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Use of Cannabinoids as a Novel Therapeutic Modality Against Autoimmune Hepatitis

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Abstract

Autoimmune hepatitis is a severe immune mediated chronic liver disease with a prevalence range between 50 and 200 cases per million in Western Europe and North America and mortality rates of up to 80% in untreated patients. The induction of CB1 and CB2 cannabinoid receptors during liver injury and the potential involvement of endocannabinoids in the regulation of this process have sparked significant interest in further evaluating the role of cannabinoid systems during hepatic disease. Cannabinoids have been shown to possess significant immunosuppressive and anti-inflammatory properties. Cannabinoid abuse has been shown to exacerbate liver fibrogenesis in patients with chronic hepatitis C infection involving CB1 receptor. Nonetheless, CB2 receptor activation may play a protective role during chronic liver diseases. Thus, differential targeting of cannabinoid receptors may provide novel therapeutic modality against autoimmune hepatitis. In this review, we summarize current knowledge on the role of endocannabinoids and exocannabinoids in the regulation of autoimmune hepatitis.

I. INTRODUCTION

Medicinal properties of *Cannabis sativa,* Marijuana have been explored for centuries. Interest in the potential medicinal use of cannabinoids grew following the isolation and characterization of delta-9-tetrahydrocannabinol (THC) as the major psychoactive component in marijuana (Gaoni and Mechoulam, 1971) and subsequently the discovery of two cannabinoid receptors, CB1 and CB2 (Matsuda *et al.,* 1990; Munro *et al.,* 1993). Cannabinoids have been used as potential therapeutic agents in alleviating such complications as intraocular pressure in glaucoma, cachexia, nausea, and pain (Watson *et al.,* 2000). Nonetheless, the clinical use of Marijuana cannabinoids has been controversial due to their psychotropic effects and potential abuse. To date, over 60 other unique phytocannabinoids have been identified from the marijuana plant, generating renewed interest in its medicinal potential.

It is well established now that cannabis acts primarily on mammalian tissue through at least two specific cannabinoid receptor subtypes which are both G protein-coupled membrane receptors. Both the receptors share little sequence homology, only 44% at the protein level

or 68% in the transmembrane domains which are thought to contain the binding sites for cannabinoids (Howlett, 2002; Lutz, 2002). The CB1 receptor is found in abundance in the brain areas which control motor activity, memory, cognition, emotions, endocrine functions, and sensory perception. It is also expressed in peripheral nerve terminals and other sites such as eye, spleen, vascular endothelium, adipose tissue, gut, liver, and testis. Its activation results in central and many peripheral effects of cannabinoids by modulating the neurotransmitter release. The CB2 receptor is primarily expressed on subsets on immune cells and several leukocyte lines of hematopoietic systems (macrophages, both B andT lymphocytes), secondary lymphoid tissues such as spleen, tonsils, Peyer's patches, Lymphatic ganglia, microglia and hepatic myofibroblastic cells (Gong *et al.,* 2006; Munro *et al.,* 1993). The precise function of CB2 receptors on immune cells is not clear. However, studies from our laboratory demonstrated for the first time that activation of CB2 can induce apoptosis in immune cells, including T-cells and dendritic cells (McKallip *et al.,* 2002b). Other studies have shown that activation of CB2 may alter the cytokine profile and promote the differentiation of Th2 cells (Roth *et al.,* 2002). Intracellular CB 1 and CB2 dependent signaling pathways include $G_{i/0}$ -dependent inhibition of adenylyl cyclase, PI3-kinase, MAP kinase and FAK pathways, and modulation of calcium and potassium channels. The discovery of expression of cannabinoid receptors in vertebrate body was followed by rapid discovery of naturally occurring endogenous ligands for these receptors. It is, however, noteworthy that endocannabinoids may also bind to vanilloid receptors and peroxisome-proliferators activated receptors or can also exhibit receptor—independent properties due to their high lipophili-city (Batkai *et al.,* 2001; Maccarrone and Finazzi-Agro, 2003).

II. The Endogenous Cannabinoid System

The discovery of cannabinoid receptors occurring naturally throughout the vertebrate body and the availability of highly selective and potent canabimimetics led to identification of a naturally occurring lipid signaling system termed as the endocannabinoid system. This comprises of a family of lipid transmitters serving as natural ligands for the cannabinoid receptors. The first endocannabinoid discovered was named as anandamide (AEA) from the Sanskrit “internal bliss” (Devane *et al.,* 1992). AEA is an amide of arachidoic acid and ethanolamine. It binds to brain cannabinoid receptor with high affinity and mimics the behavioral actions ofTHC when injected into rodents. Subsequently, 3 years later, another ligand named 2-arachidinoiylethanolamide (2-AG) was discovered independently by Mechoulam *et al.* (1995) and Sugiura *et al.* (1995), which was found to be in much higher concentration in serum and brain than anandamide. This triggered an exponential growth of studies describing the endocannabinoid synthesis, their release (Di Marzo *et al.,* 1994), transport (Beltramo *et al.,* 1997) and their degradation (Cravatt *et al.,* 1996) constituting the new ubiquitous “endogenous cannabinoid system.”

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

III. The Biosynthesis of Endocannabinoids

Endocannabinoids are not stored in vesicles or cells like neurotransmitters; rather their biosynthesis takes place on demand from lipid precursors in the cytoplasmic membrane through enzyme activation in response to elevations of intracellular calcium. They are produced by receptor-stimulated cleavage of membrane lipid precursors and then released from cells. Anandamide is the product of arachydonate and phosphoethanolamide. Arachydo-nate is released from cytoplasmic membrane through enzymatic cleavage of N-arachydonoyl phophatidiethanolamide by a Ca^{++} dependent N-acyl transferase (Schmid *et al.*, 1983). 2-AG is produced by hydrolysis of a diacyglyceride containing arachdonate in glycerol position2 released by a specific diacylglycerol lipase (Lichtman *et al.*, 2002). The endocannabinoids are then removed from the site by cellular uptake processes such as simple diffusion, through membrane associated binding proteins or by a transmembrane carrier protein. Inside the tissue, their metabolism is catalyzed by fatty acid amide hydrolase (FAAH) (Kozak and Marnett, 2002; Lichtman *et al.*, 2002) which is located on cytosolic surfaces ofSER cisternae and mitochondria. In some tissues, however, endocannabinoids also suffer an oxidative catabolism through lipoxygenases, cyclooxygenase-2 and cytochrome P450 (Kozak and Marnett, 2002), and palmitoylethanolamide-preferring acid amidase (PAA) (Goparaju *et al.*, 1999).

IV. Endocannabinoid System is Autoprotective

It certain disorders such as multiple sclerosis, cancer, intestinal disorder, cardiovascular disorder, pain, Parkinson's disease and excitotoxicity, the tissue concentration of endocannabinoids, cannabinoid receptor density and the cannabinoid receptor coupling efficiency increases resulting in reduction of symptoms of these disorders. The endocannabinoid system has been shown to be involved in various physiological processes like lipogenesis, inflammation, food intake, and nociception (DiMarzo *et al.*, 2004).

A. The endocannabinoid system and pathophysiology of liver diseases

There is increasing evidence suggesting the role of endocannabinoids in the pathophysiology ofliver diseases such as in hepatic injury and as a mediator of complications of cirrhosis. The normal adult liver hepatocytes and nonparenchymal cells have been found to produce low levels of endocannabinoids (Julien *et al.*, 2005; Teixeira-Clerc *et al.*, 2006). This can be attributed to presence ofhigh hepatocellular expression ofFAAH responsible for AEA degradation (Cravatt *et al.*, 1996). Hepatic and serum levels of AEA increase during acute hepatitis and fatty liver disease (Batkai *et al.*, 2007; Biswas *et al.*, 2003). In fatty liver, this increase in AEA was attributed to a decrease in AEA degradation by FAAH.

Although embryonic liver has been shown to express CB2 receptor mRNA, adult liver hepatocytes and endothelial cells display a faint physiological level of expression of CB1 receptors. However, both CB1 and CB2 receptors are upregulated in early stages of liver injury (Batkai *et al.*, 2007; Biecker *et al.*, 2004; Julien *et al.*, 2005; Schwabe and Siegmund, 2005; Teixeira-Clerc *et al.*, 2006). CB1 receptors have been found to be upregulated in the vascular endothelium and in myofibroblasts located in fibrotic bands of cirrhotic livers in humans and rodents (Teixeira-Clerc *et al.*, 2006). CB2 receptors are also expressed in

myofibroblasts and in inflammatory cells and biliary epithelial cells (Julien *et al.*, 2005). Thus, induction of endocannabinoids and their receptors are potentially two mechanisms rendering liver responsive to endocannabinoids in course of liver fibrogenesis.

Nonetheless, many recent studies indicate strongly the increased upregulation of the endocannabinoid system during liver diseases affecting hepatocyte injury, inflammation, fibrogenesis, hepatic encephalopathy, cirrhotic cardiomyopathy, and portal hypertension.

Liver injury is associated with Kupffer cell activation, release of proinflammatory cytokines and generation of reactive oxygen species leading to infiltration of the liver by activated polymorphonuclear leukocytes. In a recent study, hepatic ischemia-reperfusion (1/R) injury was shown to be associated with dramatic induction of hepatic expression of anadamide and 2-AG directly related to extent of liver damage (Batkai *et al.*, 2007). The data also points out the protective role of CB2 receptor activation in inflammatory response related with chronic liver diseases such as viral hepatitis and alcoholic or nonalcoholic fatty liver diseases.

Similarly CB1 and CB2 receptors are also markedly upregulated in cirrhotic human liver samples demonstrating the impact of endocannabinoid in liver fibrogenesis. Elevated circulating levels of anadamide and of hepatic 2-AG in cirrhosis and liver fibrosis, respectively, have been consistently reported (Batkai *et al.*, 2001). The role of CB2 receptors in mediating antifibrogenic response has been corroborated using CB2 knockout mice which when exposed to CC14 showed enhanced liver fibrosis and increased liver fibrogenic cell accumulation as compared to their wild type counterparts (Julien *et al.*, 2005). The role of CB2 was corroborated *in vitro* showing that CB2 receptor activation induces apoptosis in cultured liver fibrogenic cells by activation of oxidative stress (Julien *et al.*, 2005). However, CB1 receptors were found to signal profibrotic response (Teixeira-Clerc *et al.*, 2006) depicting that CB1 and CB2 receptors exert opposite effects on liver fibrosis and further suggesting that endocannabinoid system regulates both pro- and antifibrogenic responses in the liver.

In an analysis carried out on patients with chronic hepatitis C, it was observed that cannabis used over the span of the disease was an independent predictor offibrosis severity (Hezode *et al.*, 2005) indicating that cannabinoids may exacerbate liver fibrogenesis and thus CB1 antagonists may play as antifibrosing molecules. It should be noted that CB2 is expressed at higher density on monocytes and the immunosuppression exerted through CB2 may have an effect in patients with hepatitis C because such patients require an intact immune component to keep hepatitis in check. Thus, chronic marijuana consumption may promote fibrogenesis via CB2 mediated suppression of antiviral immunity (Schwabe and Siegmund, 2005).

Studies have also been carried out to evaluate the role of endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis. Reports indicate that endocannabinoids trigger vasorelaxing effects and an upregulated CB1-dependent cannabinoid tone causes enhanced mesenteric vasodialation leading to portal hypertension (Batkai *et al.*, 2001; Ros *et al.*, 2002).

B. Anti-inflammatory effects of endocannabinoids

Hepatic inflammation is linked to hepatic fibrosis in models of fibrogenesis. Kupffer cells are crucial and promote activation of hepatic stellate cells (HSCs). Activated T lymphocytes and neutrophils also contribute to inflammatory microenvironment leading to HSC activation and fibrogenesis. Kupffer cells are shown to express high CB2 mRNA which is known to mediate anti-inflammatory effect by suppression of TNF-α and IFN-γ and stimulate anti-inflammatory cytokines such as IL-l 0 and also inhibit macrophage migration at sites of inflammation leading to anti-fibrogenic effects of endocannabinoids in the liver. The selective CB2 receptor agonists JWH133 and HU-308 have also been shown to decrease TNF-α, ICAM-1, and VCAM-1 expression in human liver sinusoidal endothelial cells (HLSECs) expressing CB2 receptors and thus decreased adhesion of human neutrophils to HLSECs, thus depicting a role for CB2 receptor in endothelial cell activation and endothelial-inflammatory cell interactions (Rajesh *et al.*, 2008).

V. Autoimmune Hepatitis

Autoimmune liver disease (ALD) includes a spectrum of diseases which comprises both cholestatic and hepatitic forms: autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and the so-called “overlap” syndromes where hepatitic and cholestatic damage coexist. All these diseases are characterized by an extremely high heterogeneity of presentation, varying from asymptomatic, acute (as in a subset of AIH) or chronic (with a specific symptoms such as fatigue and myalgia in AIH or fatigue and pruritus in PBC and PSC). The incidence and prevalence of the forms of ALDs remain to be defined worldwide, although information is available for distinct ethnic populations (Berdal *et al.*, 1998). The prevalence of ALD ranges from 13 to 20/100,000 population and the incidence from 1 to *21100,000* in a Norwegian population (Boberg *et al.*, 1998).

AIH comprises a relatively diverse group ofliver diseases associated with autoantibody formation which are thought to occur as a result of an uncontrolled, self-directed inflammatory attack on hepatocytes or bile ducts. The immune injury commonly leads to rapidly progressive liver disease, progressive fibrosis, and ultimately cirrhosis. AIH occurs mainly in women and is characterized by elevated serum transaminase activity, hypergammaglobulinemia, circulating positive organ and nonorgan autoantibodies, and morphologic changes of interface hepatitis on liver biopsy. The prevalence of AIH is estimated to range between 50 and 200 cases per million in North America and accounts for 5-9% of liver transplantation in United States. Mortality rates of up to 80% have been reported in untreated patients who presented with greater than fivefold elevations in serum aspartate (AST) or alanine (ALT) aminotransferase activities (Soloway *et al.*, 1972). The cause of ALD is not known, but factors that influence the development of clinical disease include genetic predisposition, prior liver injury caused by viruses, environmental exposure to toxins, and possibly, ongoing infectious factors, including viruses. Immune reactions against host liver antigens are believed to be the major pathogenic mechanisms.

Infectious causes of ALD have been proposed, with prior viral hepatitis A, B, or C, cytomegalovirus (CMV), paramyxovirus, and most recently retrovirus infections, suggested

as possibilities (Manns and Obermayer-Straub, 1997; Mason *et al.*, 1998). The development of recurrent AIH, PBC, and PSC in liver transplant recipients provides strong support for an infectious origin for of these disorders. If environmental agents are found to be the cause or a component of disease activity and progression, advances in antibiotic and antiviral therapies may also delay or prevent progressive liver disease.

The standard therapy for AIH involves treatment with prednisone or prednisolone (40/60 mg/d) alone or a lower steroid dose (20–30 mg/d) in combination with azathioprine (1 mg/kg/d) (Murray-Lyon *et al.*, 1973). Corticosteroids and azathioprine also represent the standard therapy for AIH; both compliance and treatment outcome can be monitored by transaminase serum levels; a slow tapering of the immunosuppressive therapy is always recommended to avoid the relapse of the disease, frequently observed in cases of too fast tapering. Corticosteroids bind to corticosteroid receptor elements in the promoter regions of numerous steroid responsive genes thereby affecting transcription. They act rapidly on the immune system mainly by affecting the cytokine production and inhibition of T lymphocyte activation (Bellary *et al.*, 1995). Azathioprine acts as immunosuppressive drug by blocking the maturation oflymphocyte precursors and requires 3 months or more to be fully effective.

VI. Treatment Drawbacks

The common side effects of corticosteroids include fluid retention, obesity, diabetes mellitus, osteoporosis, psychiatric disturbances, cataracts (Beswick *et al.*, 1985; Biagini *et al.*, 1991). Azathioprine treatments can cause nausea, pancreatitis, hepatotoxicity, and bone marrow suppression (Beswick *et al.*, 1985). Additionally, about 15% patients are unresponsive to this standard therapy. Liver transplantation remains the only therapeutical approach for the end stage of liver disease with an actual survival rate over 60% after 10 years.

VII. Cannabinoid/Endocannabinoid System in Hepatitis

Cannabinoids which act as cannabinoid receptor agonists, including THC, have shown promising results in many models of inflammation (Hegde *et al.*, 2008; Pertwee, 2002; Quartilho *ct al.*, 2003). Cannabinoid compounds have been shown to possess significant immunosuppressive and anti-inflammatory properties (Croxford and Yamarnura, 2005). The histo-logical picture of AIH with its striking infiltrate of lymphocytes, plasma cells, and macrophages was the first to suggest an autoaggressive cellular immune attack in the pathogenesis of AIH. Whatever is the initial trigger, this massive recruitment of activated inflammatory cells causes damage. The T lymphocyes have been identified as the predominant cells in immunohistochemical studies.

Recently our lab has reported that both exogenous and endogenous cannabinoids can attenuate concanavalin A-induced acute hepatitis (a well-established model for viral or AIH in which liver injury is T -cell-mediated). TNF-α:, IFN-γ, and IL-2 play crucial roles in Con-induced hepatitis (Ganter *et al.*, 1995; Tagawa *et al.*, 1997). We demonstrated that a single intraperitoneal injection of THC (a *CB* 1 and CB2 receptor agonist) and anandamide (an endocannabinoid, partial agonist of CB1 and CB2 receptors) can ameliorate ConA-induced hepatitis via a negative inflammatory cytokine regulation (IL-2, TNF-α:, IFN-γ,

IL-1α:, IL-1β, IL-5, IL-6, IL-10, IL-17, GM-CSF, G-CSF, KC, MIP-1α:, and RANTES) in mice and by upregulating forkhead helix transcription factor p3(Foxp3)+ regulatory T-cells (Hegde *et al.*, 2008).

An impairment of immunoregulatory mechanisms, which would enable the autoimmune response to develop, has been repeatedly reported in the setting of both human and experimental autoimmunity. In early studies it was shown that patients with AIH have low levels of circulating T -cells (Lobo-Yeo *et al.*, 1987) and impaired suppressor cell function. Recent experimental evidence confirms an impairment of the immunoregulatory function in AIH. Thus, among recently defined T -cell subsets, with potential immunosuppressive function, $CD4^+$ T -cells constitutively expressing the interleukin two receptor α chain (CD25) (T -regulatory cells, T -regs) have emerged as a dominant immunoregulatory lymphocyte (Shevach *et al.*, 2003). In addition to CD25, which is also present on activated T -cells, T -regs express a number of additional markers such as the glucocorticoid-induced tumor necrosis factor receptor, CD62L, the cytotoxic T lymphocyte associated protein-4 (CTLA-4) and the forkhead/winged helix transcription factor FOXP3, the expression of which is closely associated with the acquisition of regulatory properties. In patients with AIH, T -regs are defective both in number and function compared to normal controls and these abnormalities relate to the stage of disease, being more evident at diagnosis than during drug induced remission (Longhi *et al.*, 2005, 2006). The percentage of T -regs inversely correlates with markers of disease severity, such as levels of antibodies to antisoluble liver antigen (Ma *et al.*, 2002), and anti-LKM-1 autoantibody titers, suggesting that a reduction in T -regs favors the serological manifestations of ALD. If loss of immunoregulation was central to the pathogenesis of ALDe, treatment should concentrate on restoring T -regs ability to expand, with consequent increase in their number and function. This is at least partially achieved by standard immunosuppression, since T -regs numbers do increase during remission (Longhi *et al.*, 2005). With reference to the reports stating that T regulatory cells play a crucial role in natural tolerance in ConA-induced hepatitis (Erhardt *et al.*, 2007), we observed that THC treatment significantly enhanced T regulatory cells over vehicle controls which may be considered as a critical factor to reduce the severity of hepatitis. Decreased IL-6 levels observed in $ConA^+$ THC-injected mice may be contributing to suppression of effector T -cell function by T -regs. We found that THC treatment in ConA-injected mice triggered a significant increase in the number of $CD4^+$ $CD25^+$ T -regs in the liver that was responsible for decreased hepatitis (Hegde *et al.*, 2008). We also analyzed Foxp3 mRNA expression by reverse transcription-PCR in splenocyte and liver cells which were obtained 4 h after ConA challenge *in vivo.* We found that ConA + TH C treated mice had higher levels of Foxp3 mRNA in the liver and spleen. Mice injected with ConA and pretreated with anti-CD25 antibodies (which effectively depleted $CD25^+$ cells) showed higher serum AST levels compared with mice pretreated with control IgG suggesting that regulatory T -cells are required for THC-mediated suppression of hepatitis. It is important to note that $CD25^+$ cell-depleted mice showed increased AST levels after ConA injection when compared to naive mice. One possible explanation for this would be that depletion of T -regs resulted in uncontrolled hepatotoxicity upon polyclonal activation of T -cells and other inflammatory cells by ConA. This supports the recent observation that T -regs mediate tolerance in ConA-induced hepatitis model (Erhardt *et al.*, :2007). Previous

studies have shown that THC suppresses cytokine secretion by its direct action on lymphocytes (Blanchard *et al.*, 1986; Klein *et al.*, 2000). In the current model, THC may be acting directly on T -cells and also by enhancing T -reg function in the presence of ConA to suppress cytokine production and disease attenuation. Suppression of M-CSF, G-CSF, RANTES, KC, and MIP-lα can be considered important to decrease chemotaxis and activation of macrophages and eosinophils which is associated with ConA-induced hepatitis (Louis *et al.*, 2002; Bonder *et al.*, 2004).

Circulating T -cells specific for liver autoantigens is found in normal subjects. However, in AIH, their frequency is at least 10-fold higher (Wen *et al.*, 2001). This finding suggests that the pool of autoreactive T -cells undergoes a significant expansion in patients with AIH and hence may be involved in the initiation and perpetuation of the immune attack to the liver. Previous studies showed that activated T- and NK T -cells are increased in liver after ConA challenge. However, these cells rapidly undergo apoptosis, which may be crucial for mice to recover from ConA-induced hepatitis (Russell, 1995). We have noted previously that THC induces apoptosis in thymic and splenic T -cells (McKallip *et al.*, 2002b). Thus, we also investigated the frequency of hepatic T lymphocytes undergoing apoptosis which we found was increased in ConA-injected mice upon THC treatment (Hegde *et al.*, 2008). Nonetheless, absolute numbers of hepatic T -cells in THC-treated hepatitis-induced mice were significantly higher. This may result from induction of T -regs, as well as, possible increased migration ofT -cells into the liver. Interestingly, we also noted that IL-2 was not decreased upon THC treatment, and it may be contributing to proliferation of T -cells. However, T -cells were functionally suppressed by THC as indicated by decreased serum cytokine levels and liver injury. This could be due to direct suppressive effect ofTHC on activated T -cells as well as an increase in $CD4^+$ $Foxp3^+$ T -regs. IL-2 is an essential growth and survival factor for T -regs, and it is required for their function (Furtado *et al.*, 2002). Sufficient levels ofiL-2 observed in ConA-injected mice treated with THC or anandamide could contribute to increased T -reg function, resulting in significant suppression of inflammatory cytokines.

Because THC acts through both CB1 and CB2 receptors, we also tested CB1/CB2 mixed agonists (CP55,940 and WIN55212) and found that these compounds were also effective to suppress ConA-induced hepatitis (Hegde *et al.*, 2008). Interestingly, we found that CB1 or CB2 activation alone had no anti-inflammatory effect on hepatitis. However, cannabinoids that bind to both CB1 and CB2 receptors (THC, CP55,940, WIN55212, and anandamide) effectively attenuated hepatitis. That CB1/CB2 mixed agonists could suppress the pathogenesis but not the coadministered CB1 and CB2 agonists indicated that both cannabinoid receptors need to be activated simultaneously to produce the observed effect and that different pharmacokinetics of the two coadministered agonists may not allow this to happen. Signaling through both receptors is important because blocking either CB1 or CB2 could reverse the effect of THC. We also noted that activation through CB1 *per se* worsened the effect of ConA, which is in agreement with previous studies showing that CB1 may contribute toward liver inflammatory disorders (Teixeira-Clerc *et al.*, 2006). However, activation of both CB1 and CB2 not only prevented the worsening effect of CB1 stimulation alone, but also resulted in a strong counteraction of inflammation. The need for

simultaneous activation of CB1 and CB2 to see the beneficial effect is consistent with some previous observations (Van Sickle *et al.,* 2005). However, these results are contradictory to previous understanding that anti-inflammatory properties of cannabinoids are mainly mediated by CB2. The finding that hepatocytes (Batkai *et al.,* 2007; Biswas *et al.,* 2003) and dendritic cells (Do *et al.,* 2004; Matias *et al.,* 2002) express CB1 and CB2 receptors may explain the contribution of both receptors toward protection observed in this study. To further confirm the role of both cannabinoid receptors, we blocked either of these receptors by pretreating mice with CB1 (AM251) or CB2 (SR144258) antagonists before injecting $ConA^{+}$ THC and observed that blocking of either receptor was sufficient to reverse the effects ofTHC (Hegde *et al.,* 2008). These data demonstrated that immunomodulatory function ofTHC in ConA-induced hepatitis is mediated by signaling through CB1 and CB2 receptors, and blocking either of these receptors was sufficient to reverse the effects ofTHC.

Similar to our findings with THC, exogenously administered anandamide in the hepatitis model also caused a decrease in hepatic injury which correlated with decreased AST and AL T levels, and inflammatory cytokines. We also observed that 12 h after anadamide treatment, in ConA injected mice, there was a significant decrease in inflammatory cytokines TNF-α, IL-1β, IL-6, IL-9, and IL-17 and in chemokines KC, eotaxin and monocyte chemoattractant protein-1. Blocking of CB1 or CB2 receptors reversed the anandamide-mediated suppression of hepatitis thereby indicating that anandamide was acting in a CB1 and CB2 dependent manner. Anandamide levels are elevated in the absence ofF AAH activity (Cravatt *et al.,* 1996). Mice which lacked FAAH enzyme (responsible for hydrolysis of anandamide) were found to be resistant to ConA-induced hepatitis and showed less severe liver tissue damage and less leukocyte infiltration (Hegde *et al.,* 2008). Similarly, increased endogenous anandamide levels caused by administering mice with FAAH inhibitors MAFP or URB532 also decreased hepatic injury with significant decrease in AST levels upon ConA challenge. These findings are exciting and suggest the potential manipulation of endocannabinoids, including the use of FAAH inhibitors in the treatment of AIH.

A. Controversies on the beneficial and deleterious roles of cannabinoid receptors in the regulation of liver disease

As seen from the discussion above, the role of endocannabinoid system in hepatitis is somewhat controversial. On one hand, human studies have revealed that chronic marijuana use is detrimental to the liver (Hezode *et al.,* 2005; Ishida *et al.,* 2008). It is known to increase the risk for progression of liver fibrosis, in patients with hepatitis C infection. These studies contradicted the experimental studies in rodents that CB2 activation may protect from fibrosis (Julien *et al.,* 2005). However, subsequent studies from the same group suggested that activation of CB l receptors promotes progression of fibrosis and therefore CB l antagonists may be useful for the treatment of liver fibrosis (Teixeira-Clerc *et al.,* 2006). In contrast, studies from our laboratory seem to suggest that cannabinoids including THC seem to protect hepatic cell injury in a rodent model of hepatitis triggered by T -cell mitogen, ConA. Our findings are consistent with another report which demonstrated that a synthetic nonpsychoactive cannabinoid derivative (PRS-211,092) was found to attenuate ConA-induced hepatitis, although it should be noted that the effect was noted to be

independent of cannabinoid receptors. These discrepancies can be explained in many ways: First, ConA-induced rodent model of hepatitis, although mimics human viral and AIH in many aspects, is an acute model of hepatitis in which activation of T -cells with the release of cytokines including TNF-α, IFN-γ, IL-6, and IL-1 seems to play a crucial role. In addition toT-cells, neutrophils, δT, NK T -cells, and Kupffer cells have been implicated in ConA-induced hepatitis and similar involvement of immune cells has also been noted in AIH. Thus, cannabinoids that are anti-inflammatory are likely to provide beneficial effects. In contrast, chronic liver injury may include additional nonimmune mechanisms. Likewise, in human chronic hepatitis C infection, a sustained immune response against the virus may be crucial for regulating the infection (Yu *et al.,* 2008) and smoking marijuana which is know to suppress the immune response is likely to promote chronic infection and liver fibrosis. Secondly, while in an acute model, cannabinoid receptors expressed on immune cells may play a crucial role, in the chronic disease, the cannabinoid receptors expressed on hepatocytes may also regulate the disease process as seen from their upregulation (Julien *et al.,* 2005; Schwabe and Siegmund, 2005). It is interesting to note that even in the acute murine model of ConA-induced hepatitis, we noted that use of CB1 select agonists at higher doses worsened the effect of ConA (Hegde *et al.,* 2008). Thirdly, in human studies using marijuana smoke, the patients are potentially exposed to a large number of other compounds besides THC; thus the net effect may be different from the use ofTHC as a single compound used in animal studies. Of particular interest is cannabidiol which has been shown be induce apoptosis and cause immunosuppression (Straus, 2000).

VIII. Conclusions and Future Directions

Taken together, our data suggest that exogenous cannabinoids such as THC, upon binding to CB1 and CB2 receptors on immune cells, mediate anti-inflammatory effects through multiple pathways including induction of apoptosis in effector T-cells (Do *et al.,* 2004; Hegde *et al.,* 2008; Jia *et al.,* 2006; Lombard *et al.,* 2005; McKallip et al., 2002a,b), upregulation of T -reg function and suppression of inflammatory cytokines (Hegde *et al.,* 2008), thereby preventing ConA-induced activated T -cell-mediated liver injury. The observation that the anandamide treatment ameliorates ConA-induced hepatitis, together with FAAH deficiency or inhibition leading to increased resistance to the disease, strongly suggests that the endocannabinoid system serves to attenuate the inflammatory response in acute model of ConA-induced hepatitis. These findings raise the promising potential of developing novel pharmacological treatments for T -cell-mediated liver diseases. Due to the controversial nature of potential deleterious effects of marijuana smoke on liver fibrosis as seen from human studies versus the beneficial effects of THC and endocannabinoids as seen in acute rodent models of hepatitis, clearly, additional studies are necessary to address the precise nature and role of cannabinoid receptors and their ligands in acute and chronic hepatitis. Better animal models that mimic chronic hepatitis need to be developed and the effect of cannabinoids addressed. Whether activation of cannabinoid receptors on immune cells found in the liver versus hepatocytes and endothelial cells, leads to differential effects needs to be investigated. While some studies, including those from our lab, have shown that CB2 activation alone is sufficient to cause immunosuppression and trigger an anti-inflammatory response (Lombard et al., 2005, 2007), in some disease models, activation

ofboth CB receptors seems to be necessary to trigger immunosuppression and attenuation of the disease (Hegde *et al.*, 2008). Thus, additional studies should be pursued that delineate the signaling events that follow activation of individual versus combined CB receptors on immune cells. Moreover, the potential manipulation of endocannabinoids through use of FAAH inhibitors to protect the liver from immune-mediated attacks, and the unique ability of CB receptor agonists to trigger regulatory T -cells (Hegde *et al.*, 2008), deserves further attention.

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