CHAPTER EIGHT

Marijuana Use and Brain Immune Mechanisms

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Contents

1. Introduction 200
2. Phytocannabinoids and Immune Function 202
3. Immune Modulation and Cannabinoid Receptors 203
4. Marijuana and Neuroimmunity 205
5. Effect of Phytocannabinoids on Microglia 207
6. Marijuana and Astrocytes 211
7. Marijuana and Infectious Agents That Target the CNS 215
8. Summary and Future Prospectives 220

References 223

Abstract

The recreational smoking of marijuana, or *Cannabis sativa*, has become widespread, including among adolescents. Marijuana contains a class of compounds known as phytocannabinoids that include cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC). THC is the major psychoactive component in marijuana, but also exhibits immunosuppressive activity. CBD, while not psychotropic, also modulates immune function, but its mechanism of action appears to differ from that of THC. Since both compounds are highly lipophilic, they readily passage the blood–brain barrier and access the central nervous system. Since CBD is not psychotropic, it has been considered as a candidate therapeutic compound for ablating neuropathological processes characterized by hyperinflammation. However, an unresolved question centers around the impact of these compounds on immune-competent cells within the CNS in relation to susceptibility to infection. There are accumulating data indicating that THC inhibits the migratory capability of macrophage-like cells resident in the CNS, such as microglia, toward nodes of microbial invasion. Furthermore, phytocannabinoids have been reported to exert developmental and long-term effects on the immune system suggesting that exposure to these substances during an early stage in life has the potential to alter the fundamental neuroimmune response to select microbial agents in the adult.

1. INTRODUCTION

The recreational smoking of *Cannabis sativa*, mainly its resin (hashish) or the chopped flowering tops of the plant (marijuana), has become widespread. Because of its lack of acute life-threatening effects, marijuana has been often thought of as a "soft drug." However, this perception may have to be revised in face of mounting scientific data that it impairs memory and learning and has residual effects on lung, immune, and reproductive function. Products derived from smoking marijuana vary in the content of Δ^9-tetrahydrocannabinol (THC), the major psychoactive component (reviewed in Nahas and Latour, 1993; Fig. 8.1). The flowering tops of the plant may contain 1–6% THC total weight, although the percentage by weight of this component may reach 8% in hashish and exceed 50% in hash oil. However, the fiber type of marijuana that is used for the manufacture of rope and twine contains little THC. In addition to THC, over 100 other cannabinoids have been identified in the particulate phase of the marijuana plant, mainly cannabidiol (CBD) and cannabinol (CBN) that are not psychoactive but are biologically active (Fig. 8.1). The term phytocannabinoid has been used to distinguish these plant-derived compounds from cannabinoids that have been synthesized in the laboratory. Upon heating, phytocannabinoids rapidly decarboxylate and at the temperature of pyrolysis (200–400 °C) undergo aromatization. Polycydic aromatic hydrocarbons have been identified in marijuana smoke for which the proportions of the higher molecular weight compounds, particularly the carcinogens benzo(α)pyrene and benz(α)anthracene, are greater than in tobacco smoke. The gas phase of marijuana smoke includes toxic substances such as carbon monoxide, hydrogen cyanide, and nitrosamines, although these are present in equivalent concentrations in tobacco smoke. THC and other cannabinoids are lipophilic and are stored in liver, lung, spleen, and neutral fat. Because THC has a half-life of 8 days in fat, it may take up to 1 month for complete elimination of a single dose. Furthermore, since THC is a polar compound it is slowly metabolized into more water-soluble, nonpsychoactive metabolites. The bioavailability of inhaled and ingested THC is 20% and 6%, respectively. Less than 1% of the bioavailable THC reaches the brain, a fact that illustrates the psychoactive potency of this compound (Chesher & Jackson, 1985).

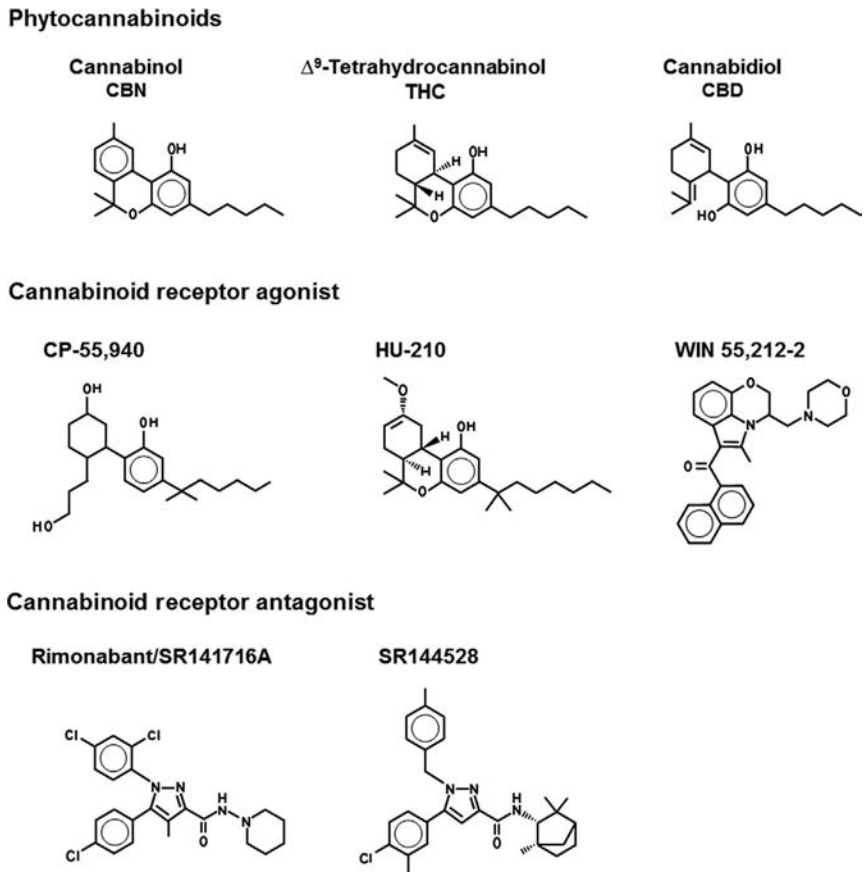

Figure 8.1 Structures of representative cannabinoids (phytocannabinoids, cannabinoid receptor agonists, and cannabinoid receptor antagonists). Cannabinol/CBN: 6,6,9-trimethyl-3-pentylbenzo[c]chromen-1-ol, Δ^9-tetrahydrocannabinol/THC; (6aR,10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromen-1-ol, Cannabidiol/CBD; 2-[(1R,6R)-6-Isopropenyl-3-methyl-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol, CP-55,940; 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol, HU-210; (6aS,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol, WIN 55,212-2; [(3R)-5-methyl-3-(morpholin-4-ylmethyl)-2,3-dihydro[1,4]oxazino[2,3,4-hi]indol-6-yl](naphthalen-1-yl) methanone, Rimonabant/SR141716A; 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide and SR144528; 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1R,3S,4S)-2,2,4-trimethyl-3-biccyclo[2.2.1] heptanyl]pyrazole-3-carboxamide. CBD, CBN, and THC are found natively in *Cannabis sativa*.

2. PHYTOCANNABINOIDS AND IMMUNE FUNCTION

THC also has been shown to alter immune function, most of its effects being immune suppressive (reviewed in Cabral & Staab, 2005). The purification and structural characterization of THC (Gaoni & Mechoulam, 1971) led to the chemical synthesis of various cannabinoid analogs that have been used extensively in structure–activity studies to characterize cannabinoid-mediated effects *in vitro* and *in vivo* related to the central nervous and immune systems and contributed to the definition of the mechanisms by which cannabinoids exert their effects. THC has been reported to suppress the antibody response of humans and animals (Klein, Friedman, & Specter, 1998) and to suppress a variety of activities of T lymphocytes (Kaminski, 1998; Klein et al., 2004). Administration of THC to mice also resulted in inhibition of natural killer (NK) cytolytic activity and in reduction of interferon-gamma (IFNγ) levels (Massi, Fuzio, Vigano, Sacerdote, & Parolaro, 2000). In addition, THC has been reported to abolish the functional activities of macrophages and macrophage-like cells, including macrophage-like cell contact-dependent cytolysis of tumor cells and the processing of antigens (Burnette-Curley, Marciano-Cabral, Fischer-Stenger, & Cabral, 1993; Klein, Kawakami, Newton, & Friedman, 1991; McCoy, Matveyeva, Carlisle, & Cabral, 1999). It has been reported also that THC alters the production of chemokines and cytokines, leading to a perturbation in the homeostatic balance between proinflammatory (Th1) and antiinflammatory (Th2) activities. Proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) promote systemic inflammation. Antiinflammatory cytokines such as IL-4 and IL-10, on the other hand, play an immunoregulatory role in controlling the proinflammatory response. Klein, Newton, Nakachi, and Friedman (2000) showed that treatment of BALB/c mice with THC resulted in a decrease in levels of the proinflammatory (Th1) cytokines IFNγ and IL-12 and an increase in the level of the antiinflammatory cytokine IL-4 in response to infection with *Legionella pneumophila*. Conversely, through the use of a mouse lung tumor model in which THC mediated a decrease in tumor immunogenicity, Zhu et al. (2000) demonstrated that levels of the immune inhibitory Th2 cytokines IL-10 and transforming growth factor were augmented, while the level of the immune stimulatory Th1 cytokine IFNγ was downregulated. These collective results suggested that THC exposure alters the Th1/Th2 cytokine profile in experimental animals such that the balance of chemokine and

cytokine functional activity shifts from that of a Th1-type proinflammatory cytokine profile to that of a Th2-type antiinflammatory cytokine profile. Such a shift in the Th1/Th2 cytokine profile could contribute to altered proinflammatory responsiveness to infection with select biological agents such as bacteria and viruses. Although the preponderance of scientific reports indicates that THC inhibits immune functional activities *in vitro* and *in vivo*, it is now recognized that other phytocannabinoids such as CBD and CBN may alter the functional activities of the immune system. However, whether the mechanism by which these phytocannabinoids alter immune function is comparable to that exerted by THC remains to be defined.

3. IMMUNE MODULATION AND CANNABINOID RECEPTORS

Studies in which synthetic cannabinoid compounds such as CP-55,940, HU-210, WIN 55,212, JWH-015, ACEA, SR141716A, and SR144528 (Fig. 8.1) have been used have provided important insights into the functional relevance and mechanism of action by which THC exerts its effects on the immune system. Furthermore, these collective studies have served as a basis for the identification of specific binding sites in mammalian brain and peripheral nonneuronal tissues which are now recognized as representing cannabinoid receptors (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990; Munro, Thomas, & Abu-Shaar, 1993). To date, two cannabinoid receptors that meet stringent pharmacological and molecular criteria have been identified (Fig. 8.2). The first of these, the cannabinoid receptor type 1 (CBR1), is a serpentine, seven-transmembranal, G protein-coupled receptor that has been found by radioligand binding and *in situ* mRNA hybridization to be distributed throughout the brain and localized predominantly in the cerebellum, cerebral cortex, hippocampus, basal ganglia, and spinal cord (Herkenham et al., 1990; Matsuda et al., 1990; Westlake, Howlett, Bonner, Matsuda, & Herkenham, 1994). The CB1R also has been found in testis (Gerard, Mollereau, Vassart, & Parmentier, 1991) and, to a lesser extent, in other tissues and cells. The psychotropic effects attributed to THC are due to activation of this receptor (Agarwal et al., 2007; Kunos, Osei-Hyiaman, Batkai, Sharkey, & Makriyannis, 2009).

A second serpentine, seven-transmembranal, G protein-coupled cannabinoid receptor, the cannabinoid receptor type 2 (CB2R), has been identified in cells and tissues of the immune system (Munro et al., 1993). This receptor possesses approximately 44% amino acid similarity to the CB1R.

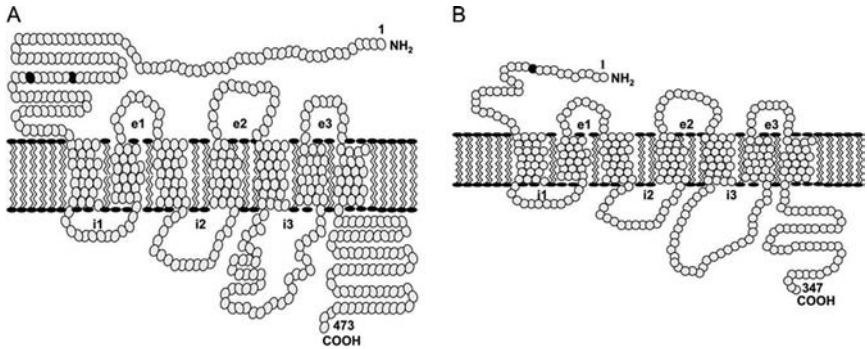

Figure 8.2 Diagrammatic representation of the CB1R and CB2R. (A) Mouse CB1R: The solid circles denote putative glycosylation sites at asparagine residues 78 and 84. The extracellular and intracellular loops are denoted as e1, e2, and e3, and i1, i2, and i3, respectively. (B) Mouse CB2R: The solid circle denotes a putative glycosylation site at asparagine residue 11 of CB2. The extracellular and intracellular loops are denoted as e1, e2, and e3, and i1, i2, and i3, respectively. The CB1Rs of different mammalian species exhibit a high level of similarity. The CB2R exhibits greater interspecies differences with the deduced amino acid sequence of the mouse CB2R differing from that of human in 60 residues (82% similarity) (Shire et al., 1996). *Panel (B) From Cabral and Griffin-Thomas (2008).*

The level of the CB2R varies among different immune cell populations, with B lymphocytes expressing the highest levels followed by macrophages, monocytes, NK cells, and polymorphonuclear cells, in that order (Galiegue et al., 1995; Schatz, Lee, Condie, Pulaski, & Kaminski, 1997). Early studies indicated that the distribution of the CB2R was confined to peripheral non-neuronal sites. However, it is now recognized that this receptor is expressed by different subsets of immune-competent cells found in the CNS (Cabral & Marciano-Cabral, 2005; Carlisle, Marciano-Cabral, Staab, Ludwick, & Cabral, 2002; Carrier et al., 2004; Fernandez-Ruiz et al., 2007; Nunez et al., 2004; Ramirez, Blazquez, Gomez del Pulgar, Guzman, & de Ceballos, 2005). Most of the immunomodulatory effects attributed to THC have been linked to activation of the CB2R.

There is evidence for the existence of additional cannabinoid receptors, based primarily on *in vivo* studies in which cannabinoid receptor knock-out mice have been used to investigate the pharmacology and pharmacokinetics of cannabinoids (Breivogel, Griffin, Di Marzo, & Martin, 2001; Di Marzo et al., 2000; Jarai et al., 1999). Included among these candidate cannabinoid receptors is GPR55, a seven-transmembranal G protein-coupled receptor first cloned and identified *in silico* from an expressed sequence tags database

(Baker, Pryce, Davies, & Hiley, 2006; Pertwee, 2007; Sawzdargo et al., 1999). GPR55 is activated by the phytocannabinoids THC and CBD, the synthetic cannabinoids abnormal CBD, HU-210, and CP-55,940, and the endogenous cannabinoids anandamide, 2-arachidonoylglycerol, and noladin ether (Ryberg et al., 2007). However, unlike the CB1R and the CB2R, GPR55 is coupled to a G-alpha (Gα) protein instead of a Gi/o protein (Ryberg et al., 2007), is not activated by the synthetic agonist WIN 55212-2, and increases intracellular calcium levels upon activation (Lauchner et al., 2008). Nevertheless, to date, a novel non-CB1, non-CB2 cannabinoid receptor (non-CB1R, non-CB2R) that meets rigid pharmacological and functional criteria as selective for cannabinoid ligands has yet to be cloned and characterized at the molecular level (Breivogel et al., 2001; Di Marzo et al., 2000; Jarai et al., 1999; Wiley & Martin, 2002).

4. MARIJUANA AND NEUROIMMUNITY

The blood–brain barrier (BBB) is a selectively permeable continuous layer of microvascular endothelial cells that is interconnected by tight junctions. It serves to regulate the traffic into the CNS of substances and cells that are present in the circulatory system. Perivascular macrophages are found in the region surrounding the endothelium of brain capillaries and are replenished through the migration of circulating monocytes. In addition, astrocytes are found in this region and play a critical homeostatic role by maintaining contact with endothelial cells and regulating permeability of the endothelial layer through the release of soluble factors. Within the CNS are found immune-competent cells such as microglia, perivascular macrophages, and pericytes. These cells, once activated, release a plethora of inflammatory factors that secondarily activate astrocytes, resulting in a cascade of chemokines and cytokines that promote expansion of the neuroinflammatory response. The recognition that these resident immune cells within the CNS also express the CB2R, and that they were responsive to activation by cannabinoids, suggested that exogenously introduced cannabinoids had the capacity to modulate their functional activities. Furthermore, they could do so in a fashion selectively differential from that resulting from activation of the CB1R that was linked to elicitation of psychotropic effects. Indeed, cannabinoids that target the CB2R have been considered as therapeutic agents for management of multiple sclerosis (Maresz et al., 2007; Zhang, Adler, et al., 2009), ischemic/perfusion injury following an induced stroke (Ni et al., 2004; Zhang et al., 2007; Zhang, Martin et al., 2009),

rheumatoid arthritis (Sumariwalla et al., 2004), inflammatory bowel disease (Storr et al., 2008, 2009), inflammatory autoimmune diabetes (Li, Kaminski, & Fischer, 2001), spinal cord injury (Adhikary et al., 2011; Baty et al., 2008), sepsis (Tschöp et al., 2009), autoimmune uveoretinitis (Xu et al., 2007), osteoporosis (Ofek et al., 2006), and systemic sclerosis (Servettaz et al., 2010). This potential for selectively targeting the CB2R is especially relevant since cannabinoids are highly lipophilic molecules, readily cross the BBB, and have the capacity to interact selectively with target cells at concentrations that do not engender overt cytotoxic effects.

However, while it has been proposed that the CB2R may have therapeutic potential, especially when considered for dampening of untoward immune responses associated with a variety of neuropathological conditions, exogenously introduced cannabinoids have the potential to place a host at greater susceptibility to infection. This potential could be realized under conditions in which THC or other exogenous cannabinoid is introduced into the CNS through the medium of marijuana smoke. However, the acquisition of direct data that unequivocally links marijuana use in humans to immune dysfunction within the CNS has been difficult to obtain. Marijuana users may partake of other drugs that affect immune function and dissecting the relative contribution of distinct phytocannabinoids has been difficult. Furthermore, because marijuana contains a plethora of phytocannabinoids, attributing a specified action as linked functionally to THC, CBD, or CBN divorced from their potential interactive action is difficult. The complexity is further amplified by the emerging scientific data that distinct phytocannabinoids may activate immune cells by receptor-mediated as well as by nonreceptor-mediated modes. Thus, in order to garner insight as to the potential linkage between marijuana use in humans and compromised neuroimmune function, investigators have resorted to the use of purified synthetic phytocannabinoid preparations in cell culture models and in experimental animals. Monnet-Tschudi et al. (2008) assessed THC accumulation, metabolism, and cell-type-specific adverse effects in aggregating brain cell cultures. Mixed-cell aggregate cultures of fetal rat telencephalon were used as an *in vitro* model, as well as aggregates enriched either in neurons or in glial cells. It was found that THC accumulated preferentially in neurons, and that glia–neuron interactions decreased THC accumulation. The quantification of 11-OH–THC and of THC–COOH showed that brain aggregates were capable of THC metabolism. No cell-type difference was found for the metabolite 11-OH–THC, whereas the THC–COOH content was higher in mixed-cell cultures. Neurons, and

particularly GABAergic neurons, were most sensitive to THC. JWH-015, a CB2R agonist, showed effects similar to THC, whereas ACEA, a CB1R agonist, had no effect. The expression of IL-6 was upregulated after treatment with THC or JWH-015, whereas the expression of TNF-α remained unchanged. These results suggested that the adverse effects of THC were related either to THC accumulation or to CBR activation and associated with IL-6 upregulation. Nevertheless, while data derivative from the use of experimental models must be interpreted with appropriate caveats, they converge on the outcome that phytocannabinoids such as THC and CBD when used at biologically relevant concentrations alter immune function.

5. EFFECT OF PHYTOCANNABINOIDS ON MICROGLIA

The preponderance of the data that are available concerning the effects of phytocannabinoids on CNS immune functional activity involves their action on microglia. Microglia are a population of glial cells that act as resident macrophages of the CNS and represent 10–15% of the total glial population. Once considered to be scavenger cells in this compartment, it is now recognized that they exert a major role in CNS remodeling and regeneration (reviewed in Moore & Thanos, 1996). Chronic activation of microglia, however, can exacerbate pathologies of the CNS including AIDS dementia and Alzheimer's disease (Dickson, Lee, Mattiace, Yen, & Brosnan, 1993). Microglia may contribute to the neurodegeneration by secreting nitric oxide (NO), IL-1β, IL-6, and TNF-α (Lee, Liu, Dickson, Brosnan, & Berman, 1993; Merrill, Ignarro, Sherman, Melinek, & Lane, 1993). In this context, select cannabinoids have been proposed as having therapeutic potential in that they may downmodulate the excessive inflammatory response. However, there is evidence that phytocannabinoids may decrease inflammatory responses that are consequent of bacterial infection with a CNS dimension. Puffenbarger, Boothe, and Cabral (2000) examined the effect of cannabinoids on the induction of cytokine messenger RNAs (mRNAs) by rat microglia in response to bacterial lipopolysaccharide (LPS), a component that is found in the outer membrane of Gram-positive bacteria. Exposure of neonatal rat cortical microglial cells to THC resulted in reduced amounts of LPS-induced mRNAs for IL-1α, IL-1β, IL-6, and TNF-α. Of these proinflammatory cytokine mRNAs, the response of that for IL-6 was exquisitely sensitive to THC. When the paired enantiomers CP-55,940 or CP-56,667 were used as a measure of enantiomeric selectivity as a hallmark of a receptor-mediated action, a similar inhibition of LPS-induced cytokine

mRNA expression was obtained. A comparable inhibitory outcome was obtained when the paired enantiomers levonantradol and dextronantradol were employed. Neither the CB1R-selective antagonist SR141716A nor the CB2R-selective antagonist SR144528 was able to reverse the inhibition of cytokine mRNA. The collective results indicated that THC down-modulated levels of proinflammatory cytokine mRNAs in rat microglia, but did so by a mode that did not involve a known cannabinoid receptor. Cutando et al. (2013) reported that microglial activation served as an underlying basis for cerebellar deficits produced by repeated use of marijuana. It was found that subchronic administration of THC to mice activated cerebellar microglia and increased the expression of neuroinflammatory markers, including that of IL-1β. The neuroinflammatory phenotype correlated with deficits in cerebellar conditioned learning and fine motor coordination. The neuroinflammatory phenotype was readily detectable in the cerebellum of mice with global loss of the CB1R (i.e., CB1R, Cb1 (−/−) mice) and in mice lacking CB1R in the cerebellar parallel fibers, suggesting that CB1R downregulation in the cerebellar molecular layer played a key role in THC-induced cerebellar deficits. Expression of CB2R and IL-1β mRNA was increased under neuroinflammatory conditions in activated CD11b-positive microglia. Furthermore, administration of the immunosuppressant minocycline or an inhibitor of IL-1β receptor signaling prevented the deficits in cerebellar function in Cb1(−/−) and THC-withdrawn mice. The results suggested that cerebellar microglial activation played a crucial role in the cerebellar deficits induced by repeated cannabis exposure. Thus, while THC affects microglial responsiveness, the mechanism through which it alters this responsiveness, and whether the altered activity is attributed to activation of a specified receptor, remains to be resolved.

Studies conducted on the effects of THC on microglia have been extended to include those involving CBD. The rationale for examining the effects of this phytocannabinoid on microglial function is based on recognition that it has immunomodulatory activity divorced from elicitation of psychotropic effects. Carrier, Auchampach, and Hillard (2006) examined the effects of both THC and CBD on microglial proliferation and found that these compounds potently inhibited [^{3}H]thymidine incorporation into a mouse microglial cell line while having no effect on cell cycle. Treatment with THC and CBD decreased [^{3}H]thymidine uptake into microglia. CBD and, less potently, THC decreased uptake of [^{3}H]adenosine to a similar extent as [^{3}H]thymidine in both mouse microglia and mouse RAW264.7

macrophage-like cells. Binding studies confirmed that CBD bound to the equilibrative nucleoside transporter 1. Because adenosine agonists have antiinflammatory effects, and because uptake of adenosine is a primary process that is involved in terminating adenosine signaling, these investigators tested the hypothesis that CBD is immunosuppressive since it enhanced endogenous adenosine signaling. *In vivo* treatment with CBD decreased TNF-α production in LPS-treated mice, an effect that was reversed with an A2A adenosine receptor antagonist and abolished in A2A receptor knock-out mice. This G protein-coupled receptor has been reported to negatively regulate overreactive immune cells so as to protect tissues from collateral inflammatory damage (Ohta & Sitkovsky, 2001). It was suggested that CBD had the ability to enhance adenosine signaling through inhibition of its uptake and that a non-cannabinoid receptor-mediated mechanism accounted for the CBD-induced decrease in inflammation. Kozela et al. (2010) reported that THC and CBD differentially inhibited the LPS-activated NF-κB and interferon-β/STAT proinflammatory pathways in BV-2 microglial-like cells. It was found that THC and CBD, the two major cannabinoids present in marijuana, decreased the production and release of proinflammatory cytokines, including IL-1β, IL-6, and IFN-β from LPS-activated microglial cells. The cannabinoid antiinflammatory action did not appear to involve the CB1R and the CB2R or the abn-CBD-sensitive receptor, a putative receptor that is sensitive to a synthetic regioisomer of CBD (abn-CBD). In addition, it was found that THC and CBD acted through different, although partially overlapping, mechanisms. CBD, but not THC, reduced the activity of the NF-κB pathway, a primary pathway regulating the expression of proinflammatory genes. Moreover, CBD, but not THC, upregulated the activation of the STAT3 transcription factor, an element of homeostatic mechanism(s) inducing antiinflammatory events. Following CBD treatment, a decreased level of mRNA for the suppressor of cytokine signaling (SOC3) gene, a main negative regulator of STATs and particularly of STAT3, was observed. However, both CBD and THC decreased the activation of the LPS-induced STAT1 transcription factor, a key player in IFN-β-dependent proinflammatory processes. Thus, CBD and THC appeared to vary in their effects on antiinflammatory pathways, including the NF-κB and IFN-β-dependent pathways. Wu et al. (2012) investigated the proapoptotic effect of CBD on primary microglial cells. Treatment of mouse primary microglial cultures with CBD resulted in a time- and concentration-dependent induction of apoptosis, as shown by increase in hypodiploid cells and DNA strand breaks, and marked activation

of both caspase-8 and -9. Mechanistic studies revealed that antioxidants such as *N*-acetyl-L-cysteine and glutathione, that the putative GPR55 agonist abn-CBD, and that specific antagonists for the vanilloid receptor, the CB1R, and the CB2R did not counteract the apoptosis induced by CBD. In contrast, methyl-β-cyclodextrin (MCD), a lipid raft disruptor, potently attenuated CBD-induced microglial apoptosis and caspase activation. Furthermore, CBD-induced lipid raft coalescence and augmented the expression of GM1 ganglioside and caveolin-1, all of which were attenuated by MCD. The results suggested that CBD induced a proapoptotic effect in primary microglia through lipid raft coalescence and the elevation of expression of GM1 ganglioside and caveolin-1.

The anti-neuroinflammatory effects of phytocannabinoids may be exerted differentially in male versus female animals and may be linked to age differences. For example, adolescent exposure to THC may exert sex-dependent long-term effects on neuroinflammation, serotoninergic, and cannabinoid systems. Lopez-Rodriguez, Llorente-Berzal, Garcia-Segura, and Viveros (2014) examined the long-term effects of THC and 3,4-methylenedioxymethamphetamine (MDMA) on diverse neuroinflammation and neurotoxic markers in male and female Wistar rats. Rats were chronically treated with increasing doses of THC and/or MDMA during adolescence. The effects of THC and/or MDMA on glial reactivity and on serotoninergic and cannabinoid systems were assessed by immunohistochemistry in the hippocampus and parietal cortex. THC was shown to significantly increase the glial fibrillary acid protein (GFAP), or astrocytic, stained area in both sexes. In males, both drugs either separately or in combination induced a significant increase of the percentage of reactive microglial cells based on staining for the microglial marker-ionized calcium adapter molecule 1(Iba-1, also referred to as allograft inflammatory factor 1). In contrast, in females, each drug, when administered alone produced a significant decrease of this percentage, whereas the combination of both drugs resulted in a "normalization" to control values. In males, MDMA reduced the number of serotonin transporter-positive (SERT+) fibers. THC induced an opposite effect and the group receiving both drugs did not differ significantly from the controls. In females, MDMA reduced the number of SERT+ fibers and the combination of both drugs counteracted this effect. THC induced a significant reduction of the CB1R in females, an effect that was aggravated by the combination with MDMA. The collective results indicated that adolescent exposure to THC and/or MDMA induced long-term, sex-dependent neurochemical

and glial alterations. Furthermore, the results indicated that combination drug exposure may lead to additive or synergistic immunomodulatory effects in the affected host.

6. MARIJUANA AND ASTROCYTES

Astrocytes are the most abundant cells in the CNS and provide biochemical support of endothelial cells that form the BBB, nutrients to nervous tissue, maintenance of extracellular ion balance, and repair of neuronal tissue. Astrocytes are sensitive to factors released from microglia and macrophage-like cells and, in response, elicit inflammatory factors that have the potential to generate a "cytokine storm." Because astrocytes, coexpress CB1R and CB2R (Stella, 2010), they have been considered as candidate targets for therapeutic manipulation of untoward inflammatory responses. However, by virtue of the fact that they express the CB1R, they have limited therapeutic potential for immune modulation using ligands that target the CB1R, the activation of which would engender psychotropic effects. Nevertheless, various investigators have conducted experiments to determine the level to which this receptor plays a functionally relevant role in cannabinoid-mediated modulation of astrocyte activities. Sanchez, Galve-Roperh, Canova, Brachet, and Guzman (1998) reported that THC-induced apoptosis in rat C6.9 glioma cells, as determined by DNA fragmentation and loss of plasma membrane asymmetry. THC stimulated sphingomyelin hydrolysis in these cells. However, THC and *N*-acetylsphingosine, a cell-permeable ceramide analog, induced apoptosis in several transformed neural cells but not in primary astrocytes or neurons. Neither the THC-induced apoptosis nor the THC-induced sphingomyelin breakdown was prevented by the CB1R-selective antagonist SR141716A. The investigators suggested that THC-induced apoptosis in glioma C6.9 cells relied on a CB1R-independent stimulation of sphingomyelin breakdown. Sanchez, Galve-Roperh, Rueda, and Guzman (1998) extended these studies to show that sphingomyelin hydrolysis and the mitogen-activated protein kinase (MAPK) cascade were involved in the THC-induced stimulation of glucose metabolism in primary rat astrocytes. THC increased the rate of glucose oxidation to CO_2 as well as the rate of glucose incorporation into phospholipids and glycogen. These effects of THC were mimicked by HU-210, a synthetic THC analog, and prevented by forskolin, pertussis toxin, and the CB1R antagonist SR141716A. THC did not affect basal cAMP levels but partially antagonized the forskolin-induced elevation of intracellular

cAMP concentration. THC stimulated p42/p44 MAPK activity, Raf-1 phosphorylation, and Raf-1 translocation to the particulate cell fraction. In addition, the MAPK inhibitor PD 098095 and the phosphoinositide 3-kinase inhibitors wortmannin and LY 294002 were able to antagonize the THC-induced stimulation of glucose oxidation to CO_2, phospholipid synthesis, and glycogen synthesis. In addition, the involvement of sphingomyelin breakdown in the metabolic effects of THC was studied. THC produced a rapid stimulation of sphingomyelin hydrolysis that occurred concomitant to an elevation of intracellular ceramide levels. This effect was prevented by the CB1R antagonist SR141716A. Moreover, the cell-permeable ceramide analog D-erythro-*N*-octanoylsphingosine as well as exogenous sphingomyelinase were able to stimulate MAPK activity, to increase the amount of Raf-1 bound to the particulate cell fraction, and to stimulate glucose metabolism. The latter effect was prevented by PD 098059, a selective mitogen-activated protein (MAP) kinase inhibitor, and was not additive to that exerted by THC. The collective results suggested that THC produced a cannabinoid receptor-mediated stimulation of astrocyte metabolism that appeared to rely on sphingomyelin hydrolysis and MAPK stimulation. There is accumulating evidence that THC may exert a wide range of effects on a diverse array of gene products. Bindukumar et al. (2008) undertook genomic and proteomic analysis of the effects of cannabinoids on normal human astrocytes (NHA) to determine the global molecular effects of cannabinoids on NHA using genomic and proteomic analyses. NHA were treated with THC and assayed using gene microarrays and two-dimensional (2D) difference gel electrophoresis coupled with mass spectrometry to elucidate their genomic and proteomic profiles, respectively. The results showed that the expression of more than 20 translated protein gene products from NHA was differentially dysregulated by treatment with THC.

Pre- and perinatal exposure to THC may lead to long-term effects in both neurons and glial cells. Suárez, Bodega, Ramos, Fernandez-Ruiz, and Fernandez (2000) evaluated the responses of neurons and astroglial cells to pre- and perinatal exposure to THC in the substantia nigra (SN) of male and female rats, at three postnatal ages (PD21, PD30, and PD70), by immunohistochemical detection of tyrosine hydroxylase (TH) in dopaminergic neurons and of GFAP in astrocytes. The results showed that the effects of pre- and perinatal exposure to THC on neuronal and astroglial immunoreactivities in the SN (compacta and reticulata) varied with sex, with male rats being more susceptible than females. Prenatal exposure to THC decreased

TH immunoreactivity in the SN of males on PD21 when compared to both their controls and THC-exposed females of the same age. Furthermore, the TH expression decreased with age in THC-exposed males in the SN pars compacta, whereas it increased in controls. In contrast, TH expression was maintained at a stable level in the SN pars compacta of THC-exposed females from PD21. These differences in neuronal development caused by prenatal THC exposure were associated with significant differences in GFAP expression by astroglial cells in both sexes. On PD21, GFAP immunoreactivity decreased in the SN in THC-exposed male rats. Although GFAP expression increased in THC-exposed males with age, it did not reach control levels by PD70. In contrast, significantly increased GFAP expression in THC-exposed females on PD21 was observed, compared to controls and also to THC-exposed male rats. It was suggested that these THC-induced changes in glial development indicated that this cannabinoid accelerated the maturation of astrocytes in female rats, whereas astrocytic maturation was delayed in THC-exposed males. Suárez et al. (2002) also analyzed the responses of cerebellar astroglial cells to pre- and perinatal THC exposure in three postnatal ages and both sexes. To determine whether THC directly modified astroglial growth during development, the effects of this cannabinoid on astroglial morphological changes and on the expression of specific astroglial markers (GFAP and glutamine synthetase: GS) were investigated. It was demonstrated that the administration of THC during development had deleterious effects on astroglial maturation in the cerebellum. These results also confirmed that THC could interfere with astroglial differentiation in a mode that was dependent on sex. The data suggested that pre- and perinatal THC exposure directly interfered with astroglial maturation by disrupting normal cytoskeletal formation, as indicated by the irregular disposition of GFAP and the lower GFAP expression observed at all the ages studied. It was indicated that THC exposure during development could also modulate glutamatergic nervous activity since GS expression was reduced in THC-exposed brains. GS expression increased progressively after THC withdrawal, but this expression failed to reach control values even after 2 months following THC withdrawal. These results suggested that glutamate uptake was lower in glial cells exposed to THC. Furthermore, it was suggested that glutamatergic neurotransmission was affected by THC exposure during gestation and that cannabinoids exerted developmental toxicity, at least on astroglial cells, which could contribute to fetal brain growth retardation. The collective data suggest that prenatal and/or postnatal exposure to THC has potential to hinder maturation of

astrocytes, an outcome that may predispose these cells to compromised responsiveness to neuroinvasive agents.

The effect of CBD on astrocyte function also has been examined. The data that are available suggest that CBD has a general protective effect within the CNS. Thus, CBD may act to counteract the potential deleterious effects of THC on astrocytes. For example, Lafuente et al. (2011) reported that CBD reduced brain damage and improved functional recovery after acute hypoxia-ischemia (HI) in newborn pigs. In their studies, newborn piglets exposed to acute HI received intravenous CBD (HI + CBD) or vehicle (HI + VEH). In HI + VEH, 72 h post-HI brain activity as assessed by amplitude-integrated EEG (aEEG), near-infrared spectroscopy (NIRS) parameters remained lower than normal, and neurobehavioral performance was abnormal. In the brain, there were fewer normal and more pyknotic neurons, while astrocytes were less numerous and swollen. Cerebrospinal fluid concentration of neuronal-specific enolase (NSE) and S100β protein (a multifunctional protein found in large amounts in astrocytes) and brain tissue percentage of TNF-α-positive cells were higher. In contrast, in HI + CBD, aEEG had recovered to 86 ± 5%, NIRS parameters increased, and the neurobehavioral score normalized. HI-induced histological changes, and NSE and S100β concentration and TNF-α-positive cell increases were suppressed by CBD. It was concluded that post-HI administration of CBD-protected neurons and astrocytes, leading to histological, functional, biochemical, and neurobehavioral improvements. Nabissi, Morelli, Santoni, and Santoni (2013) suggested that CBD could have a potential therapeutic application in that triggering of the transient receptor potential vanilloid type 2 (TRPV2) channel by CBD-sensitized glioblastoma multiforme (GBM) cells to cytotoxic chemotherapeutic agents. Several chemotherapeutic agents such as temozolomide (TMZ), carmustine (BCNU), or doxorubicin (DOXO) have been employed for treatment of GBM, but display limited efficacy. Activation of the TRPV2 has been found to inhibit human GBM cell proliferation and overcome BCNU resistance of GBM cells. Thus, the involvement of CBD-induced TRPV2 activation, in the modulation of glioma cell chemosensitivity to TMZ, BCNU, and DOXO was evaluated. It was found that CBD increased TRPV2 expression and activity. CBD, by triggering TRPV2-dependent Ca(2+) influx, increased drug uptake and synergized with cytotoxic agents to induce apoptosis of glioma cells. Moreover, as the pore region of transient receptor potential channels is critical for ion channel permeation, it was demonstrated that deletion of the TRPV2 pore domain inhibited CBD-induced Ca(2+)

influx, drug uptake, and cytotoxic effects. Thus, coadministration of cytotoxic agents together with CBD increased drug uptake and potentiated cytotoxic activity in human glioma cells. These latter observations highlight the fact that distinct chemotherapeutic agents, and by implication cannabinoids, may interact to modulate glial cell functional activities. They also emphasize the difficulty of assignation of a particular mechanistic effect to a specified phytocannabinoid divorced from the milieu presented by other components in marijuana.

7. MARIJUANA AND INFECTIOUS AGENTS THAT TARGET THE CNS

The observation that select phytocannabinoids such as THC and CBD can alter immune function, including that attributed to immune-competent cells resident in the CNS, suggested that these compounds also had the capability to alter immune responsiveness to infectious neurotropic agents. In this context, by ablating the inflammatory response to an invasive microbial agent THC and CBD could place a host at increased risk of infection. The extent to which a phytocannabinoid mediates immune dysfunction may be linked to the nature of the immune response elicited against the invasive agent. This area of phytocannabinoid research has been understudied, principally due to the fact that cannabinoids that have immune modulatory capacity and little psychotropic activity, such as CBD, or selective agonists for the CB2R, have come under scrutiny as potential agents for moderating neuropathological processes that are characterized by hyperinflammatory responses. The data that are available converge on the outcome that THC compromises host resistance to protozoan infections of the CNS and effects migratory responses of CNS-related macrophage-like cells to select components of the human immunodeficiency virus type-1 (HIV-1). Studies by Marciano-Cabral, Ferguson, Bradley, and Cabral (2001) and Cabral and Marciano-Cabral (2004) demonstrated, using a mouse model of Granulomatous Amebic Encephalitis (GAE), that THC exacerbated CNS infection by the opportunistic ameba *Acanthamoeba culbertsoni* (*A. culbertsoni*). Mice administered THC and infected with these amebae exhibited dose-related higher mortalities than infected vehicle controls. The greater severity of disease for THC-treated mice was accompanied by decreased accumulation of macrophage-like cells at focal sites of infection in the brain (Fig. 8.3). Analysis of the migratory capability of macrophage-like cells toward extracellular products secreted by these amebae was inhibited by

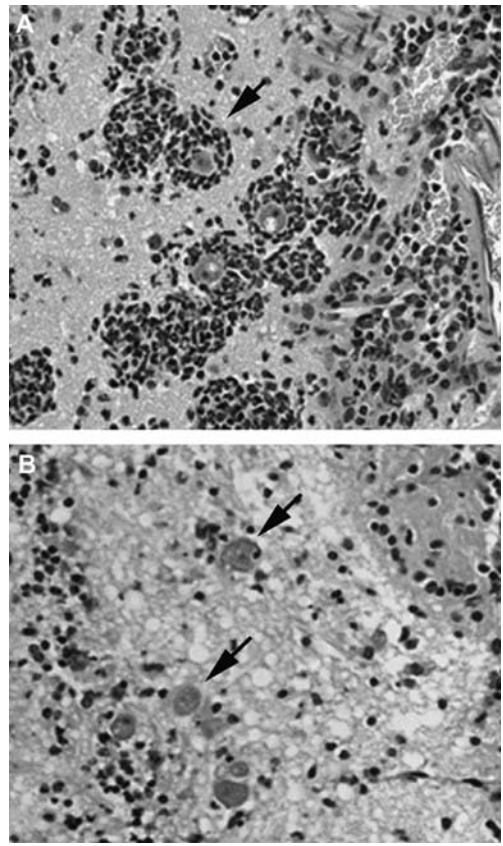

Figure 8.3 THC downregulates accumulation of macrophage-like cells at focal sites of *Acanthamoeba* in mouse brain. $(B_6C_3)F_1$ mice were treated once intraperitoneally with THC (25 mg kg^{-1}) or vehicle (ethanol:emulphor:saline, 1:1:18), inoculated intranasally with three 50% lethal doses (3 LD_{50}) of *A. culbertsoni*. Paraffin sections of brain were stained with hematoxylin and eosin. (A) Section from vehicle-treated mouse depicting accumulation of macrophage-like cells around *Acanthamoeba* (arrow). (B) Section from THC-treated mouse depicting *Acanthamoeba* in the brain in the absence of macrophage-like cell accumulation (arrows). THC, $\triangle^9$-tetrahydrocannabinol. *From Cabral, Raborn, Griffin, Dennis, and Marciano-Cabral (2008).*

THC in a mode (Fig. 8.4) that was found to be linked pharmacologically to the CB2R (Fig. 8.5). In addition, THC administration resulted in decreased levels of mRNA for the proinflammatory cytokines IL-1α, IL-1β, and TNF-α for neonatal rat microglia co-cultured with the *Acanthamoeba*. However, whether these latter events are linked functionally to a cannabinoid receptor has not been resolved. These results indicated a potential for the

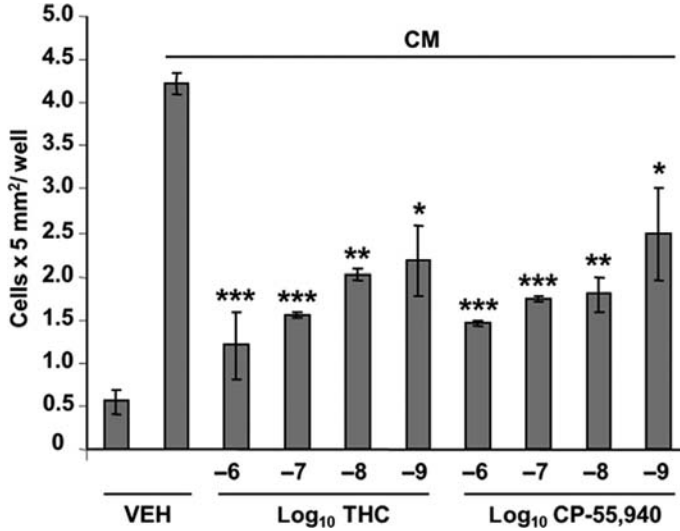

Figure 8.4 THC inhibits chemotaxis of microglia. Microglia were isolated from neonatal Sprague–Dawley rats and purified as described (Waksman, Olson, Carlisle, & Cabral, 1999), treated (3 h) with cannabinoid or vehicle (0.01% ethanol) and assessed (2 h) for migration against *Acanthamoeba*-conditioned medium (CM). The CB1R/CB2R partial agonist THC has a $K_i = 46$ nM at the CB2R, while the potent full agonist CP-55,940 has a $K_i = 0.9$ nM at the CB2R. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $n = 3$/group. CB1R, CB1 receptor; CB2R, CB2 receptor; CM, *Acanthamoeba*-conditioned medium; THC, $\triangle^9$-tetrahydrocannabinol. *Acanthamoeba* were maintained in culture for 24 h to generate the *Acanthamoeba*-conditioned medium (CM) that harbors proteases and other factors released from amebae that serve as chemotactic stimuli for attracting microglia. The full agonist CP-55,940 elicited a concentration-related inhibition of chemotaxis comparable to that of the partial agonist THC. *From Cabral et al. (2008).*

phytocannabinoid THC to dampen the capacity of brain macrophage-like cells to mount a full complement of immune responsiveness to brain infection by these opportunistic amebae. Fraga, Raborn, Ferreira, and Cabral (2011) also reported that cannabinoids altered the responsiveness of microglial-like cells to the transactivating protein Tat of the HIV-1. This protein is an early RNA-binding protein that plays a crucial role in the replication of the HIV-1. At initiation of infection, large amounts of Tat, and other viral-regulatory proteins, are synthesized and released from infected cells (Ensoli et al., 1993). The Tat protein then can translocate across cell membranes and localize in the nucleus of cells where it drives virus replication (Watson & Edwards, 1999). In addition, Tat modulates expression of genes that regulate cellular activities related to survival and growth, inflammation, and angiogenesis (Chang, Gallo, & Ensoli, 1995); promotes chemotaxis and invasive behavior

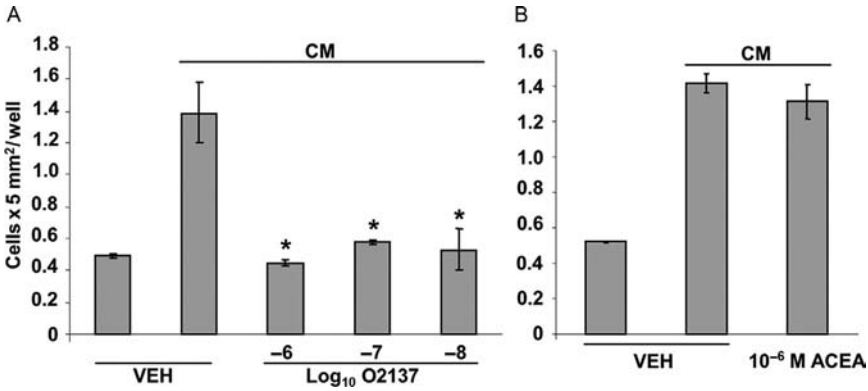

Figure 8.5 Inhibition of chemotaxis of microglia in response to *Acanthamoeba*-conditioned medium (CM) is linked pharmacologically to the CB2R. Microglia were treated (3 h) with cannabinoid or vehicle (0.01% ethanol) and assessed (2 h) for migration against CM. (A) O2137 has greater than 200-fold selectivity for the CB2R: CB1R $K_i = 2700$ nM, CB2R $K_i = 11$ nM; (B) ACEA CB1R $K_i = 1.4$ nM, >1400-fold selectivity over the CB2R. $^*P < 0.05$. $n = 3$/group. ACEA, *N*-(2-chloroethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide; CB1R, CB1 receptor; CB2R, CB2 receptor; CM, ameba-conditioned medium. *From Cabral et al. (2008).*

by monocytes, T-helper lymphocytes, neutrophils, and microglia (Albini et al., 1998; Benelli et al., 1998; de Paulis et al., 2000; Pu et al., 2003); and induces production of chemokines (D'Aversa, Yu, & Berman, 2004) and cytokines (Nath, Conant, Chen, Scott, & Major, 1999; Pu et al., 2003).Tat also harbors a β-chemokine ligand motif articulating a mode by which it recruits uninfected immunocytes to focal areas of HIV infection (de Paulis et al., 2000). Thus, it is not surprising that HIV-1 infection is associated with brain damage (Gendelman et al., 1997) typified by cerebral atrophy, neuronal loss, gliosis, infiltration of inflammatory cells, and microglial activation (New, Maggirwar, Epstein, Dewhurst, & Gelbard, 1998; Orsini, Debouck, Webb, & Lysko, 1996). Fraga et al. (2011) used a mouse BV-2 microglial-like cell model to demonstrate that THC exerted a concentration-related reduction in the migration of BV-2 cells toward Tat. The CB2R antagonist SR144528, but not the CB1R antagonist SR141716A, blocked this inhibition of migration. Similarly, CB2R knockdown with small interfering RNA reversed the cannabinoid-mediated inhibition. In addition, the level of the β-chemokine receptor CCR-3 was reduced and its intracellular localization was altered. These collective results suggested that THC-mediated inhibition of BV-2 microglial-like cell migration to Tat was linked functionally to the CB2R and, in that fashion could ablate HIV-associated neuroinflammatory expansion.

While phytocannabinoids such as THC may have an effect on immune responsiveness *in vitro* to select gene products of the HIV-1, *in vivo* studies that have been conducted to date show no major effect on virus replication and/or associated disease progression. In fact, Molina et al. (2011) reported that THC administration attenuated the progression of simian immunodeficiency virus in the rhesus macaque that is used as a primate model of human HIV infection. Ramirez et al. (2013) implied that cannabinoids that activate the CB2R played a role in limiting HIV-1 infection in the CNS. It was speculated that the prevention of HIV-1 entry into target cells was not a central mechanism for CB2R-mediated suppression in viral replication but that activation of the CB2R was involved in HIV-1 replication. This speculation was supported by results from a single-round infection with a pseudotyped virus that revealed a marked decrease in HIV-1 LTR activation by the CB2R ligands. Williams et al. (2014) reported that THC treatment during human monocyte differentiation reduced macrophage susceptibility to HIV-1 Infection. THC treatment of primary human monocytes during differentiation reduced HIV-1 infection of subsequent macrophages. In contrast, treatment of macrophages with THC immediately prior to, or continuously following, HIV-1 exposure failed to alter infection. Through the use of selective receptor agonists, it was indicated that the THC effect during monocyte differentiation was mediated primarily through the CB2R. Based on these data, it was indicated that THC suppressed HIV-1 infection by a reduction in cell surface HIV receptor expression that diminished efficiency of entry.

Cells of monocyte/macrophage lineage are prime targets for infection with the HIV-1 and serve as replication-competent hosts for the HIV-1. In this capacity they act as "carriers" of virus to distal sites, and upon activation play a central role in dissolution of the BBB leading to expansion of disease into the CNS (Gendelman et al., 2012). HIV-infected monocytes/macrophages produce virus, release virus-specified gene products such as the transactivating protein Tat and the major envelope glycoprotein gp120, and emit a plethora of cell-specified inflammatory products such as NO, chemokines, and cytokines (Ensoli et al., 1993; Schneider, Kaaden, Copeland, Oroszlan, & Hunsmann, 1986; Zagury et al., 1998; reviewed in Haughey & Mattson, 2002; Li, Lee, Cheung, & Lau, 2005; Nath et al., 1999; Yeh et al., 2000; reviewed in Lee et al., 2003). These products also serve as chemotactic and/or inflammatory stimuli that engender inflammatory responses from bystander uninfected immunocytes, the concerted action of which results in breakdown of the BBB, enhancement of

trafficking of immunocytes into the CNS, and expansion of the CNS inflammatory process (Mitola et al., 1997; Vené, Benelli, Noonan, & Albini, 2000; reviewed in Pugliese, Vidotto, Beltramo, & Torre, 2005). Indeed, it has been postulated that HIV-associated neuropathogenesis is due mainly to effects of cytokines and neurotoxins that are produced by activated monocytes, perivascular macrophages, and microglia in the CNS rather than to direct cytotoxic effects of the HIV (Genis et al., 1992; Nath et al., 2000; Wesselingh et al., 1993; Williams, Turchan, Lu, Nath, & Drachman, 2005; reviewed in Mattson, Haughey, & Nath, 2005; van de Bovenkamp, Nottet, & Pereira, 2002). These inflammatory events are salient in the post highly active antiretroviral therapy (HAART) era and have been attributed mostly to Tat, which is not targeted by protease inhibitor and deoxynucleoside analog composites of HAART, and/or to gp120 that is emitted from cells that serve as virus reservoirs (reviewed in Nath & Sacktor, 2006; Rumbaugh et al., 2006; reviewed in Navia & Rostasy, 2005; reviewed in Anthony & Bell, 2008; reviewed in Alexaki, Liu, & Wigdahl, 2008). Thus, phytocannabinoid dampening of hyperinflammatory responses that are associated with select neuropathological processes such as those engendered by HIV could prove beneficial to the human host. On the other hand, phytocannabinoid exposure could prove detrimental to the host by inhibiting macrophage-like cell migration to, and subsequent sequestration of, a pathogen such as *A. culbertsoni*. In this context, the nature of neuroimmune response to the invasive agent may predicate whether a phytocannabinoid plays a role in mediating disease progression. Nevertheless, the study of the effects of marijuana or its major phytocannabinoid components on the neuroimmune response to infectious agents is in its infancy.

8. SUMMARY AND FUTURE PROSPECTIVES

Marijuana is a complex substance that harbors a group of terpenoid-like compounds known as phytocannabinoids, of which the psychoactive compound THC and the nonpsychoactive compound CBD have been the most studied. These phytocannabinoids have been shown to alter the functional activities of a variety of immune cells at peripheral sites and in the CNS. While there is a large body of data from animal models that the effects of THC on immunity are linked to decreased resistance to infectious agents, a comparable linkage in humans has yet to be obtained, especially at least as relates to long-terms effects. A major confound in resolving this issue is that individuals who use marijuana in a recreational mode also partake of

other substances that have immune-suppressing potential. On the other hand, individuals who use marijuana, or select cannabinoid formulations, for therapeutic purposes already have underlying health conditions that may render them immune compromised or susceptible to infection. Finally, the presence of CBD in marijuana may counteract the effects of THC and temper the overall immune dysfunctional outcome *in vitro* and in experimental animals. In this context, the effect of THC on a given immune functional system may depend on the marijuana formulation and its included concentration of THC. Many of the activities of THC are mediated through activation of cannabinoid receptors. The CB1R appears to be functionally relevant for the overall homeostatic balance and regulation of the CNS (Marsicano et al., 2003; reviewed in Cota, 2007). It has been suggested that the CB1R has potential as a molecular target for therapeutic attenuation of cognitive impairment and degeneration in select CNS disorders (Pryce et al., 2003; Pryce & Baker, 2007; Shen & Thayer, 1998). However, a drawback to this consideration is the recognition that activation of the CB1R also engenders psychotropic effects. On the other hand, while many neuropathogenic processes are characterized by progressive decline in cognitive functions, a major hallmark of CNS pathologies is inflammation. Since the CB2R has been linked to the modulation of immune responses, and its expression by macrophages and microglia appears to be upregulated in response to various stimuli (Carlisle et al., 2002), this receptor has potential to serve as a selective molecular target for ablating untoward inflammatory responses. Finally, the body of scientific data that is available suggests that THC has a major effect on macrophage-like cell migratory activity. This recognition may provide a rationale for distinctive THC-mediated effects on the responsiveness of macrophage-like cells to select pathogens that invade the CNS. For example, microglia play a critical role in sequestering invasive opportunistic amebae such as *A. culbertsoni* within the CNS. THC-mediated inhibition of the capacity of microglia to migrate to nodal sites of infection could compromise this process of sequestration and contribute to dissemination of amebae. On the other hand, microglia serve as replication-competent hosts for certain viruses such as the HIV-1. In this context, inhibition of microglial migration to sites of HIV infection within the CNS could hinder dissemination of the virus. Thus, the functional consequences of THC exposure on immune responsiveness within the CNS to a specified pathogen may be linked to the fundamental nature of the host–pathogen interaction. Resolution of these potential distinctive differences requires further investigation.

Finally, the question of whether select cannabinoids, including those that are native to marijuana, alters immune homeostatic balance within the CNS remains unresolved. This issue is particularly salient since use of marijuana may place a human at increased risk of infection with neurotropic infectious agents. The data that are available suggest that casual use of marijuana is not associated with overt perturbations of cellular and humoral immunity in humans. However, habitual marijuana smokers could experience a different outcome. Furthermore, comparatively little is known about the effects of marijuana or its included phytocannabinoids on immune responsiveness within the CNS. Infectious agents may engender disparate immune responses within different compartments that may be selectively responsive to the action of cannabinoids. Such a distinction may be manifest in terms of responsiveness to infection with an opportunistic ameba such as *A. culbertsoni* versus a viral agent such as the HIV-1. While it is becoming apparent that these infectious agents engender distinctive cellular and soluble factor responses within the CNS, a potential commonality of action on the part of cannabinoids is that they target cell migratory responses that may be linked to these agents rather than targeting replication of the agent. In addition, there is increasing recognition that cannabinoid exposure may exert long-term effects on immune responsiveness and that these may be linked to sex differences or developmental age. For example, Lombard, Hegde, Nagarkatti, and Nagarkatti (2011) reported that perinatal exposure to THC had a profound effect on the mouse fetus as evidenced by a decrease in thymic cellularity on gestational days 16, 17, and 18 and postgestational day 1 and marked alterations in T cell subpopulations. These outcomes were reversed by CB1R/CB2R antagonists, suggesting that the THC-mediated effects were due to activation of cannabinoid receptors. Thymic atrophy induced in the fetus correlated with caspase-dependent apoptosis in thymocytes. Thymic atrophy was the result of direct action of THC and not based on maternal factors since THC was able to induce T cell apoptosis *in vitro* in fetal thymic organ cultures. These results indicated that mouse perinatal exposure to THC-triggered T cell dysfunction, suggesting translationally that the offspring of marijuana abusers who have been exposed to THC *in utero* could be at a higher risk of exhibiting immune dysfunction and contracting infectious diseases. In addition, Moretti et al. (2014) reported recently that THC exposure has an impact on the immune system during adolescence. THC exposure during adolescence triggered immune dysfunction that lasted long after the end of exposure to this cannabinoid, a dysfunction that was characterized by induction of a proinflammatory macrophage

phenotype. This dysfunction was characterized by a decrease in levels of both the Th1 and Th2 cytokines, indicating a general immune dysregulatory rather than one directed directly against Th1/Th2 bias. It was speculated that exposure to THC at a young age could place the affected individual at higher risk of developing autoimmune or chronic inflammatory disease. The recognition that phytocannabinoids can exert developmental and long-term effects on the immune system suggests that exposure to these substances, or marijuana, during an early stage in life has the capacity to alter host resistance to select microbial agents in the adult.

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