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Review article

Cannabinoids and the immune system: Potential for the treatment of inflammatory diseases?

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Abstract

Since the discovery of the cannabinoid receptors and their endogenous ligands, significant advances have been made in studying the physiological function of the endocannabinoid system. The presence of cannabinoid receptors on cells of the immune system and anecdotal and historical evidence suggesting that cannabis use has potent immuno-modulatory effects, has led to research directed at understanding the function and role of these receptors within the context of immunological cellular function. Studies from chronic cannabis smokers have provided much of the evidence for immunomodulatory effects of cannabis in humans, and animal and in vitro studies of immune cells such as T cells and macrophages have also provided important evidence. Cannabinoids can modulate both the function and secretion of cytokines from immune cells. Therefore, cannabinoids may be considered for treatment of inflammatory disease. This review article will highlight recent research on cannabinoids and how they interact with the immune system and also their potential use as therapeutic agents for a number of inflammatory disorders.

Keywords: Cannabinoids; Inflammation; Infection; Treatment

Contents

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1. Introduction

Historically, the use of cannabis as a therapeutic agent dates back thousands of years when it was known to induce alterations in the mood, cognitive functions, memory, and perception of the user (Vincent et al., 1983). However, there was little clear scientific evidence to support cannabinoids as therapeutic agents until the discovery of the endocannabinoid system. Amongst the 60 or so different cannabinoids produced by *Cannabis sativa*, Δ^9-tetrahydrocannabinol (THC), first structurally described in 1964, is the major psychoactive constituent (Gaoni and Mechoulam, 1964). Since then a number of synthetic cannabinoid analogs have been shown to induce similar in vivo effects such as analgesia, anti-emesis, immunosuppression and changes in psychomotor activity. Cannabinoids mediate their effects through the G-protein-coupled cannabinoid receptors (Devane et al., 1988; Munro et al., 1993), which are negatively coupled to the enzyme adenylyl cyclase. In addition, CB_1 receptors but not CB_2 receptors, are coupled to ion channels and can mediate both positive and negative effects, thereby inhibiting N and P/Q-type Ca2+ currents and D-type K+ channels, and activating A-type and inward rectifying K+ currents (Pertwee, 1997; Howlett and Mukhopadhyay, 2000; Mu et al., 1999). It is thought that cannabinoids signal through CB_1 via a progressive and transient activation of the mitogen activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway (Valjent et al., 2001).

CB_1 receptor expression is localized to a number of functional structures in the brain which are associated with important neurological processes. The inhibitory effects of cannabinoid signaling coupled to the location of CB_1 expression within the central nervous system (CNS) provides a rationale for the use of cannabinoids as therapeutic agents for a number of neurological disorders. However, the expression of CB_1 in these regions also mediates the side-effects of cannabis use (reviewed by Croxford, 2003). A second cannabinoid receptor, CB_2, is expressed preferentially in the periphery, particularly in lymphoid organs (Munro et al., 1993). Currently our understanding of the role of cannabinoids on the immune system is limited. This review article will focus on recent studies investigating the role of cannabinoids in the immune system and also their potential therapeutic use in a number of inflammatory disorders.

2. Function of the endocannabinoid system

The endocannabinoid system has a broad spectrum of influence on both excitatory and inhibitory neuronal circuits, owing to the wide distribution of CB_1 receptor expression in vital areas of the CNS. The endocannabinoid system is thought to control the regulation of physiological functions such as movement, memory and learning, cognition, neuroendocrine secretion, appetite, emesis, regulation of body temperature, pain and immune system modulation. The ability of endocannabinoids to regulate synaptic neurotransmission means that it has great potential as a symptomatic and/or therapeutic agent in diseases where inappropriate neurotransmission induces disease pathology. However, the presence of cannabinoid receptors on immune system cells is less well understood. Although studies have demonstrated immunosuppressive effects on immune function following administration of a number of different cannabinoids the precise function of the endocannabinoid system on immune system development remains unclear.

2.1. Cannabinoid receptor localization

Although currently there are two known cannabinoid receptors, CB_1 and CB_2 there is evidence to suggest others may exist (reviewed by Croxford, 2003). Both known receptors are $G_{i/o}$-protein coupled and signaling through these receptors can affect cellular regulation as they have an inhibitory effect on adenylate cyclase activity and cAMP accumulation, which can be blocked by pertussis toxin (Howlett et al., 1986; Pacheco et al., 1993). It is likely that CB receptors have important roles as CB_1 has been evolutionarily conserved between a number of primitive species including fish, hydra, mollusk, leech and sea urchin (Yamaguchi et al., 1996; De Petrocellis et al., 1999; Stefano et al., 1997; Chang et al., 1993; Bisogno et al., 1997a). A splice variant of CB_1, CB_1A also has been identified (Shire et al., 1995). CB_1 has been identified in many tissues both of the CNS and in the periphery. In the CNS, CB_1 is predominantly found presynaptically, on neurons in areas of the brain which are consistent with the behavioral and pharmacological effects seen following cannabinoid usage, such as loss of short-term memory, dizziness, ataxia and sedation (reviewed by Croxford, 2003). Although CB_1 is highly expressed throughout the brain it is primarily

expressed in the cerebellum and hippocampus (Herkenham et al., 1991; Glass et al., 1997). Peripheral expression of CB_1 has been described in vascular endothelium, small intestine, peripheral nerve synapses, testis and cells of the immune system (reviewed by Pertwee, 1997; Croxford, 2003).

CB_2, often termed the "peripheral" cannabinoid receptor, is highly expressed on cells of the immune system and is found in abundance in the pancreas and tissues of the lymphoid system including the thymus, tonsils, bone marrow, spleen (Munro et al., 1993; Galiegue et al., 1995; Lynn and Herkenham, 1994). In addition, CB_2 is also found in other peripheral structures such as the retina (Lu et al., 2000) and CB_2-like receptors have been described on the peripheral nerve terminals in mouse vas deferens (Griffin et al., 1997). Northern analysis, quantitative RT-PCR analysis and autoradiography could not detect quantifiable CB_2 in the brain and therefore CB_2 is thought to not be expressed in the CNS (Munro et al., 1993; Schatz et al., 1997). However, recent studies have demonstrated CB_2 expression in human astrocytes by immunohistochemistry and Western blot analysis (Sheng et al., 2005) although this may be due to the presence of contaminating microglial cells (Walter and Stella, 2003). Furthermore, both mRNA and protein coding for CB_2 has been demonstrated in human, mouse and rat microglial cells (Walter et al., 2003; Franklin and Stella, 2003; Carrier et al., 2004; Klegeris et al., 2003). In addition, macrophage and microglial CB_2 expression levels are dependent upon the activation state of the cell (Carlisle et al., 2002). Therefore, it is possible that CB_2 expression may be expressed in the CNS in response to infection or during periods of stress. To support this, CB_2 receptor mRNA expression has been shown to be upregulated in restricted areas of the spinal cord following peripheral nerve injury but not peripheral inflammation (Zhang et al., 2003). Therefore, the presence of CB_2 and other non-CB_1 cannabinoid receptors in the CNS is still unclear and requires further study.

2.2. Cannabinoid agonists

The first endogenous cannabinoid receptor agonist identified was anandamide (AEA), a derivative of arachidonic acid, and was isolated from porcine brain (Devane et al., 1992). Both neurons and immune cells secrete AEA, which can mediate a number of typical cannabis-like effects, such as nociception, catalepsy and hypoalgesia. AEA is selective for CB_1 compared to CB_2 (inhibition constant (Ki) 89 nmol/L and 371 nmol/L, respectively) (Showalter et al., 1996). Interestingly, areas of high CB_1 receptor expression such as the hippocampus, striatum and cerebellum also produce the highest levels of AEA (Devane et al., 1992; Schmid et al., 1995; Sugiura et al., 1996; Felder et al., 1996). Peripherally, AEA is expressed in structures such as the spleen, kidney, skin and uterus (Felder et al., 1996; Yang et al., 1999; Deutsch et al., 1997; Schmid et al., 1997; Giuffrida and Piomelli, 1998). A second identified endocannabinoid ligand, 2-arachidonoylethanolamide (2-AG), was originally isolated from canine intestinal tissue (Mechoulam et al., 1995). Compared to AEA, 2-AG is present in greater quantities in the CNS although it has a lower affinity for CB_1 (Ki=472 nmol/L) (Mechoulam et al., 1995; Stella et al., 1997). It has been suggested that 2-AG is the natural ligand for CB receptors (Sugiura and Waku, 2000). Another potential endocannabinoid is palmitoylethanolamide (PEA) which is produced by neurons and immune cells (Facci et al., 1995; Calignano et al., 2001). However, it is thought not to bind to CB_1 or CB_2 receptors although its cannabinoid-like effects can be inhibited with CB_2 receptor antagonists (Hanus et al., 2001; Di Marzo et al., 1994). Recently other ligands such as virodhamine, noladin ether, *N*-arachidonoyldopamine (NADA) and docosatetraenylyethanolamide (DEA) have been isolated from the CNS and have been proposed to be potential endocannabinoid receptor ligands (Porter et al., 2002; Fezza et al., 2002; Walker et al., 2002). However, the classification of noladin ether as an endocannabinoid has been challenged by a recent study that demonstrated that noladin ether could not be detected in the brain of a number of different mammalian species including rat, mouse and pig (Oka et al., 2003).

Further evidence for the presence of a mammalian endocannabinoid system was provided with the discovery of endocannabinoid transport/breakdown mechanisms. Following depolarization-induced synthesis and secretion of endocannabinoids from neurons, it is thought that a diffusion-facilitated transport mechanism removes AEA from the extracellular space. However, it has been suggested that AEA uptake is not mediated by a specific membrane-associated AEA carrier but instead by simple diffusion (Glaser et al., 2003). AEA is then hydrolyzed to arachidonic acid and ethanolamine by an enzyme, fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996; Deutsch et al., 2001). FAAH is present in neurons, astrocytes and a number of immune system cells including lymphocytes and macrophages (Beltramo et al., 1997; Egertova et al., 1998; Maccarrone et al., 2000a; Di Marzo et al., 1999).

Apart from the endocannabinoid ligands a number of other ligands exist which can bind and signal through the cannabinoid receptors. The most commonly known of these is THC, which has a binding affinity for both CB_1 and CB_2. Administration of THC to animals induces a number of behavioral effects such as hypothermia, catalepsy and hypomobility (Gaoni and Mechoulam, 1971; Howlett et al., 2002). However, other compounds from the *Cannabis sativa* plant including cannabinol (CBN) and cannabidiol (CBD), the major non-psychoactive compound, which has very low affinity for cannabinoid receptors, can also mediate anti-inflammatory effects (Malfait et al., 2000). In addition to endogenous and naturally-occurring cannabinoids such as THC, synthetic cannabinoids have been shown to mediate cannabimimetic effects via stereoselective receptor-mediated mechanisms, although these

compounds differ structurally from the endocannabinoids. R(+)WIN55,212 is an aminoalkylindole with both CB_1 and CB_2 specificity, which induces cannabimimetic effects in vivo. Other non-CB receptor selective agonists include CP 55,940 and HU-210 which are more potent than THC.

There are rapidly increasing numbers of cannabinoid ligand analogues with different CB receptor specificities and binding affinities. Although most ligands induce similar effects there are subtle differences between those mediated by the different classes of cannabinoids. As an example, DNA microarray analysis studies in R(+)WIN55,212 or THC-treated mice, demonstrated that although many CNS genes showed similar responses, a significant number of different genes were affected depending upon which ligand was used (Parmentier-Batteur et al., 2002).

3. Function of cannabinoids in the immune system

Although immune cells express both CB_1 and CB_2 receptors, secrete endocannabinoids and have functional cannabinoid transport and breakdown mechanisms, and are thought to play a role in immune homeostasis and control, the specific role of endocannabinoids in the development and function of the immune system is still unclear (Pestonjamasp and Burstein, 1998; Bisogno et al., 1997b). However, a number of studies have demonstrated potent effects upon cytokine production in immune cells (Table 1). Although the majority of studies show that administration of cannabinoids have inhibitory effects on immune cells, a number of recent studies have demonstrated that the endocannabinoids may have some stimulatory impact on the immune system and may actually be important in homeostasis or control of immune reactions. This apparent contradiction may be due in part to a biphasic response relative to the cannabinoid ligand concentration. In addition, plant-derived cannabinoids such as THC, may act as partial agonists at the receptor level, and therefore antagonize the effects of 2-AG, which acts as a full agonist. Many of the inhibitory effects of cannabinoids in vitro are in the micromolar concentration range, whereas stimulatory concentrations are in the nanomolar range. Furthermore, concentrations in the micromolar range have been estimated to be at least 10 fold higher than observed in the blood of marijuana smokers. The use of different types and doses of cannabinoid ligands, experimental protocols and stimulatory conditions have made comparisons between studies difficult. The next part of this review will discuss the immunological effects of cannabinoids on different immune system cell types (Fig. 1).

3.1. Differential expression of cannabinoid receptors on immune system cells

CB_1 and CB_2 mRNA expression is present in human and mouse immune cells in the order B cells > natural killer (NK) cells > monocytes > neutrophils > CD8 leukocytes > CD4 leukocytes (Galiegue et al., 1995; Klein et al., 1995; Lee et al., 2001). However, both human and mouse immune cells express CB_2 at higher levels than CB_1 (Schatz et al., 1997; Galiegue et al., 1995). The level of CB receptor expression appears to be dependent upon the activation state of the cell and the type of activating stimuli. Following lipopolysaccharide (LPS) stimulation of splenocytes, CB_2 mRNA expression is downregulated, whereas in contrast anti-CD40 co-stimulation upregulates CB_2 expression (Lee et al., 2001). CB_1 expression is also upregulated on activated mouse macrophages (Klein et al., 1995) and the human Jurkat T-cell line (Daaka et al., 1996). There are also differences between cell types as to how stimuli affect CB receptor expression. T cell mitogen signaling decreases CB_1 expression whereas B cell mitogen signaling increases CB_1 expression (Noe et al., 2000). Furthermore, administration of THC was unable to inhibit the activation of T helper cells from CB_2-deficient mice, suggesting a role for the CB_2 receptor in immunomodulation (Buckley et al., 2000). In addition, THC may affect T helper cells via their inhibitory effects on antigen presenting cells.

Table 1
Effects of cannabinoids on cytokine production

Cytokine	System	Drug	Reference
Increases in cytokine production			
IL-1	In vitro mouse macrophages	THC	Zhu et al., 1994
	In vivo mouse serum	THC	Klein et al., 1993
	In vitro mouse macrophages	THC	Newton et al., 1998
TNF	In vitro human monocytes	THC	Shivers et al., 1994
	In vivo mouse serum	THC	Klein et al., 1993
	In vitro mouse macrophages	THC	Newton et al., 1998
IL-4	In vitro human T cell dendritic cell co-culture	THC	Yuan et al., 2002
IL-6	In vivo mouse serum	THC	Klein et al., 1993
IL-12	In vitro/ex vivo mouse macrophages	CBD	Sacerdote et al., 2005
Decreases in cytokine production			
IFN-γ	Ex vivo mouse spleen	THC	Blanchard et al., 1986
	In vitro human NK cells	THC	Srivastava et al., 1998
	In vitro mouse splenocytes	THC	Blanchard et al., 1986
	In vitro human PBMC	THC/CBD	Watzl et al., 1991
	In vitro human T cell dendritic cell co-culture	THC	Yuan et al., 2002
	In vitro mouse splenocytes	THC	Newton et al., 1998
TNF	Macrophage cell lines	THC	Zheng et al., 1992
	In vitro human NK cells	THC	Kusher et al., 1994
	In vitro human PBMC	CBD	Watzl et al., 1991
	In vitro human NK cells	THC	Srivastava et al., 1998
IL-1	In vitro human PBMC	CBD	Watzl et al., 1991
IL-2	In vitro mouse spleen	THC	Nakano et al., 1992
IL-10	In vitro human T cells	THC/CBD	Srivastava et al., 1998
	In vitro/ex vivo mouse macrophages	CBD	Sacerdote et al., 2005
IL-12	In vitro mouse splenocytes/macrophages	THC	Newton et al., 1998

THC—Δ^9-tetrahydrocannabinol; CBD—cannabidiol; PBMC—peripheral blood monocytes.

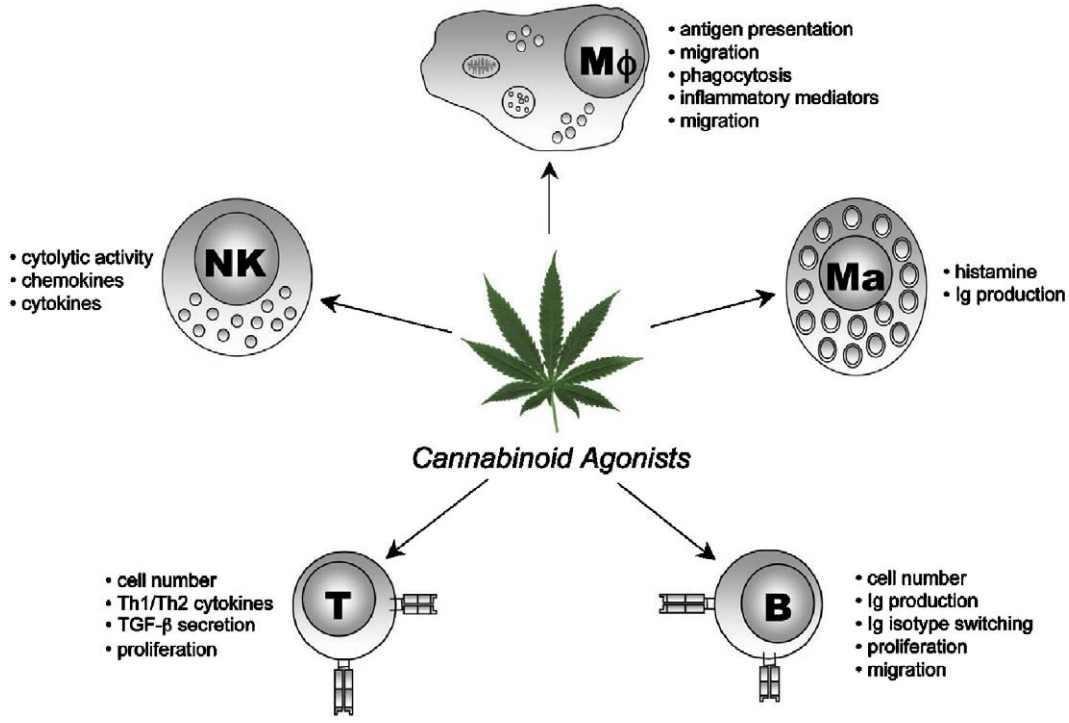

Fig. 1. Schematic diagram to represent the effects of cannabinoids on different functions of cells of the immune system. Abbreviations: NK — natural killer cell; Mϕ—macrophage; Ma—mast cell; T—T lymphocyte; B—B lymphocyte.

3.2. T-lymphocytes

Early studies demonstrated the in vitro effects of cannabinoids on individual immune cell types and their ability to function following inflammatory stimuli. A variety of cannabinoids including AEA, THC, R(+)WIN55,212, CBD, 2-AG and CP55,940 (which binds CB_1 and CB_2) have all been shown to affect various immune cell type functions (Fig. 1) from both human and animal subjects (reviewed by Klein et al., 1998, 2000a). Initial experiments to determine the effects of smoked cannabis on human immunity were undertaken by measuring both the number and function of immune cells isolated from subjects who smoked marijuana regularly. Peripheral blood T lymphocytes, which are important in cell-mediated immunity, were isolated from marijuana smokers and studied to determine whether smoking had affected their ability to proliferate. In some subjects T cell proliferation was not affected (Lau et al., 1976; White et al., 1975), whereas others demonstrated a marked decreased in sensitivity (Nahas et al., 1974). A more recent study demonstrated that ingestion of cannabinoids in the form of "bhang" over a period of 6 to 36 months, resulted in a decreased number of T lymphocytes (El-Gohary and Eid, 2004). One major problem in these types of studies is the variability between human subjects such as type and quantity of marijuana used, route of administration, concentration of THC in these preparations, frequency of smoking and duration of inhalation, which makes it difficult to compare results between individuals.

The in vitro effects of cannabinoids on human and mouse T cells was also studied and demonstrated decreased responses to LPS, T cell mitogens and anti-CD3 antibody induced activation (reviewed by Klein et al., 1995, 1998, 2000a). However, in some cases these effects were biphasic. Low doses of THC appeared to stimulate T cells whereas higher doses appeared to inhibit these responses (Klein et al., 1985; Pross et al., 1992; Luo et al., 1992).

Th1 type cytokines interferon (IFN)-γ, and tumor necrosis factor (TNF)-α regulate cell-mediated immune reactions whereas Th2 type cytokines interleukin (IL)-4 and IL-5 mediate humoral immunity. Th1 cytokines have been implicated in the pathogenesis of a number of autoimmune diseases, such as Multiple Sclerosis (MS) (Panitch et al., 1987) and its animal model experimental autoimmune encephalomyelitis (EAE), and inhibition of Th1 cytokines or a shift to a Th2 type response is thought to provide therapeutic benefit (Croxford et al., 1998, 2000, 2001). The effects of cannabinoids on T cell cytokine production are contradictory and a number of studies have demonstrated that both Th1- and Th2-type cytokines can be either inhibited or induced (Table 1) (reviewed by Klein et al., 1995, 1998, 2000a). IFN-γ is an important anti-viral agent, and is vital in delayed-type hypersensitivity reactions. A recent study demonstrated that the addition of THC to a co-culture system containing human T cells and dendritic cells could significantly inhibit T cell proliferation and IFN-γ secretion in a CB_2-dependent way (Yuan et al., 2002). Cannabinoids have also been shown to inhibit a number of other pro-inflammatory cytokines, IL-1, IL-2, IL-6, IL-12

and TNF-α (Table 1). However, in contrast, other studies have demonstrated the stimulatory properties of cannabinoids which can induce Th1-type cytokines (Table 1).

In addition to inhibiting Th1-type cytokines, cannabinoid administration has been reported to increase the expression of Th2 cytokines such as IL-4 and IL-10, which are important for humoral immunity (Table 1) (Smith et al., 2000; Newton et al., 1994; Klein et al., 2000b) and transforming growth factor (TGF)-β which has immunosuppressive properties (Gardner et al., 2002). However, in contrast CBD or THC could inhibit IL-10 production from human T cells (Srivastava et al., 1998). Blockade of Th1 cytokines and the administration of Th2 cytokines and TGF-β have been shown to be effective in inhibiting inflammatory diseases in a number of animal models including EAE (Croxford et al., 1998, 2000, 2001; Racke et al., 1991) and rheumatoid arthritis (Mageed et al., 1998; Triantaphyllopoulos et al., 1999) suggesting that cannabinoids could be useful therapeutic agents for inflammatory disease. As Th1 and Th2 cytokines are inhibitory towards each other cannabinoids may potentially inhibit Th1 type responses either directly or indirectly through the induction of Th2 type cytokines.

3.3. B-lymphocytes

B cells express high levels of CB_2 therefore a number of studies have investigated the potential for the immunomodulation of B cells, the immunoglobulin producing cells of the immune system, by cannabinoids (Fig. 1). A number of studies have demonstrated a reduction in antibody production and B cell proliferation (reviewed by Klein et al., 1995, 1998). In human subjects', oral ingestion of "bhang" decreased the number of B lymphocytes and serum levels of immunoglobulins, IgG and IgM, and C3 and C4 complement proteins (El-Gohary and Eid, 2004). Serum analysis from chronic marijuana smokers demonstrated that numbers of T and B lymphocytes and serum IgG and IgM levels were within the normal range although IgE levels were increased (Rachelefsky et al., 1976). In another study, serum levels of IgG were decreased, IgD was increased and IgA and IgM were unaffected in serum from marijuana smokers (Nahas and Osserman, 1991). It has been demonstrated that cytokines can modulate serum immunoglobulin levels and therefore this particular effect may be mediated by the effect of cannabinoids on Th cell cytokine secretion/polarization rather than directly on B cells themselves.

Studies have also identified other effects of cannabinoids on B cells. Similar to the biphasic response to cannabinoids in T cell studies, low dose treatment of B cells with CP55,940, R(+)WIN55,212 or THC caused a dose-dependent increase in B cell proliferation (Derocq et al., 1995), whereas in other studies B cell proliferation in response to LPS could be effectively inhibited by cannabinoids (Klein et al., 1985). Interestingly, whereas many studies have demonstrated inhibitory effects upon B cells using synthetic ligands, endocannabinoids may induce positive effects. The endocannabinoid 2-AG, but not synthetic cannabinoids, R(+)WIN55,212, or plant-derived cannabinoids, THC, could stimulate the migration of splenocytes in a CB_2-dependent manner (Jorda et al., 2002). In addition, CB_2 stimulation may be associated with B cell differentiation (Carayon et al., 1998) and may suggest a positive role for endocannabinoids in mobilizing B cells during immune responses. Currently, it is not clear whether cannabinoids solely have a direct effect upon the B cells themselves or indirectly through T cells and macrophages which are required for B cell activation.

3.4. Macrophages

Macrophages are important mediators in innate and adaptive immunity. They mediate their effects through phagocytosis of infectious agents, presentation of antigenic peptide fragments to T cells and the secretion of acute phase proteins such as nitric oxide (NO), TNF-α, IL-1 and IL-6. The LPS-induced expression of pro-inflammatory mediators can be inhibited by cannabinoid ligands in murine RAW264.7 macrophages and microglia (Puffenbarger et al., 2000; Fischer-Stenger et al., 1993; Jeon et al., 1996). TNF-α, is an important mediator of inflammation and has been implicated in the pathology of many inflammatory disorders such as MS (Maimone et al., 1991; Selmaj et al., 1991) and rheumatoid arthritis (Tracey and Cerami, 1994). Anti-TNF therapies have been exploited in animal models for MS (Croxford et al., 1998, 2000), and rheumatoid arthritis (Mageed et al., 1998; Triantaphyllopoulos et al., 1999), therefore the anti-TNF properties of cannabinoids provides a rationale for their use as anti-inflammatory agents.

Alveolar macrophages line the epithelial surface of the lung and provide first-line defense against bacterial pathogens. Bacterial infection induces phagocytosis and the production of NO from macrophages to kill bacteria. Although early studies demonstrated no effect of chronic marijuana smoking on alveolar macrophage phagocytosis function in human and rat subjects (Mann et al., 1971; Drath et al., 1979) there appears to be some inhibitory effect on NO production (Roth et al., 2004) and anti-microbial activity (Shay et al., 2003). In vitro studies (Fig. 1) demonstrated that cannabinoid ligands could suppress phagocytosis, cell spreading, and antigen presentation (Lopez-Cepero et al., 1986; Cabral and Mishkin, 1989; Burnette-Curley et al., 1993; McCoy et al., 1995). More recently, a comparison of cannabinoid ligands demonstrated that THC and AEA could inhibit LPS-induced NO and IL-6 from J774 macrophages whereas 2-AG inhibited IL-6 secretion but slightly increased inducible nitric oxide synthase (iNOS)-dependent NO production (Chang et al., 2001). LPS-induced NO production was also shown to be inhibited by R(+)WIN55,212 treatment in vitro (Ross et al., 2000). In contrast, another study showed that 2-AG could inhibit the in vitro production of TNF-α but not NO (Gallily

et al., 2000). Administration of CBD either in vitro to murine peritoneal macrophages or in vivo to mice was shown to increase IL-12 and decrease IL-10 production (Sacerdote et al., 2005). In addition, CBD decreased the response of macrophages to chemotactic stimuli (Sacerdote et al., 2005). The same group also demonstrated that migration of rat macrophages both in vitro and in vivo could be significantly inhibited by the administration of CP55,940 (Sacerdote et al., 2000) whereas macrophage proteolytic and lysosome processing can be inhibited by THC (Matveyeva et al., 2000). These studies demonstrate that macrophages are highly susceptible to the effects of cannabinoids including migration, phagocytosis of foreign particles to the process and presentation of peptide antigens to cytokine secretion.

3.5. Natural killer cells

Natural killer (NK) cells are involved in host defense against infectious pathogens and limit the spread of infection by killing infected target cells. Very few studies in human subjects have addressed the effects of cannabinoids on NK cell function (Fig. 1). In the study mentioned previously where ingestion of "bhang" reduced numbers of T and B cells, NK cell numbers were also reduced (El-Gohary and Eid, 2004). In another study THC had little effect upon NK cell function in human subjects (Dax et al., 1989). In vitro studies of human NK cells demonstrated that THC could inhibit the constitutive expression of chemokines, IL-8, MIP-1α, MIP-1β, and RANTES and also phorbol ester stimulated TNF-α, GM-CSF and IFN-γ (Srivastava et al., 1998).

In contrast, in vitro studies have demonstrated that THC can suppress NK cell function such as cytolytic activity, in rats, mice and humans (Patel et al., 1985; Klein et al., 1987; Specter et al., 1986). A recent study demonstrated that subcuteaneous administration of THC could inhibit the in vivo cytolytic activity of NK cells in mice which could be reversed by both CB receptor antagonists although the CB_1 receptor seemed to impart the greater inhibitory effect (Massi et al., 2000).

3.6. Neutrophils

Neutrophils play an important role in early anti-microbial responses. Initial studies demonstrated that various cannabinoid ligands could induce a dose-dependent and non-cytotoxic release of lysosomal enzymes from neutrophils. In addition, cannabinoids also modulated responses to chemotactic peptides (Naccache et al., 1982). Although the presence of cannabinoid receptors on neutrophils was first described in 1993 there have been very few studies since on the effects of cannabinoids on these cells. A recent study demonstrated that low dose THC treatment of human polymorphonuclear leukocytes from healthy individuals, failed to inhibit migration of phagocytosis, and was associated with the failure to find CB_2 expression on neutrophils by Western analysis (Deusch et al., 2003) in contrast to CB_2 mRNA expression previously demonstrated in neutrophils (Galiegue et al., 1995). The most recent study has shown that superoxide production could be inhibited in neutrophils by CP55,940 treatment but not AEA. It was suggested that this effect was not CB mediated (Kraft et al., 2004).

3.7. Mast cells

Mast cells are bone-marrow-derived cells, which populate connective and mucosal tissue as well as the nervous system, and are involved in inflammatory reactions. Activation of mast cells results in the secretion of a variety of pro-inflammatory mediators including cytokines, chemokines, histamine and proteases. Mast cells also express high-affinity IgE receptors and are involved during allergy reactions. At present there is some controversy as to whether mast cells express cannabinoid receptors or not and whether they mediate the actions of cannabinoid ligands. Some of the known effects of cannabinoids on mast cells are shown in Fig. 1. Although one study has reported that human mast cells do not express cannabinoid receptors, they have been shown to transport and hydrolyze AEA by the action of FAAH (Maccarrone et al., 2000b). Recently however, a study demonstrated both mRNA and protein expression of CB_1 and CB_2 receptors in two mast cell lines (Samson et al., 2003). In this study the application of both CB_2-selective and non-selective CB_1/CB_2 agonists could induce the activation of extracellular signal-regulated kinase (Samson et al., 2003). In support of this, another study demonstrated that rat peritoneal mast cells expressed CB_2 mRNA (Facci et al., 1995) although THC administration induced a non-lytic, energy- and concentration-dependent histamine release, which occurred irrespective of the presence of CB receptors (Bueb et al., 2001). Similar to THC treatment, the endogenous ligand AEA induced a significant level of histamine secretion in rat mast cells (Lau and Chow, 2003). In addition, the synthetic ligands R(+)WIN55,212 and HU-210, enhanced anti-IgE mediated histamine release whereas AEA, PEA and CP 55,940 treatment had no effect (Lau and Chow, 2003). In this study the effects of cannabinoids were mediated independently of cannabinoid receptors. In contrast, it has been demonstrated that 2-AG and CP 55,940 mediated suppression of histamine release from guinea pig mast cells could be reversed by an unselective nitric-oxide synthase inhibitor or a CB_2 receptor antagonist (Vannacci et al., 2004). Clearly there is much work to be done to understand more clearly the role of cannabinoids in mast cell immunology.

3.8. Dendritic cells

Dendritic cells play a key role in both the initiation of immune responses and the development of T cell responses.

Recent studies have observed the expression of both CB_1 and CB_2 receptors present on human dendritic cells, by Western blotting and RT-PCR (Matias et al., 2002). In addition, AEA, 2-AG and PEA were observed in lipid extracts from immature dendritic cells (Matias et al., 2002). The dendritic cells also expressed FAAH suggesting they have a fully functioning endocannabinoid system present (Matias et al., 2002). Upon LPS activation, the quantity of 2-AG but not AEA or PEA was increased in dendritic cells (Matias et al., 2002). However, cell activation did not increase the expression of either CB_1, CB_2 receptor or FAAH (Matias et al., 2002). This study suggests that dendritic cells may be important peripheral targets for the therapeutic use of cannabinoids in a number of inflammatory conditions.

3.9. Cannabinoids and immune responses to infection

One particular concern in the use of cannabinoids as therapeutic agents is the possibility of increased infectious susceptibility due to their immuno-modulatory potential. Herpes simplex virus (HSV) is a double stranded DNA enveloped virus of the Herpesviridae family, which is contracted through direct skin contact, and infects skin or mucous membranes causing lesions anywhere on the body, but especially near the mouth or genital areas. *Legionella pneumophilia* is a gram-negative bacterium bacillus of the genus *Legionella* and the causative agent of Legionnaires' disease, which usually presents as pneumonia, with a 25% fatality rate. Outbreaks of *Legionella pneumophilia* usually occur around areas of contaminated water. In animal models of HSV and *Legionella pneumophilia* infection, enhanced progression of infection was observed following THC administration (Klein et al., 1994; Newton et al., 1994; Morahan et al., 1979; Cabral et al., 1986; Specter et al., 1991). The increased susceptibility to infectious agents may be due in part to the effect of cannabinoids on interferons. These cytokines are important inflammatory mediators produced by numerous cell types including T cells, NK cells and fibroblasts, in response to pathogenic stimuli and mediate anti-viral responses. THC or R(+)WIN55,212 administration can inhibit both IFN-α, IFN-β and IFN-γ (Croxford and Miller, 2003; Newton et al., 1994; Klein et al., 2000; Cabral et al., 1986). Administration of either R(+)WIN55,212 or HU-210 prior to LPS injection in mice, reduced serum TNF-α and IL-12 levels and increased IL-10 levels (Smith et al., 2000). In addition, *Cornebacterium parvum* infected mice treated with R(+)WIN55,212 or HU-210 were protected from the lethal effects of LPS which could be reversed with the CB_1 antagonist, SR141716A (Smith et al., 2000). In a viral study, administration of R(+)WIN55,212 significantly reduced IFNα, β and γ, following CNS infection of mice with Theiler's murine encephalomyelitis virus (TMEV) and viral titers were significantly higher in the cannabinoid treated groups (Croxford and Miller, 2003). Furthermore, other pro-inflammatory cytokine responses, IL-1β and IL-6, were also diminished. In studies investigating the use of cannabinoids to treat Chagas' heart disease, R(+)WIN55,212 was found to inhibit the invasion of cardiac myoblasts by the parasite *Trypanosoma cruzi* (Croxford et al., in press). However, although cardiac inflammation was significantly reduced in vivo, this protective effect was found to be counterbalanced by the inhibitory effect of R(+)WIN55,212 on anti-parasite immune response, including delayed-type hypersensitivity and reduced serum anti-parasite immunoglobulin levels, which led to an increased parasitaemia (Croxford et al., in press). Therefore increased susceptibility to infection may be due to a decrease in direct inhibition of infectious agent by interferons, reduction of innate immune responses by macrophages and NK cells and/or anti-microbial cell mediated immunity by T and B leukocytes. This may have implications for the use of cannabinoids as therapeutic agents.

However, many immunomodulatory studies use high doses of cannabinoids, which may be many times higher than serum cannabinoid levels following oral ingestion (as used in most clinical trials of cannabinoids) or inhalation.

4. Potential therapeutic anti-inflammatory uses of cannabinoids

Recent studies have demonstrated the great potential for the use of cannabinoids for neurological disorders such as spasticity and tremor in MS (Baker et al., 2000), cerebral trauma (Panikashvili et al., 2001) and other neurological disorders due to the widespread expression of CB_1 receptors in disease-relevant regions of the brain, as reviewed recently (Croxford, 2003). However, it has long been suggested, from anecdotal evidence and more recently from animal studies, that cannabinoids may also have a potent effect upon the immune system. Therefore, we will review recent studies, which have focused on the use of cannabinoids for the treatment of immune-mediated diseases (Table 2), mostly in experimental models of disease, but where applicable we have added information on human clinical trials.

4.1. Multiple sclerosis

Multiple Sclerosis (MS) is an autoimmune inflammatory disease of the CNS, which affects roughly 1 in 1000 people. Symptoms of MS usually include muscle stiffness and spasticity, tremor, fatigue, pain, incontinence and sexual dysfunction, which can lead to increased anxiety and depression. Control of these MS-associated symptoms can be difficult and current drug therapies for MS-associated spasticity including oral or intrathecal baclofen infusion, dantrolene, diazepam, tizanidine (Noth, 1991) and gaba-

Table 2
The effects of cannabinoids in inflammatory disease

Disease	Drug	Route	Effects	Reference
Multiple Sclerosis	THC	Oral	↑serum TNF-α, IL-12p40	Killestein et al., 2003
EAE	THC	Oral, i.p.	↓disease and CNS inflammation	Lyman et al., 1989
	Δ^8-THC	Oral	↓disease,↑serum corticosterone	Wirguin et al., 1994
	HU-211	i.v.	↓disease and CNS inflammation	Achiron et al., 2000
TMEV-IDD	R(+)WIN55,212	i.p.	↓disease,↓Th1 cytokines and T cell proliferation	Croxford and Miller, 2003
	R(+)WIN55,212 ACEA JWH-015	i.p.	↓disease,↓microglial activation and MHC expression	Arevalo-Martin et al., 2003
Collagen Induced Arthritis	CBD	Oral, i.p.	↓disease,↓IFN-γ, TNF-α and T cell proliferation	Malfait et al., 2000
	HU-320	i.p.	↓disease,↓TNF-α in vitro	Sumariwalla et al., 2004
MLDST-induced diabetes	THC	Oral	↓insulitis, IFN-γ, TNF-α, IL-12 mRNA	Li et al., 2001
OVA-induced asthma	CBN, THC	i.p.	↓IL-2, IL-4, IL-5 and IL-13 mRNA in lungs,↓serum IgE,↓mucus in lungs	Jan et al., 2003

THC—Δ^9-tetrahydrocannabinol; CNS—central nervous system; EAE—experimental autoimmune encephalomyelitis; TMEV-IDD—Theilers murine encephalomyelitis virus induced demyelinating disease; CBD—cannabidiol; CBN—cannabinol; MLDSTZ—multiple low dose streptozotocin; i.p.—intraperitoneal.

pentin (Cutter et al., 2000) can have considerable side-effects including hallucinations, hypotension, seizures, anxiety, weakness, nausea and flu-like symptoms (reviewed by Goodkin, 1997). Although current disease-modifying therapeutic agents, IFN-β and copaxone, reduce relapses in a portion of relapsing-remitting MS patients, their effectiveness in reducing disability progression in relapsing-remitting MS patients is unclear (Goodin et al., 2002). A recent study in Britain demonstrated that whilst IFN-β therapy reduces the number of relapse episodes by 41%, in 40% of MS patients the ongoing disease progression is unaffected (Dubois et al., 2003).

Therefore there is a need for novel therapeutic agents which are effective but yet induce fewer side-effects. Many MS sufferers have reported the beneficial effects of marijuana on spasticity, tremor, pain, and anxiety (Consroe et al., 1997). Currently a number of clinical trials to test the potency of cannabinoid preparations to relieve MS-related symptoms have been undertaken with varying degrees of success (reviewed by Pertwee, 2002; Croxford, 2003; Croxford and Miller, 2004). However, in a small clinical trial to test the ability of orally administered cannabinoids to alleviate symptoms of MS it was found that oral THC actually induced a modest increase in TNF-α, following LPS-stimulation of whole blood, and an increase in plasma IL-12 p40 (Killestein et al., 2003). The apparent increase in Th1 cytokines in this study is in contrast to a number of previous human studies. However, MS is thought to be a disease of immune dysfunction and this may account for the differences seen between cannabis use in healthy subjects and clinical trials of THC in MS patients.

Cells of the immune system and the cytokines they secrete are thought to play a major role in the pathogenesis of MS and the animal model of relapsing-remitting MS, experimental autoimmune encephalomyelitis (EAE). As previously discussed, cannabinoids have a potent inhibitory effect on immune cell function, especially that of macrophages and T cells, the primary cells thought to be involved in pathogenesis of MS/EAE. Using this rationale cannabinoid administration in EAE was studied. Preventative oral THC administration in Lewis rats or intraperitoneal (i.p.) injection to strain 13 guinea pigs with EAE was effective in inhibiting severity of disease and delaying onset of disease as well as CNS inflammation (Lyman et al., 1989). A second study used Δ^8-THC, a more stable and less psychoactive cannabinoid analogue than Δ^9-THC, in Lewis rat EAE. Oral but not i.p. administration reduced the severity and incidence of EAE (Wirguin et al., 1994) and increased circulating corticosterone levels twofold. However, Δ^8-THC treatment did not prevent the number and tissue penetrance of inflammatory infiltrate in the CNS. HU-211 (Dexanabinol), is a non-psychotropic cannabinoid which does not bind either of the known CB receptors, has been shown to inhibit TNF-α secretion from LPS-stimulated macrophages (Burnette-Curley and Cabral, 1995). A recent study found that intra-venous (i.v.) administration of HU-211 in Lewis rat EAE inhibited disease severity when administered at the onset of disease but not prophylactically (Achiron et al., 2000). Cannabinoids may also protect from EAE by inhibiting glutamate release. Glutamate toxicity has been suggested as a possible mediator of CNS damage to neurons and oligodendrocytes during MS and EAE (Werner et al., 2000; Pitt et al., 2000) and CB_1 receptor agonists and PEA, which does not bind either of the CB receptors, have been demonstrated to protect cerebellar granule cells from glutamate toxicity (Skaper et al., 1996). Although these studies hint at possible mechanisms of disease amelioration the mechanism of action is yet to be elucidated and requires further study. More recently two studies have developed the therapeutic use of cannabinoids in a viral model of MS, induced by Theiler's murine encephalomyelitis virus (TMEV). TMEV-induced demyelinating disease is a good model for primary-progressive MS, and is mediated by $CD4^+$ myelin-specific T cells which become activated by bystander activation and/or epitope spread, following the induction of virus specific T cell responses (Miller et al., 1997). Treatment of TMEV-induced demyelinating disease with R(+)WIN55,212 either at the time of infection, at onset

of clinical disease or during established disease significantly inhibited clinical disease (Croxford and Miller, 2003). Associated with the suppression of disease symptoms both viral- and myelin-specific T cell secretion of IFN-γ was inhibited. In addition, T cell proliferative responses and delayed type hypersensitivity reactions were also significantly decreased (Croxford and Miller, 2003). Furthermore, a number of other pro-inflammatory Th1-type cytokines, IL-1β, IL-6 and TNF-α, were inhibited in the CNS of TMEV-infected mice (Croxford and Miller, 2003). A second study demonstrated that R(+)WIN55,212 (CB_1 and CB_2 agonist), ACEA (a CB_1-selective agonist) or JWH-015 (a CB_2-selective agonist) could improve the neurological deficit in TMEV-infected mice by suppressing microglial activation, MHC class II expression and the number of CD4+ T cells infiltrating the CNS (Arevalo-Martin et al., 2003).

Further study is required to determine the cell types involved in the cannabinoid-mediated inhibition of EAE and TMEV and whether this is due to direct effects upon the immune system cells. It is likely that protection is due to a combination of effects upon both CNS resident cells and the peripheral immune system.

4.2. Rheumatoid arthritis

Rheumatoid arthritis (RA) is an inflammatory disease of the joints, which leads to their eventual destruction, deformity of affected limbs and the loss of limb function. Other symptoms associated with RA include pain and stiffness and swelling of joints, which are thought to be mediated by complex mechanisms including cells of the immune system such as T cells, macrophages and dendritic cells. A key cellular mediator in RA is TNF-α, as anti-TNF therapy has proved to be successful in treating the symptoms in RA clinical trials (Elliott et al., 1994; Moreland et al., 1997). To determine the potential of cannabinoids to treat RA, studies have been performed in an animal model, collagen-induced arthritis (CIA), which has similar pathology to RA and is mediated by CD4+ T cells and TNF (Mauri et al., 1996; Piguet et al., 1992; Williams et al., 2000). The first study demonstrated that either daily oral (25 mg/kg) or i.p. injection (5 mg/kg) of CBD, the major non-psychoactive component of cannabis which has low affinity for either cannabinoid receptor, could suppress the progression of CIA (Malfait et al., 2000). This suppressive effect was associated with a reduction in collagen-specific CD4+ T cell proliferation and IFN-γ production. In addition, CBD inhibited TNF production from synovial cells. In vitro studies determined that CBD could inhibit the oxygen-burst from zymosan-stimulated granulocytes and LPS-induced serum TNF (Malfait et al., 2000). Another study from the same group demonstrated similar effects using a novel non-psychotropic synthetic cannabinoid, HU-320, which has a low binding affinity for both CB receptors, in CIA. Daily systemic injection of 1 or 2 mg/kg HU-320 ameliorated established CIA (Sumariwalla et al., 2004). In vitro studies determined that HU-320 could inhibit TNF secreted from macrophages and reactive oxygen intermediates from RAW 264.7 cells (Sumariwalla et al., 2004). Cannabinoid therapy of RA could provide symptomatic relief of joint pain (Smith et al., 1998) and swelling as well as suppressing joint destruction and disease progression.

4.3. Diabetes

Diabetes mellitus is a group of diseases which are characterized by defects in insulin secretion and/or action resulting in hyperglycemia. Pathogenesis of diabetes is associated with the autoimmune destruction of pancreatic β cells and the subsequent loss of insulin production. A recent study investigated the use of THC to suppress an animal model of diabetes, multiple low dose streptozotocin (MLDSTZ)-induced autoimmune diabetes. Oral treatment of MLDSTZ-treated mice with THC (150 mg/kg) for 11 days, suppressed insulitis and IFN-γ, TNF-α and IL-12 mRNA expression (Li et al., 2001). Furthermore, THC treatment inhibited the MLDSTZ-induced elevation of serum glucose and the loss of pancreatic insulin. Other studies have investigated the use of cannabinoids to treat diabetic related tactile allodynia. Diabetic neuropathic pain is a complication of diabetes and is only partially treated by current medications. Treatment of rats with streptozocin induced diabetes and neuropathic pain in the hindlimbs (Ulugol et al., 2004). Tactile allodynia could be effectively suppressed following injection of diabetic rats with R(+)WIN55,212 i.p. suggesting that cannabinoids may provide protection against pancreatic destruction but also some alleviation of neuropathic pain in diabetic patients (Ulugol et al., 2004; Dogrul et al., 2004). Interestingly, it has been demonstrated that cannabinoids inhibit neuropathic pain in part via a CB2-mediated pathway (Ibrahim et al., 2003).

4.4. Allergic asthma

Allergic asthma is characterized by an increase in serum allergen-specific IgE levels, recruitment of eosinophils into the lung, activation of mucus secretion by goblet cells and airway hyperresponsiveness. Although the pathogenesis of asthma is unclear animal models suggest that CD4+ T cells with a Th2 phenotype (IL-4, IL-5, IL-13) may play a role. Th2 cytokines play a vital role in humoral immune responses. IL-4 promotes the differentiation of B cells and immunoglobulin secretion, whereas IL-5 can mediate eosinophil recruitment. Both IL-4 and IL-13 can mediate immunoglobulin isotype switching from IgM to IgE which can trigger allergic reactions (Burrows et al., 1989). In an ovalbumin (OVA)-induced model of asthma IL-4 has proved to be important in the pathogenesis, as anti-IL-4 treatment or induction of disease in IL-4 deficient mice has been successful in ameliorating allergic responses to OVA (Corry et al., 1996; Coyle et al., 1995; Brusselle et al.,

1995). Treatment of OVA-sensitized A/J mice with either CBN or THC (50 mg/kg i.p.) for 3 days resulted in the amelioration of IL-2, IL-4, IL-5 and IL-13 mRNA in the lungs (Jan et al., 2003). Furthermore, cannabinoid treatment inhibited the elevation of serum IgE and overproduction of mucus in the lungs. Therefore, cannabinoids maybe beneficial in the treatment of asthma. In addition to the immunomodulatory effects in models of asthma, cannabinoids may also play a role in bronchodilation. Early studies using Δ9-THC implicated cannabinoids in having bronchodilatory effects in asthmatic patients when administered either orally or by aerosol (Abboud and Sanders, 1976; Hartley et al., 1978). The endocannabinoid system likely plays a role in lung function as AEA has been shown to be synthesized in lung tissue and CB_1 is expressed on axon terminals of nerve fibers in bronchiolar smooth muscle cells (Calignano et al., 2000). A recent report suggests that blockade of AEA-induced CB_1 activity with a CB_1-selective receptor antagonist, SR141716A, enhances capsaicin-induced bronchospasm in a rodent model (Calignano et al., 2000). However, administration of AEA can have dual effects. Following administration of AEA capsaicin-induced bronchospasm is significantly reduced whereas in the absence of vagus nerve constricting tone, AEA induces bronchospasm (Calignano et al., 2000). Therefore the endocannabinoid system may induce constrictive tone in relaxed airway smooth muscle and vice versa. Further investigation into the effects of cannabinoids in airway responsiveness and their route of delivery may lead to more selective therapeutic agents for asthma.

5. Summary

Although the function of cannabinoid receptors on immune cells and the cross-talk between the endocannabinoid and immune systems is as yet unclear one could hypothesize that endocannabinoid signaling in lymphoid tissue may provide tonic control of immune cell activation and therefore limit spontaneous activation of immune function cells. The effect of cannabinoids on immune functions appears to be transient which would allow the inhibitory effect to be overcome when the immune system needs to be activated in response to infection. This would seem to be supported by the loss of receptor expression following activation of some immune cell types. In addition, the transient nature of cannabinoids on immune system cell functions suggests that although side-effects may be minimal, any potential therapy may require long-term administration of the cannabinoid agents. However, recent studies suggest a difference between synthetic or plant-derived cannabinoids and endocannabinoids in their effects upon the immune system. Indeed, endocannabinoids have been implicated in the induction of some cytokines when present at low doses and also in the migration of immune cells such as B cells. Therefore, it is important that precise dose-response studies with specific cannabinoid ligands are undertaken prior to clinical use for inflammatory disease.

Currently a phase II clinical trial has demonstrated that dexanabinol (HU-211), a synthetic cannabinoid agent which is a non-psychoactive enantiomer of the potent HU-210, appears to be a promising therapy for neuroprotection following both head injury and stroke (Knoller et al., 2002). Furthermore, cannabinoids have been demonstrated to be very effective therapeutic agents in an increasing number of animal disease models. The effects of cannabinoids on the expression of costimulatory molecules, adhesion molecules and chemokines require further study and may increase our understanding of the pleiotropic effects of cannabinoids on the immune system. In addition, further studies into differential cannabinoid receptor signaling, using specific receptor ligands or CB receptor knockout mice, and the identification of other cannabinoid-like receptors, may allow the circumvention of side-effects associated with CB_1 receptor mediated side-effects, whilst increasing their therapeutic efficacy. Although current studies suggest that cannabinoids may prove to be useful alternatives to some current therapeutic agents in treating a variety of human inflammatory disorders in the future, a thorough evaluation of the immunomodulatory effects of cannabinoids needs to be undertaken in the coming years.

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