ELSEVIER

Chemistry and Physics of Lipids
108 (2000) 169–190

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

www.elsevier.com/locate/chemphyslip

Review

Cannabinoid receptors and the regulation of immune response

E.V. Berdyshev *

The Hormel Institute, University of Minnesota, 801 16th Avenue N.E., Austin, MN 55912, USA

Received 27 April 2000; received in revised form 16 June 2000; accepted 16 June 2000

Abstract

Cannabinoid research underwent a tremendous increase during the last 10 years. This progress was made possible by the discovery of cannabinoid receptors and the endogenous ligands for these receptors. Cannabinoid research is developing in two major directions: neurobehavioral properties of cannabinoids and the impact of cannabinoids on the immune system. Recent studies characterized the cannabinoid-induced response as a very complex process because of the involvement of multiple signalling pathways linked to cannabinoid receptors or effects elicited by cannabinoids without receptor participation. The objective of this review is to present this complexity as it applies to immune response. The functional properties of cannabinoid receptors, signalling pathways linked to cannabinoid receptors and the modulation of immune response by cannabinoid receptor ligands are discussed. Special attention is given to 'endocannabinoids' as immunomodulatory molecules.

Keywords: Cannabinoids; Cannabinoid receptors; Tetrahydrocannabinol; Anandamide; *N*-acylethanolamines; Immune response

1. Introduction

Marijuana (*Cannabis sativa*) is one of the oldest drugs of abuse but its medicinal value has also been known by many cultures throughout human history. Indications for the medicinal use of cannabis can be found in ancient Chinese and Egyptian civilizations. Cannabis preparations were included in British and US pharmacopoeias and were used extensively up to the 1930s for treatment of convulsive disorders and as analgesics. In the 1930s, marijuana started to lose medical attention as new, pure pharmaceutical drugs began to appear such as opiates, aspirin and barbiturates which could be given in standard doses with reliable effects. As marijuana's medicinal use began to wane, its recreational use started to increase in Western countries. Shortly thereafter, abuse of marijuana led to withdrawal of cannabis preparations from pharmacopoeias and laws were passed prohibiting its use. The identification of

* Tel.: +1-507-4379611; fax: +1-507-4379606.
E-mail address: eberdyshev@hotmail.com (E.V. Berdyshev).

0009-3084/00/$ - see front matter
PII: S0009-3084(00)00195-X

Δ^9-tetrahydrocannabinol (Δ^9-THC) as a major psychoactive principle in marijuana and its chemical synthesis by Gaani and Mechoulam, 1964 opened a new era of synthetic cannabinoids as pharmacological agents. Δ^9-THC preparations have been used, albeit occasionally, for pain relief, as an anti-emetic and appetite stimulant during cancer chemotherapy, as anti-convulsant and analgesic.

A significant increase of interest in cannabinoids developed shortly after pharmacological identification (Devane et al., 1988) and subsequent cloning (Matsuda et al., 1990) of a central CB1 cannabinoid receptor which is expressed mainly by neuronal cells. Subsequent identification of a peripheral CB2 cannabinoid receptor (Munro et al., 1993), which is expressed predominantly by immune cells, revealed a basis for the known, while modest, immunomodulatory effects of cannabis preparations. Thus, the molecular basis for cannabinoid action on the central nervous and immune systems was established.

The identification of *N*-arachidonoylethanolamine, referred to as anandamide (Devane et al., 1992), and more recently 2-arachidonoylglycerol (2-AG) (Lee et al., 1995; Mechoulam et al., 1995; Sugiura et al., 1995) as endogenous ligands for cannabinoid receptors raised questions about the role of 'endocannabinoids' and cannabinoid receptors in the maintenance of physiological homeostasis and in the development of some neuronal and immune system disorders.

Since cannabinoid research initially was primarily of sociological interest, one of its objectives has been to clarify the mechanism of action of natural cannabinoids and their synthetic derivatives. Information concerning cannabinoid-induced responses tends to characterize cannabinoid-induced signalling as an extremely complex event. This complexity is determined by the linkage of multiple signalling cascades to cannabinoid receptors as well as by the apparent non-receptor-mediated action of high doses of cannabinoids. Also, the physiological consequence of cannabinoid receptor activation may depend on the type of cell, the presence of other concomitant signalling events, and the chemical nature of the agonist. In contrast to studies on Δ^9-THC and its synthetic analogs, questions regarding the intrinsic role of 'endocannabinoids' and their receptor-inactive congeners in cell signalling have been less explored. Nevertheless, a better understanding of the functioning of the endogenous cannabinoid system may help to clarify its possible role in the development of some pathologies like autoimmune disorders and propagation of HIV infection. It is the objective of this review to present information concerning the complexity of cannabinoid receptor-mediated signalling and the resulting modulation in the functioning of the immune system.

2. Cannabinoid receptors

2.1. Variability and distribution of cannabinoid receptors

Presently, two main subtypes of cannabinoid receptors have been characterized. The first pharmacological evidence for cannabinoid receptors (CB1 cannabinoid receptor) was reported by the Howlett group (Devane et al., 1988) which characterized the binding of radioactive synthetic cannabinoid CP 55, 940 to rat brain homogenates. Two years later, Matsuda et al., (1990) isolated and cloned a complementary DNA that encodes rat brain cannabinoid receptor. Subsequently, human (Gérard et al., 1991) and mouse (Chakrabarti et al., 1995; Abood et al., 1997) brain-type cannabinoid receptors were also cloned. The cDNA sequences of the rat and human CB1 cannabinoid receptors are 90% identical at the nucleic acid level and 98% identical at the amino acid level. A mouse CB1 genomic clone had 95% nucleic acid identity with the rat (99.5% amino acid identity) and 90% nucleic acid identity (97% amino acid identity) with the human receptor.

Soon after identification of the central nervous system-type CB1 cannabinoid receptor, a second major form (CB2) of cannabinoid receptors was isolated and cloned from the human promyelocytic cell line HL60 (Munro et al., 1993). Both subtypes of cannabinoid receptors had a 68%

nucleotide identity within transmembrane regions, but only 44% identity throughout the whole protein. In spite of this difference between the two types of cannabinoid receptors, most cannabinoids as well as endogenous ligands for cannabinoid receptors showed similar binding affinity to both receptor subtypes (Pertwee, 1999). Recently, mouse (Shire et al., 1996) and rat (Griffin et al., 2000) CB2 receptors were cloned. Sequence analysis indicated 90% nucleic acid identity (93% amino acid identity) between rat and mouse and 81% nucleic acid identity (81% amino acid identity) between rat and human peripheral (CB2) cannabinoid receptors.

The CB1 receptor is primarily expressed in the CNS (Herkenham et al., 1990, 1991a,b; Westlake et al., 1994; Glass et al., 1997) and peripheral neurons (Ishac et al., 1996), as well as in the retina of different vertebrates (Straiker et al., 1999). It is also present in testes (Gérard et al., 1991), in guinea pig small intestine (Pertwee et al., 1996a), the mouse vas deferens (Pertwee et al., 1996b) and urinary bladder (Pertwee and Fernando, 1996). CB1 receptor mRNA was detected in the human adrenal gland, heart, lung, prostate, ovary, bone marrow, thymus and tonsils (Bouaboula et al., 1993; Galiegue et al., 1995), in mouse spleen but not in mouse thymus or rat spleen (Kaminski et al., 1992; Schatz et al., 1997), and in preimplantation mouse embryo (Paria et al., 1995). Within the immune cells, CB1 mRNA transcripts are modestly expressed by human B-cells, T-cells, and monocytes (Bouaboula et al., 1993), in Raji, THP-1, Burkett's lymphoma cell lines of human origin and in mouse natural killer-like NKB61A2 cell line (Daaka et al., 1996), in human Daudi B-lymphoblastoid cell line and in cultured rat microglial cells (Sinha et al., 1998). Jurkat cells did not reveal detectable levels of CB1 mRNA transcript in the unstimulated state but expressed this receptor after mitogen activation (Daaka et al., 1996).

CB2 cannabinoid receptor, also referred to as peripheral cannabinoid receptor, is completely absent in CNS (Griffin et al., 1999) and is expressed predominantly by the cells of the immune system where it is particularly abundant (Pettit et al., 1996; Schatz et al., 1997). Blood cell subpopulations differ in the degree of CB2 receptor expression and are ranked in the following order: B-cells > NK-cells > monocytes > polymorphonuclear neutrophils > $CD8^+$ T-lymphocytes > $CD4^+$ T-lymphocytes (Bouaboula et al., 1993; Galiegue et al., 1995). Quantitative analysis of CB2 mRNAs in different immune cell lines revealed their relative expression level as: Daudi > HL60 > U937 > MOLT-4 cell lines (Galiegue et al., 1995). CB2 cannabinoid receptor mRNAs were also detected in preimplantaion mouse embryo (Paria et al., 1995), macrophage/monocytes of the marginal zone of the spleen (Munro et al., 1993), in the cortex of the lymph nodes and the nodular corona of Peyer's patches (Lynn and Herkenham, 1994) and in rat basophilic leukemia RBL-2H3 mast cells (Facci et al., 1995).

In addition to classical CB1/CB2 cannabinoid receptors, a human splice variant of CB1 cannabinoid receptor (CB1A receptor) was also described (Shire et al., 1995).

2.2. Cannabinoid receptors are linked to G-proteins and are constitutively active

Cannabinoid receptors belong to a superfamily of G-protein-coupled receptors, are single polypeptides with seven transmembrane α-helices, and have an extracellular, glycosylated *N*-terminus and intracellular C-terminus. Both CB1 and CB2 cannabinoid receptors are linked to $G_{i/o}$-proteins. Several studies on cannabinoid ligand binding (Houston and Howlett, 1998; Kearn et al., 1999) or regulation of [^{35}S]-GTPγS binding (Burkey et al., 1997; Breivogel et al., 1998; Griffin et al., 1998; Kearn et al., 1999) in different membrane fractions raised questions of possible differential G-protein coupling to cannabinoid receptors. Recent in situ reconstitution experiments using human CB1 and CB2 cannabinoid receptors expressed in *Spodoptera frugiperda* cells clearly showed a difference between CB1 and CB2 receptors in their selective interaction with G_i and G_o proteins (Glass and Northup, 1999). While the activation of CB1 receptor resulted in high-affinity saturable receptor interaction with both G_i and G_o proteins, CB2 receptor interacted effi-

ciently only with G_i protein. Also, this work presented important evidence that different groups of ligands have different potency to stimulate coupling of CB1 or CB2 receptors to G_o or G_i proteins. HU 210, WIN 55, 212–2 and anandamide elicited maximal CB1-mediated activation of G_i, whereas Δ^9-THC caused only partial activation. In contrast, only HU 210 elicited maximal CB1-mediated activation of G_o, other ligands being 25–40% less active. As to CB2 receptor-mediated activation of Gi, HU 210 was the only compound able to elicit maximal activation. Thus, differential activation of G_i/G_o proteins depending on the type of the receptor and the nature of the agonist presents a partial molecular explanation for the observed complex picture of physiological responses elicited by cannabinoid receptor ligands of different origin.

It was recently shown that CB1 cannabinoid receptor has a constant tonic activity. The Sanofi group has shown in a series of publications that SR 141716A, initially described as a selective antagonist for CB1 receptor (Rinaldi-Carmona et al., 1994), behaves as an inverse agonist (Bouaboula et al., 1995a,b, 1997) that indicates the intrinsic constitutive activity of the receptor. Pan et al. (1997) studied voltage-dependent Ca^{2+} currents in neurons with normal and mutant K192A CB1 receptor and also showed a constitutive activity of CB1 receptor with the use of SR 141716A. Cell preincubation with SR 141716A increased the Ca^{2+} current in cells expressing normal CB1 receptor. SR 141716A also increased Ca^{2+} current under Ca^{2+}-free conditions which are supposed to prevent Ca^{2+}-dependent anandamide and 2-AG generation. The expression of a mutant K192A cannabinoid receptor, which has no affinity for anandamide, demonstrated that this mutant receptor still has tonic activity but SR141716A could not decrease the tonic Ca^{2+} current and was still able to antagonize the effect of WIN 55, 212–2. These data demonstrated the importance of K192 receptor site for the inverse agonistic properties of SR 141716A. Glass and Northup (1999) confirmed the constitutive activity of CB1 cannabinoid receptor in their in situ reconstitutive experiments. The authors showed that CB1 receptor exhibits a spontaneous activation of both G_i and G_o proteins which can be increased by additional magnesium. SR 141716A completely blocked this spontaneous activity. It was also shown that CB1 receptor expressed in CHO cells had increased constitutive MAP kinase-activating properties that could be blocked by SR 141716A (Bouaboula et al., 1997).

Apparently, CB2 cannabinoid receptor possesses constitutive activity similar to that of CB1 receptor. The inverse agonist SR 144528, described initially as a selective antagonist for cannabinoid receptors (Rinaldi-Carmona et al., 1998), was shown to decrease the [^{35}S]-GTPγS binding to human CB2 receptor expressed in CHO cells thus confirming a constitutive activity of the receptor (Bouaboula et al., 1999a). MAP kinase was also shown to be constitutively activated by CB2 receptor (Bouaboula et al., 1999a). Δ^9-THC, which was shown to act at CB2 receptor as a neutral antagonist, (Bayewitch et al., 1996), was unable to modulate MAP kinase activity in unstimulated cells. However, when cells were treated with CP 55, 940 or SR 144528, Δ^9-THC could antagonize compound-triggered stimulation or inhibition of MAP kinase activity. These data also indicate that the observed constitutive activity of the receptor is not due to the possible presence of endogenous ligands in the cell culture media (Bouaboula et al., 1999a).

In addition to the linkage to G_i/G_o-proteins, CB1 cannabinoid receptor was recently shown to be linked simultaneously to G_i- and G_s-proteins (see next section). Thus, cannabinoid receptors are able to transmit opposite signals depending on the type of agonist and other factors which still need to be identified. For more information concerning CB1/CB2 receptor genes, receptor distribution and functional properties the reader is referred to the detailed review by Matsuda (1997).

3. Signalling events following activation of cannabinoid receptors

A significant part of information concerning signalling events triggered by cannabinoid receptor activation was obtained using CHO, Cos and AtT cells transfected with CB1/CB2 receptors.

While transfected cells offer a useful tool to explore functional properties of receptors, one should take into account the artificial nature of transfected cells and carefully extrapolate the obtained data when compared with cells naturally expressing these receptors. In fact, the number of receptors expressed in transfected cells is several orders of magnitude greater than in primary cells that may alter the stoichiometry of critical regulatory proteins and thus result in responses distinct from those found in primary cells. Also, CHO or Cos cells often used for cannabinoid receptor transfection are not of myeloid or lymphoid lineage and do not possess a immunologically relevant functional response, and this fact should also be considered when discussing signalling events in relation to modulation of immune cell functioning.

3.1. Cannabinoid receptors as regulators of cAMP-dependent signalling

One of the most extensively studied properties of cannabinoid receptors is the ability of activated CB1/CB2 receptors to block forskolin-induced accumulation of intracellular cyclic adenosine 3′,5′-monophosphate (cAMP). This property was well demonstrated using both cannabinoids and 'endocannabinoids' as ligands and cells with naturally expressed CB1/CB2 receptors (Koh et al., 1997; Schatz et al., 1997; Herring et al., 1998) as well as CHO or AtT20 cells tranfected with CB1/CB2 receptors (Felder et al., 1995; Glass and Felder, 1997; Rinaldi-Carmona et al., 1998; Roche et al., 1999). This inhibitory effect of cannabinoid receptor ligands is abrogated by cell pretreatment with pertussis toxin which ADP-ribosylates $GTP_{i/o}$-proteins, thus confirming the $G_{i/o}$-protein linkage to cannabinoid receptors (Felder et al., 1995). The intracellular cAMP level is critical for PKA-mediated signalling as cAMP regulates PKA activation and the release of catalytical subunits which phosphorylate multiple intracellular targets including the cAMP-response element binding protein/activation transcription factor (CREB/ATF) family of transcriptional regulators. The PKA-dependent signalling cascade has major importance for gene regulation in immune cells, and the role for cannabinoid receptors in modulation of cAMP-dependent immune response was shown with the example of interleukin-2 (IL-2) gene transcription.

The IL-2 gene transcription is known to be regulated by several regulatory proteins including activator protein-1 (AP-1), the nuclear factor of activated T-cells (NF-AT) and the nuclear factor for immunoglobulin κ chain in B-cells (NF-κB). All these nuclear factors are inducible and cross-regulated by both PKA and PKC. The role for cAMP in positive regulation of IL-2 gene transcription was demonstrated in experiments where the induction of cAMP formation by forskolin was shown to enhance the phorbol ester/ionophore (PMA/Io)-induced amount of transcriptional-regulatory proteins bound to the AP-1 proximal site of the IL-2 promoter (Novak et al., 1990). When present during stimulation of EL4.IL-2 cells expressing CB2 cannabinoid receptor, cannabinol and Δ^9-THC inhibited forskolin-induced cAMP formation and PKA activation, and this inhibition was closely correlated with the repression of IL-2 transcription and secretion (Condie et al., 1996). This inhibition of IL-2 transcription by cannabinol was mediated by a sustained and remarkable down-regulation of NF-AT and by the transient inhibition of AP-1 DNA binding (Yea et al., 2000). However, not all effects elicited by cannabinoid receptor activation are cAMP-dependent. Thus, in mouse splenocytes the endogenous ligand for cannabinoid receptors, 2-AG, dose-dependently reduced both NF-AT-binding and promoter activity but did not influence cAMP response element (CRE) binding activity or that of AP-1 and octamer (Ouyang et al., 1998). Thus, these data demonstrate the possible diversity of the signalling response elicited by different cannabinoid receptor ligands and the cell-dependent variability of the responses.

Independent of IL-2 gene transcription, cannabinoid receptor activation by Δ^9-THC was shown to inhibit forskolin-induced binding of PKA-dependent transcription factor to CRE present in cAMP-responsive genes of mouse splenocytes (Koh et al., 1997). Cannabinol also downregulated adenylate cyclase and PKA activity and decreased transcription factor binding to CRE and κB motifs in mouse splenocytes and

thymocytes (Herring et al., 1998). A more detailed study using mouse thymocytes revealed that cannabinoid receptor activation by cannabinol inhibits the formation of PMA/Io-induced CREB-1 homodimer, CREB/ATF-2 heterodimer complex, and the formation of two κB DNA binding complexes of which only one complex is PMA/Io-inducible (Herring and Kaminski, 1999). The observed inhibition of nuclear factor DNA binding was due to the decrease in phosphorylation of CREB/ATF nuclear proteins and to the decrease in phosphorylation-dependent degradation of the NF-κB inhibitory protein IκB-α.

In the macrophage cell line RAW 264.7 the inhibition of CREB/ATF and NF-κB/Rel DNA binding by Δ^9-THC was responsible for the observed down-regulation of LPS-induced inducible NO synthase (iNOS) activity and nitrite production (Jeon et al., 1996). Similar to splenocytes and thymocytes, cannabinoid receptor activation by Δ^9-THC in macrophages strongly inhibited forskolin-induced cAMP accumulation thus confirming that the observed inhibition of iNOS gene expression is regulated through cAMP-dependent PKA-mediated signalling cascade.

CB1 cannabinoid receptor expressed in cerebellar granule cells was also shown to inhibit iNOS upon stimulation with WIN 55, 212–2, CP 55, 940 and HU 210 (Hillard et al., 1999). Interestingly, SR 141716A was able not only to antagonize the action of WIN 55, 212–2 but also to stimulate NO synthase activity by itself (Hillard et al., 1999). The most plausible explanation for such properties of SR 141716A is the blockage of the CB1 receptor constitutive activity (see previous section).

In a series of publications by Kaminski and coworkers (Kaminski et al., 1994; Condie et al., 1996; Schatz et al., 1997; Herring et al., 1998; Herring and Kaminski, 1999), extensively discussed in several reviews (Kaminski, 1996, 1998a,b), the axiom that cAMP-dependent signalling pathways have a downregulatory role in the immune response is questioned. In fact, there is an increasing evidence that a rapid upregulation of adenylate cyclase is an early event in lymphocyte activation (Kaminski et al., 1994; Watson et al., 1994; Condie et al., 1996) and that cAMP-dependent signalling pathways may positively or negatively regulate cytokine mRNA transcription in macrophages depending on the cytokine tested (Feng et al., 2000). Also, it was shown that, in relation to immune response, cAMP analogs can be inhibitory at high (>100 μM) concentrations and stimulatory at more physiological (<100 μM) concentrations (Kaminski et al., 1994; Koh et al., 1995). On the other hand, the inhibition of LPS-induced nitrite production in RAW264.7 cells by Δ^9-THC (Jeon et al., 1996) or NO synthase activity in rat microglial cells by CP 55,940 (Waksman et al., 1999) could be reversed by the addition of 8-bromo-cAMP (Jeon et al., 1996) or dibutyryl cAMP or cholera toxin (Waksman et al., 1999). Similarly, the inhibition of T-cell-dependent antibody responses by cannabinoids could be reversed by permeable cAMP analogs (Kaminski et al., 1994). All these data support the hypothesis that the cAMP-dependent signalling also has a positive stimulatory role in the establishment of immune response and that cannabinoid receptor stimulation can provide a signal which antagonizes the early events in immune cell activation.

CB1 cannabinoid receptor was also shown to possess a known property of $G_{i/o}$-coupled receptors, namely, to increase adenylyl cyclase activity after chronic agonist treatment. This phenomenon was selective for specific adenylyl cyclase types I, III, V, VI and VII isozymes and this sensitization could be blocked by pertussis toxin, implying a role for G_i/G_o proteins in the observed effect (Rhee et al., 2000).

Several recent publications have revealed a dual linkage of CB1 but not CB2 cannabinoid receptor to G_i- and G_s-binding proteins. This makes the pattern of intracellular signals triggered by receptor activation significantly more complicated than it was initially expected. Glass and Felder (1997) have shown that concurrent stimulation of CB1 receptor and D2 dopamine receptor in striatal neurons in primary culture leads to an accumulation of cAMP in contrast to inhibition of cAMP accumulation when the stimuli were applied separately. In addition, the use of pertussis toxin has unmasked a CB1 receptor-mediated property of HU 210 to stimulate forskolin-induced cAMP

accumulation which could be blocked by SR 141716A even in the presence of pertussis toxin. When transfected to CHO cells, activated CB1 but not CB2 cannabinoid receptor maintained the ability to increase cAMP level in the presence of pertussis toxin thus providing evidence for the simultaneous link of G_s- and G_i-proteins to CB1 receptor. This property was later confirmed in CHO cells expressing CB1 receptor with the use of the specific CB1 receptor antagonist LY320135 and anandamide as agonist (Felder et al., 1998) as well as with the use of low concentrations of CP 55, 940 (Calandra et al., 1999). Although immune cells express significantly less CB1 receptors in comparison to CB2 receptors, the dual link of CB1 receptor to G_s- and G_i-proteins should be considered when evaluating immunomodulatory properties of the ligands for cannabinoid receptors.

A mutational study identified the carboxyl-terminal segment of the third intracellular loop of the CB1 receptor as responsible for the link to G_s-mediated signalling. A double mutation in this receptor region resulted in partial constitutive activation of the receptor and an agonist-independent enhancement of cAMP levels (Abadji et al., 1999). In another study the first and second intracellular loops were shown to link the CB1 receptor with cAMP-dependent signalling (Calandra et al., 1999). More work is needed to firmly identify the regions of the receptor responsible for its link to different G-proteins. Also, it is still unclear, if the same cannabinoid CB1 receptor is simultaneously linked to G_s- and G_i-proteins or whether there are two populations of receptors.

In conclusion, it is clear that cannabinoid receptors supply signals leading to upregulation or downregulation of DNA binding of different nuclear factors, and part of these transduction pathways are mediated by cAMP. Ligands for cannabinoid receptors provide a very complex pattern of cAMP-dependent signalling events due to the link of the CB1 receptor to multiple transduction pathways and the presence of both types of cannabinoid receptors in immune cells. Also, cannabinoid receptors trigger a G_i-mediated cAMP-independent MAP kinase activation (see below) that completes a picture of signalling pathways regulating gene transcription.

3.2. Cannabinoid receptors and activation of MAP kinases

Besides downregulation of cAMP formation, G_i-proteins link cannabinoid receptors to the MAP kinase signalling cascade. Such a link was demonstrated by Wartmann et al., (1995) who showed the ability of anandamide and Δ^9-THC to stimulate MAP kinase activity and to increase phosphorylation of the arachidonate-specific cytoplasmic phospholipase A_2. Bouaboula et al. (1995b), using CHO cells transfected with CB1 receptor, showed that the link of CB1 cannabinoid receptor to MAP kinase activation is blocked by SR141716A and pertussis toxin but is independent of the receptor-mediated inhibition of forskolin-induced cAMP accumulation. Similar properties of the CB1 cannabinoid receptor were observed in the human astrocytoma cell line U373 MG (Bouaboula et al., 1995b). CB2 cannabinoid receptor has the same potency to stimulate MAP kinase activity, independent of regulation of adenylate cyclase, that was shown with CB2 receptor-transfected CHO cells and with human promyelocytic cells HL60 (Bouaboula et al., 1996). As a result, CB2 receptor may provide a MAP kinase-dependent mechanism for control of the membrane potential in immune cells as was shown for the membrane key electroneutral transmembrane transporter Na^+/H^+ exchanger (Bouaboula et al., 1999b).

Recent studies revealed more complex effects of cannabinoid receptor ligands on MAP kinases. For example, some ligands for cannabinoid receptors can modulate MAP kinase activity without receptor participation. Several lines of evidence were recently presented in favor of this hypothesis. Thus, anandamide was demonstrated to strongly stimulate MAP kinase activity in CHO cells overexpressing CB2 receptor even after a complete blockage of the receptor by the inverse agonist SR 144528 (Derocq et al., 1998). In ECV304 cells derived from human aorta, synthetic cannabinoid receptor agonist HU 210 and anandamide induced MAP kinase activation but only the effect of HU 210 could be blocked by SR 141716A. The transfection of ECV304 cells with CB1 receptor antisense oligonucleotides also

blocked the effect of HU 210 but not that of anandamide. In addition to MAP kinases, anandamide was shown to activate c-Jun and p38 kinases in these cells (Liu et al., 2000). These authors also found that only anandamide could stimulate MAP kinases through genistein-sensitive tyrosine kinases and protein kinase C (PKC) thus raising the possibility that activation of MAP kinases by anandamide is not mediated by cannabinoid receptors. If these data are confirmed in other models, it will mean that anandamide, and possibly other receptor-inactive *N*-acylethanolamines, could participate in a signalling pathway which does not require cannabinoid receptors.

In contrast to all the data showing positive regulation of MAP kinases by cannabinoid receptor ligands, Faubert and Kaminski (2000) have recently demonstrated the inhibitory effect of cannabinol on ERK MAP kinases in mouse splenocytes. It should be noted that, in addition to the difference in cell type used, experimental conditions in this work were significantly different from those used by other investigators (Bouaboula et al., 1995b; Liu et al., 2000). Namely, Faubert and Kaminski used PMA/Io-stimulated mouse splenocytes grown in the presence of serum and others used non-stimulated serum-starved cells. It is evident that, as in the case of cAMP-dependent signalling, the real picture of cannabinoid receptor ligand regulation of MAP kinases is complex and may differ depending on cell type, agonist and experimental conditions.

3.3. Desensitization and internalization of cannabinoid receptors

Cannabinoid receptor-mediated signalling is regulated also by the availability of the receptor. Following activation, cannabinoid receptors were shown to undergo phosphorylation (Garcia et al., 1998) and internalization (Hsieh et al., 1999) which may be followed by recycling into the membrane if the time of the treatment is short (Hsieh et al., 1999). The region of the carboxyl terminus of the CB1 receptor was identified as necessary for internalization (Hsieh et al., 1999). In addition to internalization, cannabinoid receptors are subject to desensitization. Different domains of CB1 receptor are responsible for receptor internalization and desensitization. Mutations in the 418–439 residue region of the CB1 receptor, which contains a target amino acid for phosphorylation by G-protein-coupled receptor kinase (GRK), do not influence internalization but completely abrogate receptor desensitization (Jin et al., 1999). The second transmembrane domain of the CB1 cannabinoid receptor also contains a functional domain responsible for receptor internalization as well as for the regulation of the inwardly rectifying potassium channel. When mutated in this region, the receptor is still able to bind agonists, to inhibit cAMP production and Ca^{2+} current and to activate p42/p44 MAP kinases, but it can no longer be internalized or modulate inwardly rectifying potassium channel (Roche et al., 1999).

The CB2 cannabinoid receptor was found to be constitutively active, phosphorylated at serine 352 and internalized at the basal level in CHO cells transfected with CB2 receptor (Bouaboula et al., 1999c). CP 55, 940 treatment induced a long-term receptor phosphorylation and nonresponsiveness to CP 55, 940. Surprisingly, it was found that SR 144528, an inverse agonist for CB2 cannabinoid receptor, dephosphorylated CB2 receptor making it once again receptive for agonist treatment. The autophosphorylation and CP 55, 940-induced phosphorylation of the CB2 receptor were shown to be different as constitutive phosphorylation involved an acidotropic GRK kinase which does not need $G_i\beta\gamma$ (Bouaboula et al., 1999c). Actually, it is not clear whether or not the observed dephosphorylation of CB2 receptor through SR 144528-induced mechanism occurs during 'endocannabinoid'-elicited signalling. Also, the endogenous stimuli providing such a dephosphorylation should be identified.

Finally, cannabinoid receptors were shown to modulate the transduction of signals through other types of $G_{i/o}$ protein-binding receptors. Cannabinoid receptors were shown to decrease the availability of $G_{i/o}$ proteins for other G-protein-linked receptors thus decreasing the strength of diverse stimuli (Vásquez and Lewis, 1999). This change in the availability of G_i/G_o

proteins may finally lead to a shift in cellular homeostasis and the impairment of the immune response.

4. The effect of natural and synthetic cannabinoids on immune response

Cannabis sativa preparations were used for centuries in Asian medicine to reduce the severity of pain, inflammation and asthma (Mechoulam, 1986). However, the recent tremendous increase in recreational use of marijuana revealed a deleterious effect of marijuana smoke on defense mechanisms against bacterial and viral infections (Klein, 1999). Recent investigations prompted by the discovery of cannabinoid receptors and their endogenous ligands significantly improved our knowledge concerning beneficial and deleterious effects of cannabinoid receptor ligands and depicted a complex picture of cannabinoid effect on the immune response. However, the question of the intrinsic role of the 'endocannabinoid' system is still far from being resolved.

Generally, cannabinoids have a deleterious effect on a variety of parameters of the immune response including the impairment of macrophage functions (Lopez-Cepero et al., 1986; McCoy et al., 1999) and the induction of an imbalance in T-cell CD4/CD8 ratio (Wallace et al., 1988) that may be partially responsible for the observed perturbation in immunoglobulin production (Rachelefsky et al., 1976; Nahas and Osserman, 1991; Schatz et al., 1993). Cannabinoids also provoke downregulation of NK cell activity (Specter et al., 1986; Klein et al., 1987), cytotoxic T lymphocyte activity (Klein et al., 1991) and an increase of cell receptivity to HIV-1 virus (Noe et al., 1998). All these effects can result in a serious impairment of the host defense system.

4.1. The in vivo effects of cannabinoids

Early evidence concerning immunosuppressive properties of cannabinoids was obtained by Morahan et al. (1979) who demonstrated the decreased resistance of mice to *Listeria monocytogenes* or *Herpes simplex* virus infection after a high dose (200 mg/kg) of Δ^9-THC by i.p. injection. Later, Mishkin and Cabral (1985) and Cabral et al. (1986a) Cabral et al. (1986b) showed that the Δ^9-THC-impaired cell-mediated immune response is responsible for decreased animal resistance to primary herpes virus infection. Similarly, Specter et al. (1991) demonstrated that Δ^9-THC administration to mice resulted in greater severity of disease caused by combined infection with the murine AIDS-like FLV virus and *H. simplex* virus. Resistance to bacterial infection was also shown to be impaired by cannabinoids. Relatively low doses of Δ^9-THC (1-4 mg/kg) given a day before or after i.v. injection of sublethal doses of *Legionella pneumophila* in mice increased animal susceptibility to this intracellular pathogen without affecting mortality. However, when injected with a second higher dose of bacteria several weeks later, Δ^9-THC-treated mice had a significantly higher mortality rate in comparison to non-treated animals due to obvious impairment of the development of immunity to this pathogen (Klein et al., 1994; Newton et al., 1994). Taken together, these data clearly show that host defense to viral, bacterial or protozoan infection is impaired by cannabinoids in experimental animals and they implicate macrophage/T lymphocytes as a primary target for cannabinoid action. The question of in vivo action of cannabinoids is also discussed in detail in several reviews (Friedman et al., 1995; Cabral and Dove Pettit, 1998; Klein et al., 1998a).

4.2. Cannabinoids and macrophage functions

Macrophages represent the first line of defense against bacterial infections. Numerous studies have shown that the exposure of macrophages to cannabinoids in vitro or in vivo impairs their functional capabilities. In normal lung, macrophages represent the predominant immune cell population and trigger the immune response following bacterial invasion. When administered intranasally in mice with subsequent animal challenge with aerosolized bacterial lipopolysaccharide (LPS), Δ^9-THC and WIN 55, 212–2 (0.23–0.83 mg/kg) significantly decreased TNF-α level in mouse bronchoalveolar lavage. This was

accompanied by a decrease in neutrophil recruitment (Berdyshev et al., 1998), thus showing that cannabinoids can decrease macrophage activation and bronchopulmonary inflammation in a model which mimics bacterial pulmonary invasion. In vitro, mouse peritoneal macrophages were suppressed by Δ^9-THC (1–50 μM) in their ability to phagocytize yeast (Lopez-Cepero et al., 1986). Phagocytic activity of human peripheral monocytes (Specter et al., 1991) as well as mouse macrophage cell line P388D1 (Tang et al., 1992) was also suppressed by Δ^9-THC at concentrations above 15 μM. It is important to note that these concentrations of Δ^9-THC are far above minimal concentrations needed to activate CB1/CB2 receptors, and this fact may indicate that non-specific action of Δ^9-THC is responsible, at least partially, for the observed effects. Specter et al. (1995) found that pertussis toxin cannot block the inhibitory effect of Δ^9-THC (32 μM) on NK-cell cytotoxicity, mitogen-induced lymphocyte blastogenesis or release of 12-HETE from peripheral blood mononuclear cells. Derocq et al. (1995) also showed insensitivity of CP 55, 940 (10 μM)-induced inhibition of B-cell activation to pertussis toxin. These studies, while performed on different types of immune cells, clearly demonstrate that at least some of the immunomodulatory properties of cannabinoids are not mediated by CB1/CB2 receptors.

Generation of NO by macrophages is an obligatory element of cellular attack on bacterial pathogens. In contrast to the effect of cannabinoids on phagocytosis which is at least in part cannabinoid receptor-independent, cellular synthesis of this effector molecule is negatively controlled by cannabinoid receptors. In macrophage line RAW264.7, Δ^9-THC (10–20 μM) produced a marked inhibition of iNOS transcription and nitric oxide production in response to bacterial LPS (Jeon et al., 1996). It is known that iNOS transcription is under partial control by cAMP-dependent NF-κB/Rel family of transcriptional factor (Xie et al., 1994). Consequently, the regulation of NF-κB-dependent iNOS transcription through cannabinoid receptor-mediated downregulation of PKA activity (Jeon et al., 1996; see also previous sections) may be one of the regulatory mechanisms triggered by Δ^9-THC. However, a complex nature of iNOS regulation by cannabinoids was proposed by Coffey et al., (1996). These authors have shown that in rat peritoneal cells, cannabinoid receptor-mediated regulation of NO production induced by LPS/IFN-γ comprised only a small part of Δ^9-THC-induced (0.5–7 μM) inhibition as forskolin could only partially restore NO production and only under conditions of limited iNOS induction. More direct evidence for cannabinoid receptor-mediated regulation of iNOS was recently presented. In rat microglial cells expressing CB1 cannabinoid receptor only the high-affinity cannabinoid receptor binding enantiomer (−)-CP 55, 940, but not (+)-CP 55, 940, dose-dependently inhibited LPS/IFNγ-stimulated NO release in a 0.1–8 μM concentration range. This inhibitory activity of (−)-CP 55, 940 was abolished by pertussis toxin or SR 141716A and reconstituted by dibutyryl-cAMP or cholera toxin (Waksman et al., 1999). In neuronal cells (rat cerebellar granule cells) neuronal NO synthase also was regulated through CB1 receptor. In these cells, KCl-induced activation of NO synthase was inhibited by WIN 55, 212-2, CP 55, 940 or HU 210, and this effect was reversed by SR 141716A (Hillard et al., 1999).

Macrophages fulfill an important function of antigen processing and its presentation to $CD4^+$ T-lymphocytes. Numerous in vivo studies (discussed above) point to the macrophage/T-cell cooperation as a target for cannabinoid action. Besides the direct effect of cannabinoids on T- and B-lymphocytes in terms of their receptivity to stimuli, cannabinoids were shown to impair antigen processing in macrophages but not their presentation to T-lymphocytes. McCoy et al. (1995) McCoy et al. (1999) reported that Δ^9-THC interferes with the ability of murine macrophage hybridoma cells to process hen egg lysozyme (HEL) antigen, resulting in decreased cytokine production by these cells. The preexposure of macrophages to low nanomolar concentrations of Δ^9-THC for 24 h inhibited macrophage potency to stimulate CD4+ T cell hybridoma 930.B2 clone 63 cells as was measured by IL-2 production. The use of native HEL antigen and synthetic

peptide 11–25 of HEL revealed that Δ^9-THC exerted its inhibitory properties only when macrophages were exposed to HEL but not to synthetic peptide. This finding demonstrates that Δ^9-THC impairs antigen processing and not its presentation. This inhibitory property of Δ^9-THC was CB2 receptor-mediated as these macrophages were shown to express CB2 receptor and SR 144528 but not SR 141716A completely blocked the effect of Δ^9-THC (McCoy et al., 1999). The interference of cell stimulation through CB2 receptor with macrophage-mediated T-cell activation was confirmed in the experiments with the use of CB2-knockout mice. In these experiments Δ^9-THC could inhibit macrophage-dependent T-cell activation in the T-cell co-stimulation assay with macrophages derived from wild type, but not from knockout mice, thus indicating that this effect is mediated by the cannabinoid CB2 receptor (Buckley et al., 2000).

4.3. Cannabinoids and lymphocyte response

T- and B-lymphocytes fulfill the steps of immune response subsequent to macrophages and participate in all elements of cell-mediated and humoral immunity. The inhibition of T-cell proliferating response by cannabinoids was demonstrated in numerous studies using in vivo and in vitro models (see Klein et al., 1998a,b for review). In general, this inhibitory effect was measurable after acute or chronic administration of relatively high doses of cannabinoids (up to 200 mg/kg) to experimental animals or high concentrations of cannabinoids (5–30 μM) in in vitro experiments. However, recent studies with low nanomolar concentrations of cannabinoids showed the opposite, a stimulatory effect of cannabinoid receptor ligands on human B-cell (Derocq et al., 1995) and mouse splenocyte (Luo et al., 1992) proliferation. Interestingly, the stimulatory effect of nanomolar concentrations of CP 55, 940 could be blocked by pertussis toxin but the simultaneously observed inhibitory effect of 10 μM CP 55, 940 was insensitive to pertussis toxin treatment (Derocq et al., 1995). Also, B-cells derived from tonsils which have high (virgin cells) and low (differentiated cells) levels of CB2 receptor expression were able to increase CD40-mediated proliferation rate in response to CP 55, 940. This stimulatory effect of CP 55, 940 was blocked by SR 144528 but not by SR 141716A (Carayon et al., 1998) thus confirming CB2 receptor mediation of the observed effect. These authors reported the important finding that differentiation markedly downregulated the level of CB2 receptor expression at the protein and mRNA levels, thus pointing out a possible significance for an 'endocannabinoid' system in B-cell differentiation (Carayon et al., 1998).

The observed perturbation in macrophage/T-cell cooperation and functioning has a direct impact on antibody production by B-cells. Several in vivo (Titishov et al., 1989; Nahas and Osserman, 1991; Schatz et al., 1993) and in vitro (Klein and Friedman, 1990; Kaminski et al., 1994) studies demonstrated the inhibition of antibody formation by natural and synthetic cannabinoids at micromolar concentrations. Once again, this effect of cannabinoids was shown to be mediated, at least in part, by cannabinoid receptors and cAMP-dependent signalling elements in vitro as pertussis toxin or dibutyryl-cAMP could block the suppressive effect of Δ^9-THC (22 μM) and CP 55, 940 (5.2 μM) on antibody formation in splenocyte culture (Kaminski et al., 1994). More information about cannabinoid effects on cellular and humoral immune response is available in recent detailed and comprehensive reviews by Klein et al. (1998a,b).

4.4. Cytokines as a target for cannabinoid action

The regulation of the concerted work of different types of immune cells is controlled by cytokines — signalling proteins synthesized and secreted by immune cells upon stimulation. Cytokines, together with their membrane-associated and soluble receptors, represent a complex network with positive and negative regulatory elements which play a major role in the development of TH1- or TH2-dependent immune responses. An increasing number of publications confirm that the observed impairment of macrophage/T-cell cooperation is accompanied by deregulation of cytokine production in immune cells.

Early work on the modulation of cytokine production by cannabinoids was performed 15 years ago by Blanchard et al. (1986) who studied the influence of Δ^9-THC on IFN-γ production by stimulated mouse splenocytes. In the presence of Δ^9-THC, splenocytes isolated from normal animals or mice chronically treated with Δ^9-THC showed decreased production of IFN-γ upon stimulation by phytohemagglutinin (PHA), concanavalin A (ConA) or *Escherichia coli* LPS. Zheng et al. (1992) were the first to report Δ^9-THC-induced inhibition of TNF-α production by cultured mouse peritoneal macrophages. When cultured in serum-free medium containing 0.5% BSA, high doses of Δ^9-THC (5–10 μg/ml) were shown to decrease significantly TNF-α secretion into the medium. The inhibitory effect was related to BSA concentration as much lower concentrations of Δ^9-THC (0.1–1 μg/ml) were needed to exert an inhibitory effect in protein-free medium. This decrease in released TNF-α was due to impaired processing of the presecreted form to secreted form of TNF-α but not due to a decrease in expression of mRNA for TNF-α. This finding was confirmed later by two other groups (Fischer-Stenger et al., 1993; Zheng and Specter, 1996).

In macrophages Δ^9-THC alters not only TNF-α expression but also has an impact on the expression of other proteins participating in the immune response. Cabral and Fischer-Stenger (1994) demonstrated the restructuring by Δ^9-THC of the protein profile in P388D1 and RAW264.7 macrophage-like cells stimulated by LPS, ConA or IFN-γ. Namely, Δ^9-THC reversed the protein profile of stimulated cells to that of non-stimulated cells. Moreover, in addition to TNF-α, Δ^9-THC treatment (0.1–10 μM) also downregulated the expression of class II,Ia molecules of the major histocompatibility complex (MHC) that has also a major importance for macrophage/T-cell cooperation.

However, the immunomodulatory properties of cannabinoid receptor ligands were shown to be complex and often unequivocal. In vivo Δ^9-THC (8 mg/kg) given to mice 24 h before and 24 h after injection of sublethal dose of *L. pneumophila* resulted in sudden death which resembled cytokine-mediated shock. A measurement of the blood level of acute phase cytokines TNF-α and IL-6 revealed a significant increase in cytokine content in the drug-treated animals (Klein et al., 1993). In vitro, micromolar concentrations of Δ^9-THC were shown to decrease secretion of IL-1β, IL-6 but not IL-1α by human THP-1 cells differentiated by PMA, and TNF-α level was increased by Δ^9-THC treatment (Shivers et al., 1994).

Srivastava et al. (1998) compared the effect of Δ^9-THC and cannabinol (2.5–10 μg/ml) on cytokine production in vitro by human T, B, eosinophilic and $CD8^+$ NK cell lines. Both compounds showed complex immunomodulatory properties which depended on cell line and concentration used. In a SRIK-NKL cell line, Δ^9-THC decreased constitutive production of IL-8, macrophage inflammatory protein 1α (MIP-1α), MIP-1β and PMA-induced synthesis of TNF-α, granulocyte-macrophage colony stimulating factor (GM-CSF) and IFN-γ. When applied to SR1H-B(ATL) B-cells, Δ^9-THC stimulated IL-8 synthesis without any significant effect on that of MIP-1α and MIP-1β but cannabinol strongly inhibited IL-8, MIP-1α and MIP-1β production at the highest (5–10 μg/ml) concentrations. Δ^9-THC strongly stimulated IL-8, MIP-1α and MIP-1β production by SRIS-EOSL eosinophilic cell line. Cannabinol had a moderately stimulatory effect on the level of these cytokines. Constitutive synthesis of IL-10 by T-cell line HUT-78 was strongly inhibited by both compounds. This important study depicts a complex behavior of two natural cannabinoids but does not offer any clue about possible participation of cannabinoid receptors or the effect of low doses of cannabinoids. On the contrary, recent work reported by the Sanofi group (Derocq et al., 2000) clearly showed the participation of CB2 receptors in the stimulatory effect of nanomolar concentrations of cannabinoid CP 55, 940 on the production of several cytokines in human promyelocytic cell line HL-60. The effect of CP 55, 940 (10–100 nM) was complex as it not only stimulated the synthesis of IL-8, TNF-α, MIP-1β and MCP-1 but also increased cell migration. This effect of CP 55, 940 was mediated by MAP kinase and translocation of NF-κB into the nucleus that preceded the induction of several genes responsible for cytokine

synthesis, regulation of transcription, and cell differentiation. SR 144528 completely abolished the ability of CP 55, 940 to stimulate cytokine synthesis (as shown for IL-8 and MCP-1) as well as cell migration. Interestingly, cell differentiation by DMSO significantly decreased the ability of CP 55, 940 to stimulate MCP-1 production but increased its effect on cell migration. Altogether, these data present important finding showing the possible role for cannabinoid CB2 receptor in cell differentiation. Surprisingly, the authors did not detect any effect of anandamide or 2-arachidonoylglycerol on cytokine synthesis or cell migration that questions the role of the 'endocannabinoid' system in the process of cell differentiation.

Several recent publications presented evidence that some of the observed immunomodulatory effects of cannabinoids are not mediated by cannabinoid receptors. Puffenbarger et al. (2000) described Δ^9-THC-induced (1–10 μM) inhibition of LPS-stimulated mRNA expression of IL-1α, IL-1β, IL-6 and TNF-α in cultured rat microglial cells, IL-6 being the most sensitive to cannabinoid treatment. However, paired enantiomers CP 55, 940 and CP 56, 667 as well as levonatradol and dextronatradol induced the same effect. Also, neither SR141716A nor SR 144528 could reverse the effect of the latter enantiomers thus questioning the participation of CB1/CB2 cannabinoid receptors in the observed effects. Smith et al. (2000) studied the effect of in vivo injections of WIN 55, 212–2 (3.1–50 mg/kg i.p.) and HU 210 (0.05–0.4 mg/kg i.p.) on cytokine levels in the serum of mice primed with *Corynebacterium parvum*. The authors found that both synthetic cannabinoids decreased serum levels of TNF-α and IL-12 but increased the level of IL-10 when administered before primed animals were challenged with LPS. SR 141716A but not SR 144528 antagonized the effect of both WIN 55, 212–2 and HU 210, allowing a conclusion about the importance of CB1 receptor in the observed effect of cannabinoids. At the same time, SR 141716A modulated cytokine response by itself in a manner identical to that of WIN 55, 212–2 and HU 210. This effect of SR 141716A was explained by possible partial agonistic properties of the compound at the CB1 receptor. However, no evidence was presented to exclude possible cannabinoid receptor-independent modes of action for cannabinoid receptor agonists. It is important to mention that SR 141716A and SR 144528 acting as inverse agonists helped to reveal constitutive activity for both CB1 and CB2 receptors (see previous sections). Thus, it might be possible that 'non-specific' binding sites are responsible for the described properties of these synthetic compounds. The same conclusion may be drawn from in vitro experiments with mouse splenocytes (Klein et al., 1998c) where the authors have shown that pertussis toxin could block Δ^9-THC (3–5 μg/ml)-induced increase of IL-4 production but failed to block its inhibitory effect on IFN-γ production by stimulated splenocytes. Also, SR 141716A had no effect on Δ^9-THC-induced suppression of IFN-γ synthesis. Interestingly, Δ^9-THC happened to be the sole agonist among Δ^9-THC, WIN 55, 212–2 and CP 55, 940 which could upregulate IL-4 production by splenocytes stimulated with pokeweed mitogen. Δ^9-THC also increased IL-10 synthesis under the same experimental conditions (Klein et al., 1998c).

An opposite action of nanomolar and micromolar concentrations of Δ^9-THC on cytokine production by human PBMC was also described (Berdyshev et al., 1997). Low nanomolar concentrations of Δ^9-THC (3–30 nM) decreased monocyte-dependent LPS-induced synthesis of TNF-α, IL-6 and IL-8 without affecting lymphocyte-dependent PHA-induced synthesis of IFN-γ. However, micromolar concentrations of Δ^9-THC (3 μM) stimulated the production of all these cytokines with a concomitant decrease in IL-10 and IL-4 synthesis. It is important to note that a subsequent increase in Δ^9-THC concentration up to 30 μM completely blocked TNF-α production without affecting cell viability. No attempt was made in this study to clarify a possible role for cannabinoid receptors in the observed effects.

The Kaminski group accomplished an important breakthrough in understanding the regulation by cannabinoid receptor ligands of one of the most important T-lymphocyte cytokines, IL-2 (discussed in detail in the previous sections). It was shown that the blockage of cAMP-dependent

signalling is of major importance in the observed downregulation of IL-2 transcription by micromolar concentrations of cannabinoids (Condie et al., 1996; Koh et al., 1997; Herring et al., 1998; Herring and Kaminski, 1999; Yea et al., 2000) and the endogenous cannabinoid receptor ligand 2-AG (Lee et al., 1995; Ouyang et al., 1998). The observed disturbance of the adenylate cyclase/cAMP pathway was reflected in the degree of DNA binding of several nuclear binding factors (NF-κB, NF-AT, AP-1, CREB) which regulate IL-2 gene promoter activity (Condie et al., 1996; Ouyang et al., 1998; Herring and Kaminski, 1999; Yea et al., 2000).

In summarizing all this information it is possible to conclude that cannabinoids have a very complex effect on the immune system with an influence on almost every component of immune response machinery. The effect of cannabinoids is mediated only partially by cannabinoid receptors and the degree of receptor participation depends on cell type, cannabinoid concentration and cellular environment.

5. Endocannabinoids and the immune system

N-acylethanolamines became of interest to immunologists almost 50 years ago when antiinflammatory properties of some fractions from peanut oil, soybean lecithin and egg yolk, whose active principle was later identified as *N*-palmitoylethanolamine, were discovered (Coburn et al., 1954; Long and Martin, 1956; Kuehl et al., 1957). In the late 1960s antiviral and antibacterial properties of *N*-palmitoylethanolamine were also reported (Perlík et al., 1971a,b, 1973; Rašková and Mašek, 1967; Rašková et al., 1972). However, these initial findings did not result in major research on their mechanism of action.

The discovery of cannabinoid receptors and their endogenous ligands (Devane et al., 1992; Lee et al., 1995; Mechoulam et al., 1995; Sugiura et al., 1995) revived interest in *N*-acylethanolamines as modulators of immune response. Anandamide (Schmid et al., 1997; Pestonjamasp and Burstein, 1998; Varga et al., 1998; Di Marzo et al., 1999; Kuwae et al., 1999;) and 2-AG (Varga et al., 1998; Di Marzo et al., 1999) were shown to be synthesized by different immune cells. However, anandamide, as in other tissues and cells, was shown to comprise only a few percent of endogenous *N*-acylethanolamines (Schmid et al., 1997). New evidence for an unusually high rate of turnover of *N*-acylethanolamine precursor, *N*-acyl-phosphatidylethanolamine, in macrophages (Kuwae et al., 1999) suggests the participation of all saturated and unsaturated *N*-acylethanolamines in signalling events (see also the review by Schmid in this issue).

Despite great interest in cannabinoids and cannabinoid receptors, little is known concerning the role for 'endocannabinoids' in the regulation of immune response. In reality, only a few studies were designed recently to address this question. In general, the physiological role for *N*-acylethanolamines remains obscure. In all studies with parallel use of *N*-palmitoylethanolamine and anandamide, *N*-palmitoylethanolamine, which cannot bind to either type of cannabinoid receptor (Ross et al., 1997; Pertwee, 1999; Sugiura et al., 2000), was shown to possess immunomodulatory properties similar to that of anandamide or was biologically active when anandamide was inactive (see below). It is clear that more studies are required to understand the intrinsic role of 'endocannabinoids' in immune response.

In accordance with the initial hypothesis that *N*-acylethanolamines are able to downregulate immune response, Aloe et al. (1993) demonstrated mast cell downregulation by short-chain and long-chain *N*-acylethanolamines in vivo. The authors suggested that saturated *N*-acylethanolamines like *N*-palmitoylethanolamine may behave as local autocoids capable of downregulating mast cell activation. When applied to human macrophages in vitro, anandamide (1 μM) caused cell rounding, the loss of motility, the blockage of chemotaxis and stimulation of NO release (Stefano et al., 1998). Anandamide as well as *N*-palmitoylethanolamine (0.1–1 μM) were shown to stimulate the growth of murine hematopoietic cell lines in serum-free medium (Derocq et al., 1998). This effect of both *N*-acylethanolamines could not be prevented by the inverse agonists for CB1 (SR 141716A) or CB2 (SR 144528) receptors thus

indicating the independence of the observed effect from cannabinoid receptors. In contrast to these results, Valk et al. (1997) found anandamide (0.1–3 μM), but not other *N*-acylethanolamines, to be a potent cofactor to other growth stimuli which potentiated growth of murine hematopoietic cell lines in serum-free medium. The effect of anandamide was seen only at conditions of cell serum deprivation, allowing the authors to suggest that FCS contains endogenous ligands for cannabinoid receptors. This hypothesis was supported by our recent finding that anandamide and other *N*-acylethanolamines are present at significant levels in commercial FBS preparations (Berdyshev et al., 1999, 2000). This observation makes it clear that cells are constantly exposed to 'endocannabinoids' during cell growth in culture with the exception of experiments performed under serum-free conditions.

When injected into mice (i.p.) high concentrations of anandamide (20–80 mg/kg) were shown to suppress TNF-α-dependent killing of K929 cells by macrophages isolated from animals that received *Propionibacterium acnes* for cell activation (Cabral et al., 1995). In vitro anandamide was also shown to inhibit dose-dependently the LPS- or Theiler virus-induced nitrite and TNF-α production by mouse astrocytes (Molina-Holgado et al., 1997). Unfortunately, the concentrations of anandamide used in this study were extremely high (10–100 μM), limiting the significance of the results. A subsequent study by the same group of authors revealed a potency of anandamide (10–25 μM) to stimulate IL-6 synthesis by mouse astrocytes infected with Theiler's murine encephalomyelitis virus. This effect of anandamide was completely blocked by 1 μM SR 141716A (Molina-Holgado et al., 1998).

Facci et al. (1995) published an observation showing that *N*-palmitoylethanolamine but not anandamide is able to downregulate mast cell activation and is a true ligand for CB2 cannabinoid receptors expressed in RBL-2H3 cells. This publication provoked extensive speculation concerning *N*-palmitoylethanolamine as a ligand for cannabinoid receptors. However, subsequent analysis of *N*-palmitoylethanolamine binding to CB1/CB2 receptors, including CB2 receptors expressed in RBL-2H3 cells, did not confirm its binding to any of these receptor subtypes (Ross et al., 1997; Pertwee, 1999; Sugiura et al., 2000). Meanwhile, immunomodulatory properties of *N*-palmitoylethanolamine were confirmed in other models. We compared the potency of *N*-palmitoylethanolamine and anandamide to modulate LPS- and PHA-stimulated cytokine synthesis by human PBMC in vitro (Berdyshev et al., 1997) as well as LPS-induced pulmonary inflammation in mice (Berdyshev et al., 1998). Both compounds had approximately the same ability in vitro to downregulate LPS-induced synthesis of TNF-α, IL-6, IL-8 and PHA-induced IL-4 synthesis at nanomolar concentrations (3–300 nM) but anandamide was more potent inhibitor of TNF-α synthesis at higher (3 μM) concentration. Also, anandamide was found to inhibit PHA-stimulated IFN-γ synthesis while *N*-palmitoylethanolamine was inactive. In vivo both *N*-acylethanolamines also exerted antiinflammatory properties, although different in detail. Thus, anandamide decreased TNF-α level in bronchoalveolar lavage and diminished neutrophil recruitment in the lung of mice challenged by aerosolized LPS at low (0.075 nmol/kg) dose but not at higher doses while *N*-palmitoylethanolamine decreased TNF-α production only at high (0.75 μmol/kg) dose without any effect on cell composition. Thus, there is no doubt that both anandamide and *N*-palmitoylethanolamine are able to modulate immune response but the role for cannabinoid receptors in the observed effects is completely unclear.

It has been proposed that 2-AG rather than anandamide is the true endogenous ligand for both CB1 and CB2 receptors (Sugiura et al., 1999, 2000; Gonsiorek et al., 2000). However, very little attention was given to 2-AG as an immunomodulatory molecule. Lee et al. (1995) found that 2-AG but not anandamide can inhibit mixed lymphocyte response, anti-CD3 mAb-induced T-cell proliferation and LPS-induced B-cell proliferation at relatively high (5–25 μM) concentrations and low cell density (1×10^6 cells per ml). Later, 2-AG was shown to downregulate PMA/Io-induced IL-2 synthesis by mouse splenocytes (Ouyang et al., 1998). This effect of 5–50 μM concentrations of 2-AG resulted in inhibition of

IL-2 promoter activity through the downregulation of NF-AT and NF-κB/Rel DNA binding thus showing similarity between immunomodulatory effects of 2-AG and natural cannabinoids. Unfortunately, it is too early to draw any conclusions regarding possible differences between anandamide and 2-AG as immunomodulatory molecules or their role in the induction of receptor-mediated and non-receptor-mediated signalling cascades leading to cytokine gene transcription. More experiments are needed with a comparison of these two endogenous ligands in terms of their immunomodulatory properties and their role in signalling events. Also, comparing anandamide and 2-AG to their cannabinoid receptor-inactive congeners might provide additional information necessary for understanding the role of the 'endocannabinoid' system in immune response.

6. Conclusion

Cannabinoid research has experienced tremendous progress in the last decade, and credit for this fact should be given to the identification of cannabinoid receptors and their endogenous ligands. A range of potent cannabinoid receptor ligands and their selective antagonists/inverse agonists, as well as CB1/CB2 receptor binding antibodies, is now available to ensure additional breakthroughs in our understanding of the functioning of 'endocannabinoid' system. The complexity of signalling pathways triggered by cannabinoids and 'endocannabinoids' and the sometimes conflicting examples of their immunomodulatory properties make it necessary to take into account all possible factors which may influence the final outcome of cannabinoid action. Of all these possible factors several have to attract special attention. One of them is the usual presence of both CB1 and CB2 receptors in immune cells. While the CB1 receptor is significantly less expressed in immune cells, it has the distinct property to link cannabinoid receptor to the G_s signalling pathway. Also, in addition to cAMP-dependent signalling, both CB1 and CB2 cannabinoid receptors can trigger cAMP-independent MAP-kinase activation. Then, the ligands for cannabinoid receptors have a significant variability in potency to link receptors with G_i- and G_o-proteins. And finally, cannabinoid receptors have an intrinsic constitutive activity that brings an additional complication in the elucidation of the cannabinoid-induced signalling events. It seems possible that the 'endocannabinoid' signalling system plays a negative role in the onset of the immune response, but the exact role for 'endocannabinoids' and cannabinoid receptors in the maintenance of immune system homeostasis and the development of immune system disorders still needs to be defined.

Acknowledgements

The author's work was supported in part by United States Public Health Service grant GM45741, INTAS grant 97-1297 and RFBR grant 98-04-48049 and The Hormel Foundation. I thank Dr Harald Schmid, Patricia Schmid and Dr Doug Bibus of the Hormel Institute, University of Minnesota, for helpful discussion during preparation of the manuscript.

References

Abadji, V., Lucas-Lenard, J.M., Chin, C., Kendall, D.A., 1999. Involvement of the carboxyl terminus of the third intracellular loop of the cannabinoid CB1 receptor in constitutive activation of G_s. J. Neurochem. 72, 2032–2038.

Abood, M.E., Ditto, K.E., Noel, M.A., Showalter, V.M., Tao, Q., 1997. Isolation and expression of a mouse CB1 cannabinoid receptor gene. Comparison of binding properties with those of native CB1 receptors in mouse brain and N18TG2 neuroblastoma cells. Biochem. Pharmacol. 53, 207–214.

Aloe, L., Leon, A., Levi-Montalcini, R., 1993. A proposed autocoid mechanism controlling mastocyte behavior. Agents Actions 39, C145–C147.

Bayewitch, M., Rhee, M.H., Avidor-Reiss, T., Breuer, A., Mechoulam, R., Vogel, Z., 1996. (−)-Delta9-tetrahydrocannabinol antagonizes the peripheral cannabinoid receptor-mediated inhibition of adenylyl cyclase. J. Biol. Chem. 271, 9902–9905.

Berdyshev, E.V., Boichot, E., Germain, N., Allain, N., Anger, J.P., Lagente, V., 1997. Influence of fatty acid

ethanolamides and Δ^9-tetrahydrocannabinol on cytokine and arachidonate release by mononuclear cells. Eur. J. Pharmacol. 330, 231–240.

Berdyshev, E., Boichot, E., Corbel, M., Germain, N., Lagente, V., 1998. Effects of cannabinoid receptor ligands on LPS-induced pulmonary inflammation in mice. Life Sci. 63, PL125–PL129.

Berdyshev, E.V., Schmid, P.C., Dong, Z., Schmid, H.H.O., 1999. Experimentally relevant levels of *N*-acylethanolamines including anandamide are present in serum-containing cell culture media. FASEB J. 13, A1378.

Berdyshev, E.V., Schmid, P.C., Dong, Z., Schmid, H.H., 2000. Stress-induced generation of *N*-acylethanolamines in mouse epidermal JB6 P^+ cells. Biochem. J. 346, 369–374.

Blanchard, D.K., Newton, C., Klein, T.W., Stewart, W.E., II, Friedman, H., 1986. In vitro and in vivo suppressive effects of Δ^9-tetrahydrocannabinol on interferon production by murine spleen cells. Int. J. Immunopharmacol. 8, 819–824.

Bouaboula, M., Rinaldi, M., Carayon, P., Carillon, C., Delpech, B., Shire, D., Le Fur, G., Casellas, P., 1993. Cannabinoid-receptor expression in human leukocytes. Eur. J. Biochem. 214, 173–180.

Bouaboula, M., Bourrié, B., Rinaldi-Carmona, M., Shire, D., Le Fur, G., Casellas, P., 1995a. Stimulation of cannabinoid receptor CB1 induces krox-24 expression in human astrocytoma cells. J. Biol. Chem. 270, 13973–13980.

Bouaboula, M., Poinot-Chazel, C., Bourrié, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., Le Fur, G., Casellas, P., 1995b. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. Biochem. J. 312, 637–641.

Bouaboula, M., Poinot-Chazel, C., Marchand, J., Canat, X., Bourrié, B., Rinaldi-Carmona, M., Calandra, B., Le Fur, G., Casellas, P., 1996. Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. Eur. J. Biochem. 237, 704–711.

Bouaboula, M., Perrachon, S., Milligan, L., Canat, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Pecceu, F., Lupker, J., Maffrand, J.P., Le Fur, G., Casellas, P., 1997. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. J Biol Chem. 272, 22330–22339.

Bouaboula, M., Desnoyer, N., Carayon, P., Combes, T., Casellas, P., 1999a. G_i protein modulation induced by a selective inverse agonist for the peripheral cannabinoid receptor CB2: implication for intracellular signalization cross-regulation. Mol. Pharmacol. 55, 473–480.

Bouaboula, M., Bianchini, L., McKenzie, F.R., Pouyssegur, J., Casellas, P., 1999b. Cannabinoid receptor CB1 activates the Na^+/H^+ exchanger NHE-1 isoform via G_i-mediated mitogen activated protein kinase signaling transduction pathways. FEBS Lett. 449, 61–65.

Bouaboula, M., Dussossoy, D., Casellas, P., 1999c. Regulation of peripheral cannabinoid receptor CB2 phosphorylation by the inverse agonist SR 144528. Implications for receptor biological responses. J. Biol. Chem. 274, 20397–20405.

Breivogel, C.S., Selley, D.E., Childers, S.R., 1998. Cannabinoid receptor agonist efficacy for stimulating [^{35}S]GTPγS binding to rat cerebellar membranes correlates with agonist-induced decreases in GDP affinity. J. Biol. Chem. 273, 16865–16873.

Buckley, N.E., McCoy, K.L., Mezey, E., Bonner, T., Zimmer, A., Felder, C.C., Glass, M., Zimmer, A., 2000. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB2 receptor. Eur. J. Pharmacol. 396, 141–149.

Burkey, T.H., Quock, R.M., Consroe, P., Roeske, W.R., Yamamura, H.I., 1997. Δ-9-Tetrahydrocannabinol is a partial agonist of cannabinoid receptors in mouse brain. Eur. J. Pharmacol. 323, R3–4.

Cabral, G.A., Lockmuller, J.C., Mishkin, E.M., 1986a. Delta 9-tetrahydrocannabinol decreases alpha/beta interferon response to herpes simplex virus type 2 in the $B_6C_3F_1$ mouse. Proc. Soc. Exp. Biol. Med. 181, 305–311.

Cabral, G.A., Mishkin, E.M., Marciano-Cabral, F., Coleman, P., Harris, L., Munson, A.E., 1986b. Effect of delta 9-tetrahydrocannabinol on herpes simplex virus type 2 vaginal infection in the guinea pig. Proc. Soc. Exp. Biol. Med. 182, 181–186.

Cabral, G.A., Fischer-Stenger, K., 1994. Inhibition of macrophage inducible protein expression by delta-9-tetrahydrocannabinol. Life Sci. 54, 1831–1844.

Cabral, G.A., Toney, D.M., Fischer-Stenger, K., Harrison, M.P., Marciano-Cabral, F., 1995. Anandamide inhibits macrophage-mediated killing of tumor necrosis factor-sensitive cells. Life Sci. 56, 2065–2072.

Cabral, G.A., Dove Pettit, D.A., 1998. Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. J. Neuroimmunol. 83, 116–123.

Calandra, B., Portier, M., Kerneis, A., Delpech, M., Carillon, C., Le Fur, G., Ferrara, P., Shire, D., 1999. Dual intracellular signaling pathways mediated by the human cannabinoid CB1 receptor. Eur. J. Pharmacol. 374, 445–455.

Carayon, P., Marchand, J., Dussossoy, D., Derocq, J.M., Jbilo, O., Bord, A., Bouaboula, M., Galiegue, S., Mondiere, P., Penarier, G., Fur, G.L., Defrance, T., Casellas, P., 1998. Modulation and functional involvement of CB2 peripheral cannabinoid receptors during B-cell differentiation. Blood 92, 3605–3615.

Chakrabarti, A., Onaivi, E.S., Chaudhuri, G., 1995. Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. DNA Seq. 5, 385–388.

Coburn, A.F., Graham, C.E., Hahinger, J., 1954. Effect of egg yolk in diets on anaphylactic arthritis (passive Arthus phenomenon) in the guinea pig. J. Exp. Med. 100, 425–435.

Coffey, R.G., Yamamoto, Y., Snella, E., Pross, S., 1996. Tetrahydrocannabinol inhibition of macrophage nitric oxide production. Biochem. Pharmacol. 52, 743–751.

Condie, R., Herring, A., Koh, W.S., Lee, M., Kaminski, N.E., 1996. Cannabinoid inhibition of adenylate cyclase-mediated signal transduction and interleukin 2 (IL-2) expression in the murine T-cell line, EL4.IL-2. J. Biol. Chem. 271, 13175–13183.

Daaka, Y., Friedman, H., Klein, T.W., 1996. Cannabinoid receptor proteins are increased in Jurkat, human T-cell line after mitogen activation. J. Pharmacol. Exp. Ther. 276, 776–783.

Devane, W.A., Dysarz, F.A., Johnson, M.R., Melvin, L.S., Howlett, A.C., 1988. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34, 605–613.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Derocq, J.M., Segui, M., Marchand, J., Le Fur, G., Casellas, P., 1995. Cannabinoids enhance human B-cell growth at low nanomolar concentrations. FEBS Lett. 369, 177–182.

Derocq, J.M., Bouaboula, M., Marchand, J., Rinaldi-Carmona, M., Segui, M., Casellas, P., 1998. The endogenous cannabinoid anandamide is a lipid messenger activating cell growth via a cannabinoid receptor-independent pathway in hematopoietic cell lines. FEBS Lett. 425, 419–425.

Derocq, J.M., Jbilo, O., Bouaboula, M., Segui, M., Clere, C., Casellas, P., 2000. Genomic and functional changes induced by the activation of the peripheral cannabinoid receptor CB2 in the promyelocytic cells HL-60. Possible Involvement of the CB2 receptor in cell differentiation. J. Biol. Chem. 275, 15621–15628.

Di Marzo, V., Bisogno, T., De Petrocellis, L., Melck, D., Orlando, P., Wagner, J.A., Kunos, G., 1999. Biosynthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in circulating and tumoral macrophages. Eur. J. Biochem. 264, 258–267.

Facci, L., Dal Toso, R., Romanello, S., Buriani, A., Skaper, S.D., Leon, A., 1995. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proc. Natl. Acad. Sci. USA 92, 3376–3380.

Faubert, B.L., Kaminski, N.E., 2000. AP-1 activity is negatively regulated by cannabinol through inhibition of its protein components, c-fos and c-jun. J. Leukoc. Biol. 67, 259–266.

Felder, C.C., Joyce, K.E., Briley, E.M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A.L., Mitchell, R.L., 1995. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol. Pharmacol. 48, 443–450.

Felder, C.C., Joyce, K.E., Briley, E.M., Glass, M., Mackie, K.P., Fahey, K.J., Cullinan, G.J., Hunden, D.C., Johnson, D.W., Chaney, M.O., Koppel, G.A., Brownstein, M., 1998. LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. J. Pharmacol. Exp. Ther. 284, 291–297.

Feng, W., Wang, Y., Zhang, J., Wang, X., Li, C., Chang, Z., 2000. Effects of CTx and 8-bromo-cAMP on LPS-induced gene expression of cytokines in murine peritoneal macrophages. Biochem. Biophys. Res. Commun. 269, 570–573.

Fischer-Stenger, K., Dove Pettit, D.A., Cabral, G.A., 1993. Δ^9-Tetrahydrocannabinol inhibition of tumor necrosis factor-alfa: suppression of post-translational events. J. Pharmacol. Exp. Ther. 267, 1558–1565.

Friedman, H., Klein, T.W., Newton, C., Daaka, Y., 1995. Marijuana, receptors and immunomodulation. Adv. Exp. Med Biol. 373, 103–113.

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., Casellas, P., 1995. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur. J. Biochem. 232, 54–61.

Gaani, Y., Mechoulam, R., 1964. Isolation, structure, and partial synthesis of an active constituent of hashish. J. Am. Chem. Soc. 86, 1646–1647.

Garcia, D.E., Brown, S., Hille, B., Mackie, K., 1998. Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor. J. Neurosci. 18, 2834–2841.

Gérard, C.M., Mollereau, C., Vassart, G., Parmentier, M., 1991. Molecular-cloning of a human cannabinoid receptor which is also expressed in testis. Biochem. J. 279, 129–134.

Glass, M., Felder, C.C., 1997. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a G_s linkage to the CB1 receptor. J. Neurosci. 17, 5327–5333.

Glass, M., Dragunow, M., Faull, R.L., 1997. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77, 299–318.

Glass, M., Northup, J.K., 1999. Agonist selective regulation of G proteins by cannabinoid CB1 and CB2 receptors. Mol. Pharmacol. 56, 1362–1369.

Gonsiorek, W., Lunn, C., Fan, X., Narula, S., Lundell, D., Hipkin, R.W., 2000. Endocannabinoid 2-arachidonlyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. Mol. Pharmacol. 57, 1045–1050.

Griffin, G., Atkinson, P.J., Showalter, V.M., Martin, B.R., Abood, M.E., 1998. Evaluation of cannabinoid receptor agonists and antagonists using the guanosine-5′-O-(3-[^{35}S]thio)-triphosphate binding assay in rat cerebellar membranes. J. Pharmacol. Exp. Ther. 285, 553–560.

Griffin, G., Wray, E.J., Tao, Q., McAllister, S.D., Rorrer, W.K., Aung, M.M., Martin, B.R., Abood, M.E., 1999. Evaluation of the cannabinoid CB2 receptor-selective antagonist, SR144528: further evidence for cannabinoid CB2 receptor absence in the rat central nervous system. Eur. J. Pharmacol. 377, 117–125.

Griffin, G., Tao, Q., Abood, M.E., 2000. Cloning and pharmacological characterization of the rat CB(2) cannabinoid receptor. J. Pharmacol. Exp. Ther. 292, 886–894.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. USA 87, 1932–1936.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1991a. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11, 563–583.

Herkenham, M., Lynn, A.B., de Costa, B.R., Richfield, E.K., 1991b. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. Brain Res. 547, 267–274.

Herring, A.C., Koh, W.S., Kaminski, N.E., 1998. Inhibition of the cyclic AMP signaling cascade and nuclear factor binding to CRE and κB elements by cannabinol, a minimally CNS-active cannabinoid. Biochem. Pharmacol. 55, 1013–1023.

Herring, A.C., Kaminski, N.E., 1999. Cannabinol-mediated inhibition of nuclear factor-κB, cAMP response element-binding protein, and interleukin-2 secretion by activated thymocytes. J. Pharmacol. Exp. Ther. 291, 1156–1163.

Hillard, C.J., Muthian, S., Kearn, C.S., 1999. Effects of CB1 cannabinoid receptor activation on cerebellar granule cell nitric oxide synthase activity. FEBS Lett. 459, 277–281.

Houston, D.B., Howlett, A.C., 1998. Differential receptor-G-protein coupling evoked by dissimilar cannabinoid receptor agonists. Cell Signal. 10, 667–674.

Hsieh, C., Brown, S., Derleth, C., Mackie, K., 1999. Internalization and recycling of the CB1 cannabinoid receptor. J. Neurochem. 73, 493–501.

Ishac, E.J., Jiang, L., Lake, K.D., Varga, K., Abood, M.E., Kunos, G., 1996. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. Br. J. Pharmacol. 118, 2023–2028.

Jeon, Y.J., Yang, K.H., Pulaski, J.T., Kaminski, N.E., 1996. Attenuation of inducible nitric oxide synthase gene expression by Δ^9-tetrahydrocannabinol is mediated through the inhibition of nuclear factor- κB/Rel activation. Mol. Pharmacol. 50, 334–341.

Jin, W., Brown, S., Roche, J.P., Hsieh, C., Celver, J.P., Kovoor, A., Chavkin, C., Mackie, K., 1999. Distinct domains of the CB1 cannabinoid receptor mediate desensitization and internalization. J. Neurosci. 19, 3773–3780.

Kaminski, N.E., 1996. Immune regulation by cannabinoid compounds through the inhibition of the cyclic AMP signaling cascade and altered gene expression. Biochem. Pharmacol. 52, 1133–1140.

Kaminski, N.E., 1998a. Regulation of the cAMP cascade, gene expression and immune function by cannabinoid receptors. J. Neuroimmunol. 83, 124–132.

Kaminski, N.E., 1998b. Inhibition of the cAMP signaling cascade via cannabinoid receptors: a putative mechanism of immune modulation by cannabinoid compounds. Toxicol. Lett. 102-103, 59–63.

Kaminski, N.E., Abood, M.E., Kessler, F.K., Martin, B.R., Schatz, A.R., 1992. Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. Mol. Pharmacol. 42, 736–742.

Kaminski, N.E., Koh, W.S., Yang, K.H., Lee, M., Kessler, F.K., 1994. Suppression of the humoral immune response by cannabinoids is partially mediated through inhibition of adenylate cyclase by a pertussis toxin-sensitive G-protein coupled mechanism. Biochem. Pharmacol. 48, 1899–1908.

Kearn, C.S., Greenberg, M.J., DiCamelli, R., Kurzawa, K., Hillard, C.J., 1999. Relationships between ligand affinities for the cerebellar cannabinoid receptor CB1 and the induction of GDP/GTP exchange. J. Neurochem. 72, 2379–2387.

Klein, T.W., 1999. Cannabis and immunity. In: Kalant, H., Corrigall, W.A., Hall, W., Smart, R.G. (Eds.), The Health Effects of Cannabis. Centre for Addiction and Metal Health, Toronto, Canada, pp. 349–373.

Klein, T.W., Newton, C., Friedman, H., 1987. Inhibition of natural killer cell function by marijuana components. J. Toxicol. Environ. Health 20, 321–332.

Klein, T.W., Friedman, H., 1990. Modulation of immune cell function by marijuana components. In: Watson, R. (Ed.), Drugs of Abuse and Immune Function. CRC Press, Boca Raton, FL, pp. 87–111.

Klein, T.W., Kawakami, Y., Newton, C., Friedman, H., 1991. Marijuana components suppress induction and cytolytic function of murine cytotoxic T cells in vitro and in vivo. J. Toxicol. Environ. Health 32, 465–477.

Klein, T.W., Newton, C., Widen, R., Friedman, H., 1993. Delta 9-tetrahydrocannabinol injection induces cytokine-mediated mortality of mice infected with *Legionella pneumophila*. J. Pharmacol. Exp. Ther. 267, 635–640.

Klein, T.W., Newton, C., Friedman, H., 1994. Resistance to Legionella pneumophila suppressed by the marijuana component, tetrahydrocannabinol. J. Infect. Dis. 169, 1177–1179.

Klein, T.W., Friedman, H., Specter, S., 1998a. Marijuana, immunity and infection. J. Neuroimmunol. 83, 102–115.

Klein, T.W., Newton, C., Friedman, H., 1998b. Cannabinoid receptors and immunity. Immunol. Today 19, 373–381.

Klein, T.W., Newton, C., Friedman, H., 1998c. Cannabinoid receptors and the cytokine network. Adv. Exp. Med. Biol. 437, 215–222.

Koh, W.S., Yang, K.H., Kaminski, N.E., 1995. Cyclic AMP is an essential factor in immune responses. Biochem. Biophys. Res. Commun. 206, 703–709.

Koh, W.S., Crawford, R.B., Kaminski, N.E., 1997. Inhibition of protein kinase A and cyclic AMP response element (CRE)-specific transcription factor binding by Δ^9-tetrahydrocannabinol (Δ^9-THC): a putative mechanism of cannabinoid-induced immune modulation. Biochem. Pharmacol. 53, 1477–1484.

Kuehl, F.A., Jr, Jacob, T.A., Ganley, O.H., Ormond, R.E., Meisinger, M.A.P., 1957. The identification of *N*-(2-hydroxyethyl)-palmitamide as a naturally occurring anti-inflammatory agent. J. Am. Chem. Soc. 79, 5577–5578.

Kuwae, T., Shiota, Y., Schmid, P.C., Krebsbach, R., Schmid, H.H., 1999. Biosynthesis and turnover of anandamide and

other *N*-acylethanolamines in peritoneal macrophages. FEBS Lett. 459, 123–127.

Lee, M., Yang, K.H., Kaminski, N.E., 1995. Effects of putative cannabinoid receptor ligands, anandamide and 2-arachidonyl-glycerol, on immune function in B6C3F1 mouse splenocytes. J. Pharmacol. Exp. Ther. 275, 529–536.

Liu, J., Gao, B., Mirshahi, F., Sanyal, A.J., Khanolkar, A.D., Makriyannis, A., Kunos, G., 2000. Functional CB1 cannabinoid receptors in human vascular endothelial cells. Biochem. J. 346, 835–840.

Long, D.A., Martin, A.J.P., 1956. Factor in arachis oil depressing sensitivity to tuberculin in B.C.G.-infected guinea pigs. Lancet 1, 464–466.

Lopez-Cepero, M., Friedman, M., Klein, T., Friedman, H., 1986. Tetrahydrocannabinol-induced suppression of macrophage spreading and phagocytic activity in vitro. J. Leukoc. Biol. 39, 679–686.

Luo, Y.D., Patel, M.K., Wiederhold, M.D., Ou, D.W., 1992. Effects of cannabinoids and cocaine on the mitogen-induced transformations of lymphocytes of human and mouse origins. Int. J. Immunopharmacol. 14, 49–56.

Lynn, A.B., Herkenham, M., 1994. Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. J. Pharmacol. Exp. Ther. 268, 1612–1623.

Matsuda, L.A., 1997. Molecular aspects of cannabinoid receptors. Crit. Rev. Neurobiol. 11, 143–166.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

McCoy, K.L., Gainey, D., Cabral, G.A., 1995. Δ^9-Tetrahydrocannabinol modulates antigen processing by macrophages. J. Pharmacol. Exp. Ther. 273, 1216–1223.

McCoy, K.L., Matveyeva, M., Carlisle, S.J., Cabral, G.A., 1999. Cannabinoid inhibition of the processing of intact lysozyme by macrophages: evidence for CB2 receptor participation. J. Pharmacol. Exp. Ther. 289, 1620–1625.

Mechoulam, R., 1986. The pharmacohistory of *Cannabis sativa*. In: Mechoulam, R. (Ed.), Cannabinoids as Therapeutic Agents. CRS Press, Boca Raton, pp. 1–19.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J., Vogel, Z., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50, 83–90.

Mishkin, E.M., Cabral, G.A., 1985. delta-9-Tetrahydrocannabinol decreases host resistance to herpes simplex virus type 2 vaginal infection in the $B_6C_3F_1$ mouse. J. Gen. Virol. 66, 2539–2549.

Molina-Holgado, F., Lledo, A., Guaza, C., 1997. Anandamide suppresses nitric oxide and TNF-α responses to Theiler's virus or endotoxin in astrocytes. Neuroreport 8, 1929–1933.

Molina-Holgado, F., Molina-Holgado, E., Guaza, C., 1998. The endogenous cannabinoid anandamide potentiates interleukin-6 production by astrocytes infected with Theiler's murine encephalomyelitis virus by a receptor-mediated pathway. FEBS Lett. 433, 139–142.

Morahan, P.S., Klykken, P.C., Smith, S.H., Harris, L.S., Munson, A.E., 1979. Effects of cannabinoids on host resistance to *Listeria monocytogenes* and herpes simplex virus. Infect. Immun. 23, 670–674.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61–65.

Nahas, G.G., Osserman, E.F., 1991. Altered serum immunoglobulin concentration in chronic marijuana smokers. Adv. Exp. Med. Biol. 288, 25–32.

Newton, C.A., Klein, T.W., Friedman, H., 1994. Secondary immunity to *Legionella pneumophila* and Th1 activity are suppressed by Δ^9-tetrahydrocannabinol injection. Infect. Immun. 62, 4015–4020.

Noe, S.N., Nyland, S.B., Ugen, K., Friedman, H., Klein, T.W., 1998. Cannabinoid receptor agonists enhance syncytia formation in MT-2 cells infected with cell free HIV-1MN. Adv. Exp. Med. Biol. 437, 223–229.

Novak, T.J., Chen, D., Rothenberg, E.V., 1990. Interleukin-1 synergy with phosphoinositide pathway agonists for induction of interleukin-2 gene expression: molecular basis of costimulation. Mol. Cell. Biol. 10, 6325–6334.

Ouyang, Y., Hwang, S.G., Han, S.H., Kaminski, N.E., 1998. Suppression of interleukin-2 by the putative endogenous cannabinoid 2-arachidonyl-glycerol is mediated through down-regulation of the nuclear factor of activated T cells. Mol. Pharmacol. 53, 676–683.

Pan, X., Ikeda, S.R., Lewis, D.L., 1997. SR 141716A acts as an inverse agonist to increase neuronal voltage-dependent Ca^{2+} currents by reversal of tonic CB1 cannabinoid receptor activity. Mol. Pharmacol. 54, 1064–1072.

Paria, B.C., Das, S.K., Dey, S.K., 1995. The preimplantation mouse embryo is a target for cannabinoid ligand-receptor signaling. Proc. Natl. Acad. Sci. USA 92, 9460–9464.

Perlík, F., Elis, F., Raková, H., 1971a. Anti-inflammatory activity of *N*-(2-hydroxyethyl)palmitamide. Acta Physiol. Hung. 39, 123–124.

Perlík, F., Elis, F., Rašková, H., 1971b. Anti-inflammatory properties of *N*-(2-hydroxyethyl)palmitamide. Acta Physiol. Hung. 39, 395–400.

Perlík, F., Krejci, J., Elis, F., Pekárek, J., Svejcar, J., 1973. The effect of *N*-(2-hydroxyethyl)palmitamide on delayed hypersensitivity in guinea-pig. Experientia 29, 67–68.

Pertwee, R.G., Fernando, S.R., 1996. Evidence for the presence of cannabinoid CB1 receptors in mouse urinary bladder. Br. J. Pharmacol. 118, 2053–2058.

Pertwee, R.G., Fernando, S.R., Nash, J.E., Coutts, A.A., 1996a. Further evidence for the presence of cannabinoid CB1 receptors in guinea-pig small intestine. Br. J. Pharmacol. 118, 2199–2205.

Pertwee, R.G., Joe-Adigwe, G., Hawksworth, G.M., 1996b. Further evidence for the presence of cannabinoid CB1

receptors in mouse vas deferens. Eur. J. Pharmacol. 296, 169–172.

Pertwee, R.G., 1999. Pharmacology of cannabinoid receptor ligands. Curr. Med. Chem. 6, 635–664.

Pestonjamasp, V.K., Burstein, S.H., 1998. Anandamide synthesis is induced by arachidonate mobilizing agonists in cells of the immune system. Biochim. Biophys. Acta 1394, 249–260.

Pettit, D.A., Anders, D.L., Harrison, M.P., Cabral, G.A., 1996. Cannabinoid receptor expression in immune cells. Adv. Exp. Med. Biol. 402, 119–129.

Puffenbarger, R.A., Boothe, A.C., Cabral, G.A., 2000. Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. Glia 29, 58–69.

Rachelefsky, G.S., Opelz, G., Mickey, M.R., Lessin, P., Kiuchi, M., Silverstein, M.J., Stiehm, E.R., 1976. Intact humoral and cell-mediated immunity in chronic marijuana smoking. J. Allergy Clin. Immunol. 58, 483–490.

Rašková, H., Mašek, K., 1967. Nouvelles possibilités d'augmentation de la résistance non-spécifique. Thérapie 22, 1241–1246.

Rašková, H., Mašek, K., Linet, O., 1972. Nonspecific resistance induced by palmitoylethanolamide. Toxicon 10, 485–490.

Rhee, M.H., Nevo, I., Avidor-Reiss, T., Levy, R., Vogel, Z., 2000. Differential superactivation of adenylyl cyclase isozymes after chronic activation of the CB1 cannabinoid receptor. Mol. Pharmacol. 57, 746–752.

Rinaldi-Carmona, M., Barth, F., Héaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Néliat, G., Caput, D., Ferrara, P., Soubrié, M., Brelièr, J.C., Le Fur, G., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Rinaldi-Carmona, M., Barth, F., Millan, J., Derocq, J.M., Casellas, P., Congy, C., Oustric, D., Sarran, M., Bouaboula, M., Calandra, B., Portier, M., Shire, D., Breliere, J.C., Le Fur, G.L., 1998. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J. Pharmacol. Exp. Ther. 284, 644–650.

Roche, J.P., Bounds, S., Brown, S., Mackie, K., 1999. A mutation in the second transmembrane region of the CB1 receptor selectively disrupts G protein signaling and prevents receptor internalization. Mol. Pharmacol. 56, 611–618.

Ross, R.A., Murphy, V.L., McKay, N.G., Ashford, L.J., Pertwee, R.G., 1997. Characterization of cannabinoid receptors in the spleen and in mast cells. In: Riemersma, R.A., Armstrong, R., Kelly, R.W., Wilson, R. (Eds.), Essential Fatty Acids and Eicosanoids. Invited Papers from the Fourth International Congress. AOCS Press, Champaign, IL, pp. 376–379.

Schatz, A.R., Koh, W.S., Kaminski, N.E., 1993. Δ^9-tetrahydrocannabinol selectively inhibits T-cell dependent humoral immune responses through direct inhibition of accessory T-cell function. Immunopharmacology 26, 129–137.

Schatz, A.R., Lee, M., Condie, R.B., Pulaski, J.T., Kaminski, N.E., 1997. Cannabinoid receptors CB1 and CB2: a characterization of expression and adenylate cyclase modulation within the immune system. Toxicol. Appl. Pharmacol. 142, 278–287.

Schmid, P.C., Kuwae, T., Krebsbach, R.J., Schmid, H.H., 1997. Anandamide and other *N*-acylethanolamines in mouse peritoneal macrophages. Chem. Phys. Lipids 87, 103–110.

Shire, D., Carillon, C., Kaghad, M., Calandra, B., Rinaldi-Carmona, M., Le Fur, G., Caput, D., Ferrara, P., 1995. An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. J. Biol. Chem. 270, 3726–3731.

Shire, D., Calandra, B., Rinaldi-Carmona, M., Oustric, D., Pessegue, B., Bonnin-Cabanne, O., Le Fur, G., Caput, D., Ferrara, P., 1996. Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. Biochim. Biophys. Acta 1307, 132–136.

Shivers, S.C., Newton, C., Friedman, H., Klein, T.W., 1994. Δ^9-Tetrahydrocannabinol (THC) modulates IL-1 bioactivity in human monocyte/macrophage cell lines. Life Sci. 54, 1281–1289.

Sinha, D., Bonner, T.I., Bhat, N.R., Matsuda, L.A., 1998. Expression of the CB1 cannabinoid receptor in macrophage-like cells from brain tissue: immunochemical characterization by fusion protein antibodies. J. Neuroimmunol. 82, 13–21.

Smith, S.R., Terminelli, C., Denhardt, G., 2000. Effects of cannabinoid receptor agonist and antagonist ligands on production of inflammatory cytokines and anti-inflammatory interleukin-10 in endotoxemic mice. J. Pharmacol. Exp. Ther. 293, 136–150.

Specter, S.C., Klein, T.W., Newton, C., Mondragon, M., Widen, R., Friedman, H., 1986. Marijuana effects on immunity: suppression of human natural killer cell activity of delta-9-tetrahydrocannabinol. Int. J. Immunopharmacol. 8, 741–745.

Specter, S., Lancz, G., Goodfellow, D., 1991. Suppression of human macrophage function in vitro by Δ^9-tetrahydrocannabinol. J. Leukoc. Biol. 50, 423–426.

Specter, S., Diaz, S., Liu, C., 1995. Cannabinoid stimulation of arachidonate metabolism in human peripheral blood mononuclear leukocytes. In: Sharp, B. (Ed.), The Brain Immune Axis and Substance Abuse. Plenum Press, New York, pp. 97–102.

Srivastava, M.D., Srivastava, B.I., Brouhard, B., 1998. Δ^9Tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. Immunopharmacology 40, 179–185.

Stefano, G.B., Salzet, M., Rialas, C.M., Mattocks, D., Fimiani, C., Bilfinger, T.V., 1998. Macrophage behavior associated with acute and chronic exposure to HIV GP120, morphine and anandamide: endothelial implications. Int. J. Cardiol. 64, S3–13.

Straiker, A., Stella, N., Piomelli, D., Mackie, K., Karten, H.J., Maguire, G., 1999. Cannabinoid CB1 receptors and lig-

ands in vertebrate retina: localization and function of an endogenous signaling system. Proc. Natl. Acad. Sci. USA 96, 14565–14570.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Sugiura, T., Kodaka, T., Nakane, S., Miyashita, T., Kondo, S., Suhara, Y., Takayama, H., Waku, K., Seki, C., Baba, N., Ishima, Y., 1999. Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure-activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. J. Biol. Chem. 274, 2794–2801.

Sugiura, T., Kondo, S., Kishimoto, S., Miyashita, T., Nakane, S., Kodaka, T., Suhara, Y., Takayama, H., Waku, K., 2000. Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. J. Biol. Chem. 275, 605–612.

Tang, J.L., Lancz, G., Specter, S., Bullock, H., 1992. Marijuana and immunity: tetrahydrocannabinol-mediated inhibition of growth and phagocytic activity of the murine macrophage cell line, P388D1. Int. J. Immunopharmacol. 14, 253–262.

Titishov, N., Mechoulam, R., Zimmerman, A.M., 1989. Stereospecific effects of (−)- and (+)-7-hydroxy-delta-6-tetrahydrocannabinol-dimethylheptyl on the immune system of mice. Pharmacology 39, 337–349.

Valk, P., Verbakel, S., Vankan, Y., Hol, S., Mancham, S., Ploemacher, R., Mayen, A., Lowenberg, B., Delwel, R., 1997. Anandamide, a natural ligand for the peripheral cannabinoid receptor is a novel synergistic growth factor for hematopoietic cells. Blood 90, 1448–1457.

Varga, K., Wagner, J.A., Bridgen, D.T., Kunos, G., 1998. Platelet- and macrophage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. FASEB J. 12, 1035–1044.

Vásquez, C., Lewis, D.L., 1999. The CB1 cannabinoid receptor can sequester G-proteins, making them unavailable to couple to other receptors. J. Neurosci. 19, 9271–9280.

Waksman, Y., Olson, J.M., Carlisle, S.J., Cabral, G.A., 1999. The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. J. Pharmacol. Exp. Ther. 288, 1357–1366.

Wallace, J.M., Tashkin, D.P., Oishi, J.S., Barbers, R.G., 1988. Peripheral blood lymphocyte subpopulations and mitogen responsiveness in tobacco and marijuana smokers. J. Psychoactive Drugs 20, 9–14.

Wartmann, M., Campbell, D., Subramanian, A., Burstein, S.H., Davis, R.J., 1995. The MAP kinase signal transduction pathway is activated by the endogenous cannabinoid anandamide. FEBS Lett. 359, 133–136.

Watson, P.A., Krupinski, J., Kempinski, A.M., Frankenfield, C.D., 1994. Molecular cloning and characterization of the type VII isoform of mammalian adenylyl cyclase expressed widely in mouse tissues and in S49 mouse lymphoma cells. J. Biol. Chem. 269, 28893–28898.

Westlake, T.M., Howlett, A.C., Bonner, T.I., Matsuda, L.A., Herkenham, M., 1994. Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. Neuroscience 63, 637–652.

Xie, Q.W., Kashiwabara, Y., Nathan, C., 1994. Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. J. Biol. Chem. 269, 4705–4708.

Yea, S.S., Yang, K.H., Kaminski, N.E., 2000. Role of nuclear factor of activated T-cells and activator protein-1 in the inhibition of interleukin-2 gene transcription by cannabinol in EL4 T-cells. J. Pharmacol. Exp. Ther. 292, 597–605.

Zheng, Z.M., Specter, S., Friedman, H., 1992. Inhibition by Δ^9-tetrahydrocannabinol of tumor necrosis factor alpha production by mouse and human macrophages. Int. J. Immunopharmacol. 14, 1445–1452.

Zheng, Z.M., Specter, S.C., 1996. Δ^9-Tetrahydrocannabinol suppresses tumor necrosis factor alpha maturation and secretion but not its transcription in mouse macrophages. Int. J. immunopharmacol. 18, 53–68.