimimetic eicosanoids appear to affect cytokine responses through both receptor dependent and independent mechanisms. It is particularly noteworthy that these substances working through cytokines promote both proinflammatory and anti-inflammatory effects and that they also can modulate growth factor effects and potentially hematopoiesis and tumor growth. Thus, it is highly likely that the consumption of marijuana and cannabinoids modulates the cytokine network; furthermore, it is likely that the endogenous cannabinoid system of receptors and ligands regulates many facets of the cytokine network involving both factor production and response. The next few years should provide an ever-increasing body of new information in this important area.

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