Marijuana and Cannabinoids: Effects on Infections, Immunity, and AIDS

Guy A. Cabral

SUMMARY. Marijuana and its major psychoactive component, delta-9-tetrahydrocannabinol (THC), alter resistance to bacterial, protozoan, and viral infections *in vivo* and *in vitro*. These alterations have been accompanied by modifications in functional components of the immune system. In addition, marijuana and THC, as well as other cannabinoids, have been reported to directly affect functional activities of lymphocytes, macrophages, natural killer cells, and other immunocytes. These include effects on cytokine production resulting in a shift in the balance of Th1 versus Th2 cytokines. Both receptor and non-receptor mediated modes of action have been proposed as causative of cannabinoid effects. Reports that marijuana and THC alter anti-microbial activity *in vivo* and *in vitro* indicate that its use presents a potential risk of decreased resistance to infections. However, few controlled longitudinal epidemiological and immunological studies have been undertaken to correlate the immunosuppressive effects of marijuana smoke or cannabinoids on the incidence of infections or disease in humans. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>*

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INTRODUCTION

Marijuana, *Cannabis sativa*, is a highly complex substance that contains in excess of 400 chemical entities. Among these is a group of compounds classified as cannabinoids of which some of its 66 or more members exert a variety of effects on cells of the immune system. The cannabinoid that has been linked to the majority of the immunosuppressive effects attributable to marijuana is delta-9-tetrahydrocannabinol (THC), its major psychoactive component. Studies using *in vitro* and *in vivo* experimental models have indicated that marijuana or THC affects cell-mediated immunity (Klykken et al. 1977; Smith et al. 1978), humoral immunity (Mishkin and Cabral 1985), and cellular defenses against infectious agents (reviewed in: Cabral and Dove Pettit 1998; Friedman and Klein 1999). Compromised resistance in mice, rats, and guinea pigs to infection with amebae (Burnette-Curley et al. 1993), herpes simplex virus (Morahan et al. 1979; Mishkin and Cabral 1985; Cabral et al. 1986a; Cabral et al. 1986b; Fischer-Stenger et al. 1992), Friend Leukemia virus (Specter et al. 1991), *Listeria monocytogenes* (Morahan et al. 1979), *Staphylococcus aureus* (Baldwin 1997), *Treponema pallidum*, and *Legionella pneumophila* (Klein et al., 1993; Klein et al. 1994; Newton et al. 1994) has been reported. Although there are numerous reports relating to the deleterious effects of THC, this cannabinoid also has been reported to have therapeutic potential (Munson and Fehr 1983; Dewey 1986). It exhibits anti-nociceptive properties, has the ability to reduce intraocular pressure and bronchial constriction, and acts as an anti-convulsant and anti-emetic agent. Major advances have been made in the pharmacology and molecular biology of cannabinoids, the cell biology of endogenous systems, and the expression of cognate receptors. High-affinity and low-affinity cannabinoid ligands, non-cannabinoid ligands, and receptor subtype-specific antagonists have been developed. In addition, cannabinoid receptor subtype-specific molecular probes and antibodies as well as knockout animals have become available in the last few years. These experimental tools should prove highly useful to basic scientists and clinical researchers as they assess the acute as well as long-term effects of marijuana and cannabinoids on the immune system.

EFFECTS OF CANNABINOIDS ON INFECTIONS

Other than a few early studies on host resistance in mice or guinea pigs to infections with herpes simplex viruses and *Listeria monocytogenes*, there have

been few studies of the effects of cannabinoids on infectious diseases. Experimental evidence which links directly the use of cannabis or cannabinoids in a recreational or therapeutic mode to compromised host resistance in humans is not available. Data obtained have been extrapolated from studies performed on experimental animals or using *in vitro* culture systems.

Arata et al. (1992) reported that THC affects macrophage functional activities *in vitro* against *Legionella pneumophila*, the causative agent of Legionnaires' disease. Treatment of macrophages from A/J mice with THC resulted in enhanced growth of *Legionella* within macrophages. In addition, THC treatment overcame macrophage restriction of the growth of *Legionella* that is normally induced by macrophage activation with bacterial lipopolysaccharide. Klein et al. (1994) extended these studies to demonstrate that THC induces significantly increased mortality in mice infected with *Legionella*. *Legionella*-primed mice challenged with a secondary lethal dose survived the challenge infection. However, significantly increased mortality was obtained in animals subjected to the same *Legionella* infection and challenge regimen but receiving THC three weeks prior to the *Legionella* exposure. Kusher et al. (1994) assessed the effect of THC on the synthesis of tumor necrosis factor alpha (TNF) by human large granular lymphocytes (LGL) in culture. These investigators reported that THC at physiological levels down-regulated TNF production and diminished LGL cytolytic activity against K562 tumor cells. Based on these studies, it was suggested that, since the NK/polymorphonuclear neutrophil axis represents an important early defense against the opportunistic fungus *Candida albicans,* repression of this system by THC could contribute to susceptibility to infections with opportunistic pathogens.

The few studies performed to assess the effects of marijuana or cannabinoids on resistance to infection in humans have yielded contradictory results. Gross et al. (1991) reported that marijuana consumption altered responsiveness of human papillomavirus (HPV) to systemic recombinant interferon alpha 2a treatment. Simeon et al. (1996) examined characteristics of Jamaicans who smoked marijuana before sex and their risk status for sexually transmitted diseases. The results of a national sample of 2580 randomly selected individuals administered a questionnaire indicated that more persons who smoked marijuana before sex had a history of sexually transmitted diseases than non-marijuana smokers. The difference was significant among men, but not among women. The investigators indicated that, although it was not possible to establish whether the association was causal, there was an increased risk for sexually transmitted diseases among men who smoked marijuana before sex.

On the other hand, Miller and Goodridge (2000) undertook a retrospective study to evaluate the relationship between marijuana use and sexually transmitted diseases in pregnant women. Examination of clinical records over a twelve and one-half month period of 86 women entering prenatal care, and

who used no illicit substance other than marijuana, was compared with that of 441 drug-free women. No significant differences in the prevalence of gonorrhea, chlamydia, syphilis, human immunodeficiency virus, hepatitis B virus, human papilloma virus, or herpes virus were noted. Also, no differences were found for prevalence of more than one infectious agent. It was concluded that marijuana use was not associated with sexually transmitted disease in pregnant women.

In contrast to the equivocal results obtained for marijuana and susceptibility to infections, Bass et al. (1996) indicated that a synthetic non-psychotropic cannabinoid could prove useful in the treatment of bacterial infection. The synthetic non-psychotropic cannabinoid dexanabinol (HU-211), when used in combination with antimicrobial therapy, was effective in reducing brain damage in a rat model of pneumococcal meningitis. Brain edema and blood-brain barrier impairment were significantly reduced for infected animals receiving combination ceftriaxone and HU-211 therapy as compared with control animal groups.

EFFECTS OF CANNABINOIDS ON IMMUNE CELLS

Effects of cannabis and cannabinoids on host resistance to infections have occurred in association with changes in cellular and humoral immunity, suggesting a functional linkage between these two events. Studies conducted since the early 1970s reported that cannabinoids and marijuana affect the functions of various immune cells from rodents and humans including B lymphocytes (Zimmerman et al. 1977; Smith et al. 1978; Baczynsky and Zimmerman 1983; Klein and Friedman 1990; Nahas and Osserman 1991; Kaminski et al. 1992), T lymphocytes (Nahas et al. 1974; Gupta et al. 1974; Peterson et al. 1976; Nahas et al. 1977; Klein et al. 1985; Cabral et al. 1987; Klein et al. 1991; Lee et al.1995), macrophages (Mann et al. 1971; Drath et al. 1979; Lopez-Cepero et al. 1986; Cabral and Mishkin 1989; Burstein et al. 1994), and natural killer (NK) cells (Specter et al. 1986; Patel et al. 1985; Klein et al. 1987; Kawakami et al. 1988).

Cannabinoids may affect the immune system by altering functional capabilities of immunocytes rather than affecting their relative numbers or distribution. Del Arco et al. (2000) exposed Wistar rats to the potent synthetic cannabinoid agonist HU-210 during gestation and lactation. It was found that perinatal exposure partially affected the distribution of lymphocyte subpopulations in the spleen and peripheral blood. HU-210 treatment resulted in a reduction of T-helper cells in the spleen and in a dose-related decrease in the ratio of T-helper/T-cytotoxic lymphocytes in peripheral blood. In addition, animals exhibited decreased responsiveness of the hypothalamic-pituitary-adre-

nal (HPA) axis. Basal levels of luteinizing hormone (LH) were elevated in animals receiving HU-210 while those for corticosterone were reduced. The investigators concluded that maternal exposure to cannabinoids resulted in minor changes in the development of the immune system, but could induce long-lasting alterations in the functional status of the HPA axis.

Baldwin et al. (1997) evaluated the function of human alveolar macrophages recovered from the lungs of nonsmokers and habitual smokers of tobacco, marijuana, or crack cocaine. Macrophages recovered from marijuana smokers were deficient in their ability to phagocytose *Staphylococcus aureus*, and were severely limited in the capacity to kill bacteria and tumor cells. Experiments in which NG-monomethyl-L-arginine monoacetate, an inhibitor of nitric oxide synthase, was used suggested that macrophages from marijuana smokers were not able to use nitric oxide (NO) as an antibacterial effector molecule. Furthermore, macrophages from marijuana smokers, but not from smokers of tobacco or cocaine, produced lower levels of TNF , granulocyte/macrophage colony-stimulating factor (GMC-SF), and interleukin-6 (IL-6) when stimulated with lipopolysaccharide in culture when compared with alveolar macrophages obtained from control subjects. Based on these observations, it was concluded that habitual exposure of the lung to marijuana impaired select functions of alveolar macrophages including their capacity to produce cytokines.

McCoy et al. (1995) assessed the ability of macrophages and macrophage-like cells exposed to THC to process and present soluble protein. THC was found to exert a differential effect on the capacity of macrophages to process antigens that are necessary for CD4+ T lymphocytes. THC inhibited the processing of hen egg lysozyme (HEL), augmented that of cytochrome *c*, and had no apparent effect on processing of ovalbumin. It was concluded that the nature of the effect of THC on antigen processing was dependent on the intrinsic conformation of the antigen itself. Matveyeva et al. (2000) extended these studies to demonstrate that the THC induced impairment of HEL processing was due, at least in part, to a selective increase in aspartyl cathepsin D proteolytic activity. It was suggested that upregulation of cathepsin D activity resulted in "over-processing" of HEL yielding peptides below the critical size required for antigen presentation. In addition, Clements et al. (1996) demonstrated that THC also suppressed a fixation-resistant co-stimulatory signal to helper T cells by diminishing expression of macrophage heat-stable antigen.

EFFECTS OF CANNABINOIDS ON CYTOKINES

A mode of action by which cannabinoids affect immunocyte functional activities, may be their capacity to express and process effector molecules, in-

cluding chemokines and cytokines. Newton et al. (1998) demonstrated that the addition of THC to murine splenocytes stimulated with pokeweed mitogen (PWM) resulted in increased levels of the cytokines interleukin-4 (IL-4) and interleukin-10 (IL-10), which are associated with Th2 responses. In contrast, THC treatment resulted in decreased levels of interferon gamma (IFN), interleukin-15 (IL-15), and interleukin-12 (IL-12), which are associated with Th1 responses. Thus, cannabinoids induced a shift in the expression of lymphocyte cytokines associated with cell-mediated immunity (i.e., Th1) versus humoral immunity (i.e., Th2). These investigators indicated also that macrophages produced a factor that was responsible for the IL-4 increase, suggesting that macrophages play a role in the Th1 versus Th2 effects. Furthermore, peritoneal macrophages directly exposed to THC and cultured in the presence of various stimulators exhibited decreased production of IL-12, IL-15, and IL-6 while demonstrating increased production of interleukin-1 alpha (IL-1), interleukin-1 beta (IL-1), and TNF . The results suggest that THC affects macrophages in splenocyte cultures and that these cells, as well as lymphocytes, are involved in alterations in levels of cytokines.

Srivastava et al. (1998) used human T, B, eosinophilic, and CD8+ NK cell lines as *in vitro* models to examine the effects of exposure to THC or the relatively non-psychotropic cannabinoid cannabidiol (CBD) on the production of cytokines and of constitutively-expressed, as well as inducibly-expressed chemokines. It was found that cannabinoids exerted a multiplicity of alterations in levels of cytokines from various immune cells. These effects were neither uniform in action nor consistent across cell lineages. THC decreased the constitutive production of the CXC chemokine interleukin-8 (IL-8), of the CC chemokines macrophage inflammatory protein-1 alpha (MIP-1), inflammatory protein-1 beta (MIP-1), and Regulated on Activation Normal T Cell Expressed and Secreted (RANTES) protein, and of phorbol ester-stimulated production of TNF , GM-CSF, and IFN by NK cells. THC also inhibited the expression of MIP-1 in human T-lymphotropic virus 1 (HTLV-1)-positive B lymphocytes. In contrast, THC treatment resulted in augmented levels of IL-8, MIP-1 , and MIP-1 in B lymphocytes and IL-8 and MIP-1 in eosinophils. Both CBD and THC inhibited the production of IL-10 in HUT-78 T cells.

Klein et al. (1993) were among the first to relate cannabinoid effects on levels of cytokines to a specific disease process. They reported that THC induces cytokine-mediated mortality of mice infected with *Legionella*. Mice receiving THC, before and after a sublethal injection of *Legionella*, experienced acute collapse and death. The THC-induced mortality resembled cytokine-mediated shock. Acute phase sera from THC-treated animals contained significantly elevated levels of TNF and IL-6 implicating these cytokines as causative, at least in part, of the enhanced mortalities. Mice receiving a normally sub-lethal in-

jection of *Legionella* and administered anti-TNF , anti-IL-6, or a mixture of anti-IL-1 and anti-IL-1 antibodies before the second THC injection, were protected from THC-induced mortalities. Antibodies against IL-6 were shown to be the most effective in rendering protection. Subsequent experiments performed on cultured splenocytes obtained from mice infected with *Legionella* and administered THC demonstrated alterations in levels of cytokines that were attributable to T lymphocyte subsets (Newton et al. 1994). Splenocytes from THC-treated infected animals stimulated in culture with mitogen were deficient in IFN production. In addition, increased production of antibody to *Legionella* of the IgG_1 isotype, as compared to that for the IgG_{2a} isotype, was observed in sera of infected mice treated with THC. Furthermore, THC treatment of cultured, normal splenocytes stimulated with mitogen resulted in production of relatively higher levels of IL-4 as compared with those for IFN . In additional studies, Klein et al. (2000) reported that THC treatment of mice suppressed early IFN , IL-12, and IL-12 receptor beta 2 responses to *Legionella pneumophila* infection. The Th2-promoting cytokine, IL-4, was increased upon infection with *Legionella* and this increase was augmented following THC administration. However, it was suggested that suppression of Th1 immunity to *Legionella* was not due to an increase in production of IL-4 but rather to a decrease in that of IFN and IL-12. Collectively, the studies performed using *Legionella* as an infectivity model suggest that cannabinoids cause a disruption of the network of cytokines which results in a shift from Th1 to Th2 lymphocyte subtype activity. This cannabinoid-mediated shift in Th1 versus Th2 cytokine activity could explain exacerbated infection with *Legionella*.

Massi et al. (1998) also noted that THC could cause alterations in the expression profile of cytokines. These investigators examined the effect of acute versus chronic subcutaneous administration of THC on immune functional and biochemical parameters in male Swiss mice. It was reported that acute exposure to THC had no effect on the splenocyte proliferative response to concanavalin A or on NK cell activity. However, a significant decrease in interleukin-2 (IL-2) production was noted. Chronic administration, for which mice were shown to be tolerant to THC-induced analgesia, resulted in inhibition of the splenocyte proliferative response, diminished NO activity, and reduction in levels of IL-2 and IFN .

Recent studies suggest that, in addition to cannabinoids, various endogenous fatty acid ethanolamides participate in the regulation of cytokine responses. Berdyshev et al. (1997) compared the effect of anandamide (arachidonic acid ethanolamide), palmitoylethanolamide, and THC on the production of TNF , IL-4, IL-6, IL-8, IL-10, and IFN by stimulated human peripheral blood mononuclear cells. Anandamide diminished the production of IL-6 and IL-8 at nanomolar concentrations but inhibited that of TNF , IFN , and IL-4 at

micromolar concentrations. Palmitoylethanolamide inhibited production of IL-4, IL-6, and IL-8 at concentrations similar to those of anandamide but had no effect on TNF and IFN . THC exerted a biphasic effect on the production of cytokines. Maximal inhibition of TNF , IL-6, and IL-8 occurred at nanomolar levels. However, at micromolar concentrations, THC caused an augmentation of levels of TNF , IL-6, and IL-8 as well as IFN . Molina-Holgado et al. (1997) demonstrated that the endogenous cannabinoid anandamide suppressed NO and TNF production by primary cultures of neonatal BALB/c mouse cortical astrocytes in response to exposure to Theiler's virus (TMEV) or bacterial lipopolysaccharide (LPS). These investigators suggested that anandamide might play an immunoregulatory role in the central nervous system (CNS).

Collectively, studies indicate that exogenous as well as endogenous cannabinoids affect the response profile for cytokines and that the nature of alterations is dependent on the concentration of cannabinoid applied. Immunomodulatory effects of cannabinoids on the production of cytokines may vary also as a function of age. Ramarathinam et al. (1997) reported that THC exerted a differential modulation of cytokines by lymphoid cells from young versus old mice. IL-4 and IL-10 production by lymphoid cells of older mice treated with THC was consistently up-regulated in response to stimulation with concanavalin A or anti-CD3 antibody. These observations suggest that aging may be an important variable for consideration when assessing immunomodulatory effects of cannabinoids.

The data indicating that cannabinoids can alter the expression profile of cytokines also suggest a potential for these compounds as selective modulators of pathological inflammatory processes. That is, since cannabinoids have the capacity to diminish the production of cytokines, appropriately designed analogs devoid of psychotropic properties could serve as therapeutic agents applicable of the treatment of disease marked by chronic or exacerbated production of cytokines. In this context, Shohami et al. (1997) reported that HU-211 exhibited pharmacological properties of an N-methyl-D-aspartate (NMDA)-receptor antagonist and acted as an effective cerebroprotectant in an experimental model of traumatic brain injury. The experimental model for closed head injury (CHI) exhibited edema, blood-brain-barrier disruption, motor and memory dysfunction as well as spatial and temporal induction of markers for the cytokines IL-1, IL-6, and TNF . HU-211 exerted an inhibitory effect on TNF production by affecting its post-translational maturation. It was suggested that, since cytokines may play a role in the pathophysiology of brain injury, TNF-modulating agents such as HU-211 could serve to improve final neurological outcome if administered within an early time frame following CHI. Gallily et al. (1997) extended these studies to demonstrate that HU-211 also has the ability to rescue rodents from endotoxic shock after LPS injection. HU-211 administered to BALB/c mice prior to introduction of LPS

resulted in a significant reduction in lethality. Furthermore, administration of HU-211 to Sprague-Dawley rats prior to treatment with LPS abolished the typical hypotensive response resulting from administration of endotoxin. In addition, HU-211 had a marked inhibitory effect on the ability of murine peritoneal macrophages and rat alveolar macrophages maintained in culture to produce TNF and NO in response to LPS. These data suggest that HU-211 may have therapeutic potential in the treatment of TNF -mediated pathologies. Achiron et al. (2000) indicated that HU-211 also reduces the inflammatory response in the brain and spinal cord in rats used as experimental models of autoimmune encephalomyelitis. It was suggested from these studies that HU-211 might be useful as an alternative mode of treatment of acute relapses of multiple sclerosis. In addition to the synthetic compound HU-211, the nonpsychoactive cannabis constituent cannabidiol (CBD) has been reported to act as an oral anti-arthritic therapeutic in a murine model of collagen-induced arthritis (CIA) (Malfait et al. 2000). CBD administered after the onset of clinical symptoms effectively blocked progression of arthritis and was equally effective when administered intraperitoneally or orally. Furthermore, clinical improvement was accompanied by protection of the joints against severe damage. It was postulated that CBD through its combined immunosuppressive and anti-inflammatory actions has a potent anti-arthritic effect on CIA.

MECHANISMS BY WHICH MARIJUANA AND CANNABINOIDS ALTER IMMUNE FUNCTION

Cannabis and cannabinoids exert a wide range of *in vivo* and *in vitro* effects on immune cells. Cannabinoids exert augmenting (McCoy et al. 1995; Derocq et al. 1995; Srivastava et al. 1998) as well as inhibitory effects of immune cell functions (reviewed in: Munson et al. 1976; Cabral and Dove Pettit 1998). Pross et al. (1992) reported that THC exerts concentration-dependent biphasic effects on immune cells. These investigators assessed the effect of THC on T lymphocyte stimulation with anti-CD3 antibody and revealed that lower drug concentrations increased proliferation while higher concentrations inhibited the response. Concentration-dependent augmenting effects of cannabinoids have also been observed by Derocq et al. (1995). It was reported that human tonsillar B-cells exposed to nanomolar concentrations of cannabinoid exhibited enhanced growth and that this enhancement was inhibited by pertussis toxin suggesting that a G protein-coupled receptor process was involved. The observation that SR141716A, an antagonist specific for the CB_1 cannabinoid receptor (Rinaldi-Carmona et al. 1994), had no effect on the cannabinoid-mediated increased proliferative response along with the identification of large amounts of CB_2 receptor mRNA in human B cells, suggested that the growth

enhancing activity was mediated through the CB_2 cannabinoid receptor. Biphasic effects of cannabinoids with respect to immune cell lineages have been observed by Klein et al. (1985). These investigators demonstrated that THC concentrations in the micromolar range suppressed mouse splenocyte proliferation to T cell mitogens and to the B cell mitogen LPS. However, B cells appeared to be more sensitive than T cells to the effects of THC.

Cannabinoids may alter immune cell activities by multiple modes of action. At high concentrations (i.e., 10^{-5} M or greater), THC and other cannabinoids can cause membrane perturbation and disruption. Relatively high concentrations which would account for such effects are achievable in humans in the context of immune cells which populate and circulate through the lung, an organ which would be exposed directly to marijuana smoke and hence to relatively high concentrations of exogenous cannabinoids. Physical disruption of cellular membranes could affect protein translational and post-translational events of immune cell effector molecules. Furthermore, since cannabinoids such as THC are highly lipophilic, their interaction with cellular membranes could alter membrane fluidity with consequent alterations in selective permeability (Wing et al. 1985). Such alterations in membranes may account for the reported inhibition of protein synthesis (Cabral and Mishkin 1989; Cabral and Fischer-Stenger 1994) and of molecular precursor transport by THC (Desoize et al. 1979). At lower concentrations, and at sites distal to the lung, cannabinoids may affect immune cell functions by signaling through cannabinoid receptors. Such receptors have been identified both within the brain and on cells of the immune system. The CB_1 was the first cannabinoid receptor to be identified and has been localized to neuronal tissues (Matsuda et al.1990) and testis (Galiègue et al. 1995), and to a lesser extent to immune cells (Galiègue et al. 1995; Waksman et al. 1999). The second cannabinoid receptor, the CB_2, has been observed in cells of the immune system (Munro et al. 1993; Bouaboula et al. 1993; Galiègue et al. 1995; Facci et al. 1995). Both receptors are coupled to a pertussis toxin-sensitive G_i/G_o protein (Howlett and Fleming 1984; Howlett 1985; Howlett et al. 1986; Matsuda et al. 1990). Binding of cannabinoid ligand to cannabinoid receptors results in an increase in the affinity of GTP for the G subunit of the G protein, a decrease in affinity for GDP, and dissociation of the subunit from the G protein complex. The dissociated G subunit interacts with adenylate cyclase to inhibit its activity resulting in decreases in levels of the second messenger cAMP (Howlett 1984; Howlett et al. 1990; Felder et al. 1992; Felder et al. 1995) and initiation of mitogen-activated protein kinase (MAPK) and immediate early gene signaling pathways (Bouaboula et al. 1993, 1995, 1996). In turn, the complex of the G protein can interact with phospholipase C leading to release of inositol-tris-phosphate (IP_3), activation of IP_3-gated calcium channels, and release of Ca^{++} from intracellular stores (Netzeband et al. 1999). The complex also can activate protein kinase B

through class-1_B phosphoinositide 3′ kinases (Gomez Del Pulgar et al. 2000). A similar series of events occurs for the CB_2 cannabinoid receptor except that, in contrast to the CB_1, no modulation of N-type calcium channels (Mackie and Hille 1992) has been observed (Felder et al. 1995). Thus, interaction of cannabinoid ligands with cannabinoid receptors can activate different signal transduction pathways that could affect a diverse array of cellular functions.

The presence of CB_2 receptors within immune cells suggests a role for these receptors in their functional activities. Transcripts (i.e., mRNAs) for the CB_2 have been found in spleen and tonsils (Galiègue et al. 1995; Munro et al. 1993) and other immune tissues and cells (Munro et al. 1993; Bouaboula et al. 1993). However, in all studies reported to date, levels of message for the CB_2 have been found to exceed those for the CB_1. The distribution pattern of levels of CB_2 mRNA displays major variation in human blood cell populations with a rank order of B lymphocytes > NK cells > monocytes > polymorphonuclear neutrophils > T8 lymphocytes > T4 lymphocytes (Galiègue et al. 1995). A rank order for levels of CB_2 transcripts similar to that for primary human cell types has been recorded for human cell lines belonging to the myeloid, monocytic, and lymphoid lineages (Galiègue et al. 1995). In addition, the presence of cognate protein has been demonstrated in rat lymph nodes, Peyer's patches, and spleen (Lynn and Herkenham 1994). The differential levels of cannabinoid receptors reported for different immune cell types may account, at least in part, for the distinctive levels of sensitivity to cannabinoid mediated action on the part of immunocytes of different lineages.

Initial studies to examine the role of cannabinoid receptors in cannabinoid-mediated alteration of immune cell activities were primarily of an implicative nature. Kaminski et al. (1992, 1994) noted that suppression of the humoral immune response by cannabinoids was mediated partially by inhibition of adenylate cyclase through a pertussis toxin sensitive guanine nucleotide binding protein (G protein) coupled mechanism, implicating a cannabinoid receptor in this process. THC and the synthetic bicyclic cannabinoid CP55940 inhibited the lymphocyte proliferative response and the sheep erythrocyte IgM antibody-forming cell response of murine splenocytes to phorbol-12-myristate-13-acetate (PMA) plus the calcium ionophore ionomycin. Jeon et al. (1996) suggested that LPS-inducible NO release by the murine macrophage-like cell line RAW264.7 was suppressed by THC and other agonists by mechanisms that involved cannabinoid receptors. Furthermore, attenuation of inducible NO gene expression by THC was reported to be mediated through the inhibition of nuclear factor- B/Rel activation. In addition, Burstein et al. (1994) presented data indicating that THC-induced arachidonic acid release from mouse peritoneal cells occurred through a series of events consistent with a receptor-mediated process that involved the stimulation of one or more phospholipases.

Recent studies have focused on the definition of the cannabinoid receptor subtype, which may be linked functionally to cannabinoid-mediated alterations in immune cell functions. Waksman et al. (1999) reported that cannabinoids affected the production of inducible NO by neonatal rat microglial cells and that this effect was linked to the CB_1 receptor. The inhibitory effect was stereoselective, consistent with the involvement of a cannabinoid receptor. The dose-dependent inhibition of NO release was exerted by the receptor high affinity binding enantiomer CP55940 while a lower effect for each comparable concentration tested was exerted by the low affinity binding paired enantiomer CP56667. Furthermore, reversal in CP55940-mediated inhibition of NO release was effected when microglial cells were pretreated with the CB_1 receptor-selective antagonist SR141716A consistent with a functional linkage to the CB_1 receptor. Stefano et al. (1996) reported that the CB_1 receptor was linked also to cannabinoid-mediated alterations in the production of constitutive NO. However, in contrast to effects on inducible NO, cannabinoid receptor agonists increased constitutive NO levels in cultures of human monocytes. As in the case of effects on inducible NO, the effect on constitutive NO production was reversed by the CB_1 receptor antagonist SR141716A supporting that the CB_1 receptor was involved in the augmentation process.

Smith et al. (2000) indicated that the CB_1 receptor played a role in the modulation of cytokine production in response to cannabinoid ligands. Two cannabinoid receptor agonists, WIN 55212-2 and HU-210, were examined for their effects on LPS-induced cytokine production in *Corynebacterium parvum* (*C. parvum*)-primed and unprimed mice. Both cannabinoids, when administered to mice before LPS, decreased serum levels of TNF and IL-12 while increasing those for IL-10. The two agonists also protected *C. parvum*-primed mice against the lethal effects of LPS. These cannabinoid-induced effects on cytokine production were reversed by the CB_1 receptor antagonist SR141716A, but not by the CB_2 receptor-specific antagonist SR144528, consistent with a functional linkage to the CB_1 receptor. Moreover, it was reported that SR141716A when administered alone modulated cytokine responses in a fashion comparable to that of WIN55212-2 and HU-210 suggesting that it could act as a partial agonist of the CB_1 receptor.

There is a larger body of data which supports the CB_2 cannabinoid receptor as linked functionally to cannabinoid-mediated alteration of immune functions. McCoy et al. (1999) implied that a functional linkage existed between cannabinoid-mediated inhibition of antigen processing by macrophages and the CB_2 receptor. In their studies, processing of HEL was inhibited by THC and other cannabinoid agonists. Stereoselective cannabinoid enantiomers showed a differential inhibitory effect for the bioactive enantiomer CP55940 versus that of its less bioactive paired enantiomer CP56667. Furthermore, the CB_1-selective antagonist SR141716A did not block the inhibitory effect of the

cannabinoid agonist while the CB_2-selective antagonist SR144528 (Rinaldi-Carmona et al. 1998) did. Zhu et al. (2000) reported that THC inhibits antitumor immunity by a CB_2 receptor-mediated, cytokine-dependent pathway. Accelerated growth of tumor implants was observed following intermittent administration of THC in two weakly immunogenic lung cancer mouse models. In contrast to the results obtained with immunocompetent mice, THC had no effect on tumor growth of implants in severe combined immunodeficiency (SCID) mice. It was demonstrated, in addition, that levels of the immune inhibitory cytokines IL-10 and transforming growth factor beta (TGF) were increased at the tumor site as well as in the spleens of mice administered THC. This augmentation was accompanied by a decrease in levels of IFN at both sites. The THC-augmentation of tumor growth was prevented by administration of anti-IL-10 or anti-TGF neutralizing antibodies. The investigators demonstrated further that administration of the CB_2 cannabinoid receptor antagonist SR144528 blocked the effects of THC. The collective results indicated that THC inhibited antitumor activity and that it did so through a CB_2 cannabinoid receptor-mediated, cytokine-dependent mode.

Derocq et al. (2000) suggested a role for the CB_2 cannabinoid receptor in cell differentiation. These investigators applied Affymetrix DNA chips to the investigation of the gene expression profile of human promyelocytic HL-60 cells transfected with the CB_2 receptor and activated with the synthetic cannabinoid agonist CP55940. Treatment of these cells with CP55940 resulted in activation of a mitogen-activated protein kinase cascade and a receptor desensitization consistent with a functional coupling of the transfected receptors. Activation of the CB_2 receptors at the genomic level effected an up-regulation of genes involved in cytokine synthesis, regulation of transcription, and cell differentiation. A majority of the genes affected were recognized as under the control of nuclear factor-kappa B (Nf B). Many features of the transcriptional events observed by Derocq et al. (2000) appeared to be related to activation of cell differentiation suggesting that the CB_2 receptor plays a role in the initialization of cell maturation. Buckley et al. (2000), employing CB_2 cannabinoid receptor knockout mice to assess the effect of THC on T cell co-stimulation, confirmed the role of the CB_2 cannabinoid receptor as linked functionally to immunomodulation. THC was shown to inhibit helper T cell activation through macrophages derived from wild-type, but not from knockout mice, indicative of at least this immune effect as mediated by the CB_2 receptor. In contrast, central nervous effects of cannabinoids remain unaffected in the knockout mice.

There have been few studies that have addressed the role of cannabinoid receptors in cannabinoid-mediated alterations in resistance to infectious agents. Noe et al. (1998), using syncytial formation as a barometer of infection, reported that cannabinoid receptor agonists enhanced syncytia formation in MT-2 cells infected with cell free human immunodeficiency virus MN strain

(HIV-1MN). Gross et al. (2000) implicated the CB_1 receptor as linked functionally to cannabinoid effects on *Brucella suis* growth within macrophages. The CB_1-selective antagonist SR141716A effected a dose-dependent inhibition of the intracellular multiplication of this gram-negative bacterium. The nonselective cannabinoid receptor agonists CP55940 or WIN55212-2 reversed the SR141716A-mediated effect. These results suggested that the CB_1 antagonist could be beneficial as an inhibitor of macrophage infection by the intracellular pathogen *Brucella suis*.

Because individual immune cells may express both CB_1 and CB_2 receptors, a complex network of cellular signal transductional pathways may be activated upon exposure to cannabinoids. Thus signaling through cannabinoid receptors may lead to additive effects as well as to immune cell functional events characterized by augmentation as well as inhibition within the same cell. Indeed, Massi et al. (2000) reported that both types of cannabinoid receptors are involved in mediating NK cell cytolytic activity. Inhibition of NK cell activity by THC was partially reversed by both the CB_1 and the CB_2 antagonists, although the CB_1 antagonist was more effective. These investigators demonstrated, also, that both antagonists reversed completely THC-mediated inhibition of IFN production. A similar outcome was obtained by Klein et al. (2000), who indicated that THC treatment suppressed immunity and early IFN , IL-12, and IL-12 receptor 2 responses to *Legionella pneumophila* infection. Furthermore, these investigators demonstrated that the suppressive effects were attenuated by CB_1 and the CB_2 antagonists, suggesting that suppression of the Th1-promoting cytokines was linked to both cannabinoid receptors. McCoy et al. (1995) reported that distinctive receptor-mediated functional outcomes may be operative within the same immune cell type. These investigators observed that comparable concentrations of THC induced enhancement of macrophage processing of cytochrome *c* while simultaneously inhibiting that of hen egg lysozyme (HEL). Furthermore, cannabinoids may exert their effects by both receptor- and non-receptor-mediated modes within the same cell type. Felder et al. (1992) demonstrated that cannabinoid agonists stimulated receptor- and non-receptor-mediated signal transduction pathways. Fibroblast cell lines which had been transfected with a recombinant cannabinoid receptor expression vector and which expressed cannabinoid receptors were used in their studies. Experiments using the synthetic cannabinoid receptor agonist CP55940 indicated that the cloned receptors coupled to the inhibition of cAMP accumulation as anticipated for the involvement of a cannabinoid receptor-linked event. However, CP55940 also stimulated the increase of free arachidonic acid in a non-stereoselective fashion indicative of the absence of a functional linkage to a cannabinoid receptor for this cellular activity.

Whether cannabinoids interact with target immunocytes by a receptor- or non-receptor mediated mode, the fundamental result is that basic functional

activities of cells are altered which often are mediated through second messenger systems. Herring and Kaminski (1999) indicated that cannabinol (CBN) mediated inhibition of NF B, cAMP response element (CRE)-binding protein, and IL-2 secretion by phorbol ester plus calcium ionophore (PMA/Io) stimulated thymocytes. CBN decreased CRE and NF B binding activity that had been induced by PMA/Io. Both a major CRE DNA binding complex comprised of a cAMP response element-binding protein (CREB)-1 homodimer, as well as a minor CREB-1/activating transcription factor (ATF)-2 complex, were inhibited. In addition, CBN diminished the binding activity of PMA/Io-inducible and non-inducible B DNA binding complexes. In PMA/Io-stimulated thymocytes, CBN effected a decrease in phosphorylation of CREB/ATF nuclear proteins, and prevented phosphorylation-dependent degradation of the NF B inhibitory protein I B . Herring and Kaminski (1999) suggested that these results indicated a functional link between CBN-mediated inhibition of thymocyte functional activities, including IL-2 production, and inhibition of the transcriptional factor activities of complexes in the CREB/ATF and NF B/Rel families. These studies were extended by Yea et al. (2000) to demonstrate that inhibition of IL-2 production by CBN was mediated through the inhibition of IL-2 gene transcription. Electrophoretic mobility shift assays demonstrated that CBN inhibited the DNA binding activity of nuclear factor of activated-T cells (NF-AT) and activator protein-1 (AP-1) in a time- and concentration-dependent manner in activated EL4 T-cells. Furthermore, the AP-1 activity was reported to be negatively regulated through inhibition of its protein components, *c-fos* and *c-jun* (Faubert and Kaminski 2000). Thus, the CBN inhibited binding to AP-1 containing sites from the IL-2 promoter was due, in part, to decreased nuclear expression of *c-fos* and *c-jun*. In addition, it was reported that the effects of CBN were due to post-translational modification of these phosphoproteins and that CBN inhibited the activation of ERK MAP kinases. Based on these studies, it was concluded that CBN-induced immunosuppression involved a disruption of the ERK signaling cascade. However, whether a cannabinoid receptor is involved in this transductional cascade of events remains unresolved.

CANNABINOIDS, CANNABIS, AND AIDS

Many studies using *in vitro* and *in vivo* models have addressed the effects of cannabinoids and cannabis on host resistance and immunity. However, there have been few studies that have assessed directly the effects of marijuana usage or of cannabinoid administration in humans. The scarcity of data applies particularly to the evaluation of effects of marijuana, used either in a recreational or therapeutic mode, among humans who have immune deficiencies.

Epidemiological studies similar to those that have been performed to assess effects of tobacco have not been carried out in human populations in relation to infection with the human immunodeficiency virus (HIV). The studies performed to date have yielded limited and often contradictory results as to effects of cannabinoids on human immunity and resistance to infection.

Wallace et al. (1998) examined risk factors and outcomes associated with identification of *Aspergillus* in respiratory specimens from individuals with HIV disease as part of a study to evaluate pulmonary complications of HIV infection. It was indicated that a substantially greater proportion of patients with *Aspergillus* as compared with control subjects died during the study. However, the use of cigarettes and marijuana was found not to be associated with *Aspergillus* respiratory infection. In contrast, Johnson et al. (1999) suggested that marijuana smoking could increase the risk of development of sino-orbital aspergillosis in patients with acquired immune deficiency syndrome (AIDS). DiFranco et al. (1996), through the San Francisco Men's Health Study (SFMHS), evaluated the association of specific recreational drugs and alcohol with laboratory predictors of AIDS. Participants in the study were evaluated at entry into the program in 1984 and in the context of the development of AIDS during six years of follow-up. No substantial association could be obtained between the use of marijuana and the development of AIDS among HIV-infected men. Similarly, Timpone et al. (1997) reported that cannabinoid use in a therapeutic mode exerted few deleterious effects, at least as they related to immune competence and resistance to infection. Persaud et al. (1999) conducted a cross-sectional survey among 124 street- and brothel-based female commercial sex workers in Guyana. No statistically significant association was found between HIV infection and marijuana use.

On the other hand, other studies have suggested that cannabinoids or marijuana exert deleterious effects as they relate to HIV infection. Stefano et al. (1998) reported that long-term exposure of human saphenous vein or thoracic artery endothelium to the human immunodeficiency virus (HIV) envelope protein gp120 in concert with morphine and/or anandamide increased endothelial adhesion of monocytes. It was suggested that enhancement of monocyte adherence was a result of desensitization of the endothelium to further NO release after initial exposure to either anandamide or morphine. The investigators suggested that abuse of opiates and/or cannabinoids could result in higher viral load in the central nervous system. Furthermore, they suggested that the increase in monocyte adherence and mobility indicative of a higher level of transmembrane migration could contribute to a more rapid progression of the AIDS. Tindall et al. (1988) conducted immunoepidemiological studies using univariant and multivariant analyses and implied an association between marijuana use and progression of HIV infection. Caiaffa et al. (1994) indicated that smoking illicit drugs such as marijuana, cocaine, or crack, *Pneumocystis ca-*

rinii pneumonia, and immunosuppression increased risk of bacterial pneumonia in HIV-seropositive users. More recently, Whitfield et al. (1997) examined the impact of ethanol and Marinol /marijuana usage on HIV+/AIDS patients undergoing azidothymidine, azidothymidine/dideoxycytidine, or dideoxyinosine therapy. In HIV+/AIDS patients with the lowest CD4+ counts (those not on DDI monotherapy), utilization of Marinol /marijuana did not seem to have a deleterious effect. However, Marinol /marijuana usage was associated with depressed CD4+ counts and elevated amylase levels within the DDI subgroup. Furthermore, Marinol /marijuana use was associated with declining health status in both the AZT and AZT/DDC groups.

CONCLUSION

The cumulative data obtained through cell culture studies using various immune cell populations extracted from animals or humans, together with those obtained using animal models of infection, are consistent with the proposition that marijuana and cannabinoids alter immune cell function and can exert deleterious effects on resistance to infection in humans. Both receptor- and non-receptor mediated modes of action have been proposed to account for the effects of cannabinoids. However, few controlled longitudinal epidemiological and immunological studies have been undertaken to correlate the immunosuppressive effects of marijuana smoke or cannabinoids on the incidence of infections or viral disease in humans. Clearly, additional investigation to resolve the long-term immunological consequences of cannabinoid and marijuana use as they relate to resistance to infections in humans is warranted. There is also emerging evidence that select cannabinoid compounds, particularly those devoid of psychotropic properties, may be useful for therapeutic application for pathologies characterized by chronic activation of immune cells or imbalance in expression of Th1 versus Th2 cytokines.

REFERENCES

Achiron, A., S. Miron, V. Lavie, R. Margalit, and A. Biegon. 2000. Dexanabinol (HU-211) effect on experimental autoimmune encephalomyelitis: implications for the treatment of acute relapses of multiple sclerosis. *J Neuroimmunol* 102(1):26-31.

Arata, S., C. Newton, T. Klein, and H. Friedman. 1991. Enhanced growth of *Legionella pneumophila* in tetrahydrocannabinol-treated macrophages. *Proc Soc Exp Biol Med* 199:65-7.

Baczynsky, W. O. T., and A. M. Zimmerman. 1983. Effects of delta-9-tetrahydrocannabinol, cannabinol and cannabidiol on the immune system in mice. *In vitro* investigation using cultured mouse splenocytes. *Pharmacology* 26(1):12-9.

Baldwin, G. C., D. P. Tashkin, D. M. Buckley, A. M., Park, S. M., Dubinett, and M. D. Roth. 1997. Marijuana and cocaine impair alveolar macrophage function and cytokine production. *Am J Respir Crit Care Med* 156(5):1606-13.

Bass, R., D. Engelhard, V. Trembovler, and E. Shohami. 1996. A novel nonpsychotropic cannabinoid, HU-211, in the treatment of experimental pneumococcal meningitis. *J Infect Dis* 173:735-8.

Berdyshev, E. V., E. Boichot, N. Germain, N. Allain, J. P. Anger, and V. Lagente. 1997. Influence of fatty acid ethanolamides and delta9-tetrahydrocannabinol on cytokine and arachidonate release by mononuclear cells. *Eur J Pharmacol* 330(2-3): 231-40.

Bouaboula, M., C. Poinot-Chazel, J. Marchand, X. Canat, B. Bourrie, M. Rinaldi-Carmona, B. Calandra, G. Le Fur, and P. Casellas. 1996. Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. *Eur J Biochem* 237(3):704-11.

Bouaboula, M., C. Poinot-Chazel, B. Bourrie, X. Canat, B. Calandra, M. Rinaldi-Carmona, G. Le Fur, and P. Casellas. 1995. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* 312: 637-41.

Bouaboula, M., M. Rinaldi, P. Carayon, C. Carillon, B. Delpech, D. Shire, G. Le Fur, and P. Casellas. 1993. Cannabinoid receptor expression in human leukocytes. *Eur J Biochem* 214:173-80.

Buckley, N. E., K. L. McCoy, E. Mezey, T. Bonner, A. Zimmer, C. C. Felder, M. Glass, and A. Zimmer. 2000. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. *Eur J Pharmacol* 396(2-3):141-9.

Burnette-Curley, D., F. M. Marciano, K. Fischer, and G. A. Cabral. 1993. Delta-9-tetrahydrocannabinol inhibits cell contact-dependent cytotoxicity of *Bacillus* Calmétte-Guérin-activated macrophages. *Int J Immunopharmacol* 15:371-82.

Burstein, S., J. Budrow, M. Debatis, S. A. Hunter, and A. Subramanian. 1994. Phospholipase participation in cannabinoid-induced release of free arachidonic acid. *Biochem Pharmacol* 48:1253-64.

Cabral, G. A., and D. Dove Pettit. 1998. Drugs and immunity: Cannabinoids and their role in decreased resistance to infectious disease. *J Neuroimmunol* 83:116-23.

Cabral, G. A., and K. Fischer-Stenger. 1994. Inhibition of macrophage inducible protein expression by delta-9-tetrahydrocannabinol. *Life Sci* 54:1831-44.

Cabral, G. A., and E. M. Mishkin. 1989. Delta-9-tetrahydrocannabinol inhibits macrophage protein expression in response to bacterial immunomodulators. *J Toxicol Environ Health* 26:175-82.

Cabral, G. A., P. J. McNerney, and E. M. Mishkin. 1987. Effect of micromolar concentrations of delta-9-tetrahydrocannabinol on herpes simplex virus type 2 replication in vitro. *J Toxicol Environ Health* 21(3):277-93.

Cabral, G. A., J. C. Lockmuller, and E. M. Mishkin. 1986a. Delta-9-tetrahydrocannabinol decreases alpha/beta interferon response to herpes simplex virus type 2 in the B6C3F1 mouse. *Proc Soc Exp Biol Med* 181:305-11.

Cabral, G., E. M. Mishkin, F. M. Marciano, R. E. Coleman, L. S. Harris, and A. E. Munson. 1986b. Effect of delta-9-tetrahydrocannabinol on herpes simplex virus type 2 vaginal infection in the guinea pig. *Proc Soc Exp Biol Med* 182:181-6.

Caiaffa, W. T., D. Vlahov, N. M. D. Graham, J. Astemborski, L. Solomon, K. E. Nelson, and A. Munoz. 1994. Drug smoking, *Pneumocystis carinii* pneumonia, and immunosuppression increase risk of bacterial pneumonia in human immunodeficiency virus-seropositive injection drug users. *Am J Respir Crit Care Med* 150: 1493-8.

Childers, S. R., and S. A. Deadwyler. 1996. Role of cyclic AMP in the actions of cannabinoid receptors. *Biochem Pharmacol* 52:819-27.

Clements, D., G. A. Cabral, and K. L. McCoy. 1996. Delta-9-tetrahydrocannabinol selectively inhibits macrophage co-stimulatory activity and down-regulates heat-stable antigen expression. *J Pharmacol Exp Ther* 277:1315-21.

del Arco, I., R. Munoz, F. Rodriguez De Fonseca, L. Escudero, J. L. Martin-Calderon, M. Navarro, and M. A. Villanua. 2000. Maternal exposure to the synthetic cannabinoid HU-210: effects on the endocrine and immune systems of the adult male offspring. *Neuroimmunomodulation* 7(1):16-26.

Derocq, J. M., O. Jbilo, M. Bouaboula, M. Segui, C. Clere, and P. Casellas. 2000. Genomic and functional changes induced by the activation of the peripheral cannabinoid receptor CB2 in the promyelocytic cells HL-60. Possible involvement of the CB2 receptor in cell differentiation. *J Biol Chem* 275(21):15621-8.

Derocq, J. M., M. Segui, J. Marchand, G. Le Fur, and P. Casellas. 1995. Cannabinoids enhance human B-cell growth at low nanomolar concentrations. *FEBS Letters* 369:177-82.

Desoize, B., C. Leger, and G. G. Nahas. 1979. Plasma membrane inhibition of macromolecular precursor transport by THC. *Biochem Pharmaco*l 28:1113-8.

Dewey, W. L. 1986. Cannabinoid pharmacology. *Pharmacol Rev* 38(2):151-78.

DiFranco, M. J, H. W. Sheppard, D. J. Hunter, T. D. Tosteson, and M. S. Ascher. 1996. The lack of association of marijuana and other recreational drugs with progression to AIDS in the San Francisco Men's Health Study. *Ann Epidemiol* 6(4):283-9.

Dove Pettit, D. A., D. L. Anders, M. P. Harrison, and G. A. Cabral. 1996. Cannabinoid receptor expression in immune cells. *Adv Exp Med Biol* 402:119-29.

Drath, D. B., J. M. Shorey, L. Price, and G. L. Huber. 1979. Metabolic and functional characteristics of alveolar macrophages recovered from rats exposed to marijuana smoke. *Infect Immun* 25:268-72.

Facci, L., R. Dal Tosso, S. Romanello, A. Buriani, S. D. Skaper, and A. Leon. 1995. Mast cells express a peripheral receptor and differential sensitivity to anandamide and palmitoylethanolamide. *Proc Natl Acad Sci USA* 92:3376-80.

Faubert, B. L., and N. E. Kaminski. 2000. AP-1 activity is negatively regulated by cannabinol through inhibition of its protein components, c-fos and c-jun. *J Leukoc Biol* 67(2):259-66.

Felder, C. C., K. E. Joyce, E. M. Briley, J. Mansouri, K. Mackie, O. Blond, Y. Lai, A. L. Ma, and R. L. Mitchell. 1995. Comparison of the pharmacology and signal transduction of the human cannabinoid CB_1 and CB_2 receptors. *Mol Pharmacol* 48:443-50.

Felder, C. C., J. S. Veluz, H. L. Williams, E. M. Briley, and L. A. Matsuda. 1992. Cannabinoid agonists stimulate both receptor- and non-receptor-mediated signal transduction pathways in cells transfected with and expressing cannabinoid receptor clones. *Mol Pharmacol* 42:838-45.

Fischer-Stenger, K., A. W. Updegrove, and G. A. Cabral. 1992. Delta-9-tetrahydrocannabinol decreases cytotoxic T lymphocyte activity to herpes simplex virus type 1-infected cells. *Proc Soc Exp Biol Med* 200:422-30.

Friedman, H., and T. W. Klein. 1999. Marijuana and immunity. *Sci Med* 6(2):12-21.

Galiègue, S., S. Mary, J. Marchand, D. Dussosoy, D. Carrière, P. Carayon, M. Bouaboula, D. Shire, G. Le Fur, and P. Casellas. 1995. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* 232:54-61.

Gallily, R., A. Yamin, Y. Waksmann, H. Ovadia, J. Weidenfeld, A. Bar-Joseph, A. Biegon, R. Mechoulam, and E. Shohami. 1997. Protection against septic shock and suppression of tumor necrosis factor alpha and nitric oxide production by dexanabinol (HU-211), a nonpsychotropic cannabinoid. *J Pharmacol Exp Ther* 283(2): 918-24.

Gomez Del Pulgar, T., G. Velasco, and M. Guzman. 2000. The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. *Biochem J* 347:369-73.

Gross, A., A. Terraza, J. Marchant, M. Bouaboula, S. Ouahrani-Bettache, J. P. Liautard, P. Casellas, and J. Dornand. 2000. A beneficial aspect of a CB1 cannabinoid receptor antagonist: SR141716A is a potent inhibitor of macrophage infection by the intracellular pathogen *Brucella suis*. *J Leukoc Biol* 67(3): 335-44.

Gross, G., A. Roussaki, H. Ikenberg, and N. Dress. 1991. Genital warts do not respond to systemic recombinant interferon alpha-2a treatment during cannabis consumption. *Dermatologica* 183:203-7.

Gupta, G., M. Grieco, and P. Cushman. 1974. Impairment of rosette-forming T-lymphocytes in chronic marihuana smokers. *New Eng J Med* 291:874-6.

Herring, A. C., and N. E. Kaminski. 1999. Cannabinol-mediated inhibition of nuclear factor-kappa B, cAMP response element-binding protein, and interleukin-2 secretion by activated thymocytes. *J Pharmacol Exp Ther* 291(3):1156-63.

Howlett, A. C. 1985. Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. *Mol Pharmacol* 27:429-36.

Howlett, A. C. 1984. Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. *Life Sci* 35:1803-10.

Howlett, A. C., and R. M. Fleming. 1984. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Mol Pharmacol* 26:532-8.

Howlett, A. C., T. M. Champion, G. H. Wilken, and R. Mechoulam. 1990. Stereochemical effects of 11-OH-delta 8-tetrahydrocannabinol-dimethylheptyl to inhibit adenyl cyclase and bind to the cannabinoid receptor. *Neuropharmacol* 29:161-5.

Howlett, A. C., J. M. Qualy, and L. L. Khachchatrian. 1986. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol Pharmacol* 29: 307-13.

Jeon, Y. J., K. H. Yang, J. T. Pulaski, and N. E. Kaminski. 1996. Attenuation of inducible nitric oxide gene expression by 9-tetrahydrocannabinol is mediated through the inhibition of nuclear factor- B/rel activation. *Mol Pharmacol* 50:334-41.

Johnson, T. E., R. R. Casiano, J. W. Kronish, D. T. Tse, M. Meldrum, and W. Chang. 1999. Sino-orbital aspergillosis in acquired immunodeficiency syndrome. *Arch Ophthalmol* 117(1):57-64.

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

Kaminski, N. E., W. S. Koh, K. H. Yang, M. Lee, and F. K. Kessler. 1994. Suppression of the humoral immune response by cannabinoids is partially mediated through inhibition of adenylate cyclase by a pertussis toxin-sensitive G-protein coupled mechanism. *Biochem Pharmacol* 48:1899-908.

Kawakami, Y., T. W. Klein, C. Newton, J. Y. Djeu, S. Specter, and H. Friedman. 1988. Suppression of delta-9-tetrahydrocannabinol of interleukin 2-induced lymphocyte proliferation and lymphokine-activated killer cell activity. *Int J Immunopharmacol* 10:485-8.

Klein, T. W., C. A. Newton, N. Nakachi, and H. Friedman. 2000. Delta 9-tetrahydrocannabinol treatment suppresses immunity and early IFN-gamma, IL-12, and IL-12 receptor beta 2 responses to *Legionella pneumophila* infection. *J Immunol* 164(12):6461-6.

Klein, T. W., C. Newton, and H. Friedman. 1994. Resistance to *Legionella pneumophila* suppressed by the marijuana component, tetrahydrocannabinol. *J Infect Dis* 169:1177-9.

Klein, T., C. Newton, R. Widen, and H. Friedman. 1993. Delta-9-tetrahydrocannabinol injection induces cytokine-mediated mortality of mice infected with *Legionella pneumophila*. *J Pharmacol Exp Ther* 267:635-40.

Klein, T. W., Y. Kawakami, C. Newton, and H. Friedman. 1991. Marijuana components supress induction and cytolytic function of murine cytotoxic T cells *in vitro* and *in vivo*. *J Toxicol Environ Health* 32:465-77.

Klein, T. W. and H. Friedman. 1990. *Drugs of abuse and immune function*. In R. R. Watson (Ed.). Boca Raton:CRC Press.

Klein, T. W., C. Newton, and H. Friedman. 1987. Inhibition of natural killer cell function by marijuana components. *J Toxicol Environmental Health* 20:321-32.

Klein, T. W., C. Newton, R. Widen, and H. Friedman. 1985. The effect of delta-9-tetrahydrocannabinol and 11-hydroxy-delta 9-tetrahydrocannabinol on T-lymphocyte and B-lymphocyte mitogen responses. *J Immunopharmacol* 7:451-66.

Klykken, P. C., S. H. Smith, J. A. Levy, R. Razdan, and A. E. Munson. 1977. Immunosuppressive effects of 8,9-epoxyhexahydrocannabinol (EHHC). *J Pharmacol Exp Ther* 201:573-9.

Kusher, D. I., L. O. Dawson, A. C. Taylor, and J. Y. Djeu. 1994. Effect of the psychoactive metabolite of marijuana, delta-9-tetrahydrocannabinol (THC), on the synthesis of tumor necrosis factor by large granular lymphocytes. *Cellular Immunol* 154(1):99-108.

Lee, M., K. H.Yang, and N. E. Kaminski. 1995. Effects of putative receptor ligands, anandamide and 2-arachidonyl-glycerol, on immune function in B6C3F1 mouse splenocytes. *J Pharmacol Exp Ther* 275:529-36.

Lopez-Cepero, M., M. Friedman, T. Klein, and H. Friedman. 1986. Tetrahydrocannabinol-induced suppression of macrophage spreading and phagocyte activity *in vitro*. *J Leukoc Biol* 39: 679-86.

Lynn, A. B., and M. Herkenham. 1994. Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. *J Pharmacol Exp Ther* 268:1612-23.

Mackie, K., and B. Hille. 1992. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* 89:3825-9.

Malfait, A. M., R. Gallily, P. F. Sumariwalla, A. S. Malik, E. Andreakos, R. Mechoulam, and M. Feldmann. 2000. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc Natl Acad Sci USA* 97(17):9561-6.

Mann, P. E. G., A. B. Cohen, T. N. Finley, and A. J. Ladman. 1971. Alveolar macrophages. Structural and functional differences between non-smokers and smokers of marijuana *in vitro. Lab Invest* 25:111-20.

Massi, P., D. Fuzio, D. Vigano, P. Sacerdote, and D. Parolaro. 2000. Relative involvement of cannabinoid CB(1) and CB(2) receptors in Delta(9)-tetrahydrocannabinol-induced inhibition of natural killer activity. *Eur J Pharmacol* 387(3):343-7.

Massi, P., P. Sacerdote, W. Ponti, D. Fuzio, B. Manfredi, D. Vigano, T. Rubino, M. Bardotti, and D. Parolaro. 1998. Immune function alterations in mice tolerant to delta-9-tetrahydrocannabinol: functional and biochemical parameters. *J Neuroimmunol* 92(1-2):60-6.

Matsuda, L. A., S. J. Lolait, M. J. Brownstein, A. C. Young, and T. I. Bonner. 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346:561-4.

Matveyeva, M., C. B. Hartman, M. T. Harrison, G. A. Cabral, and K. L. McCoy. 2000. Delta (9)-tetrahydrocannabinol selectively increases aspartyl cathepsin D proteolytic activity and impairs lysozyme processing by macrophages. *Int J Immunopharmacol* 22(5):373-81.

McCoy, K. L., D. Gainey, and G. A. Cabral. 1995. ^{9}-Tetrahydrocannabinol modulates antigen processing by macrophages. *J Pharmacol Exp Ther* 273:1216-23.

McCoy, K. L., M. Matveyeva, S. J. Carlisle, and G. A. Cabral. 1999. Cannabinoid inhibition of the processing of intact lysozyme by macrophages: Evidence for CB2 receptor participation. *J Pharmacol Exp Ther* 289:1620-5.

Miller, J. M. Jr., and C. Goodridge. 2000. Antenatal marijuana use is unrelated to sexually transmitted infections during pregnancy. *Infect Dis Obstet Gynecol* 8(3-4): 155-7.

Mishkin, E. M., and G. Cabral. 1985. Delta-9-tetrahydrocannabinol decreases host resistance to herpes simplex virus type 2 vaginal infection in the $B_6C_3F_1$ mouse. *J Gen Virol* 66:2539-49.

Molina-Holgado, F., A. Lledo, and C. Guaza. 1997. Anandamide suppresses nitric oxide and TNF-alpha responses to Theiler's virus or endotoxin in astrocytes. *Neuroreport* 8(8):1929-33.

Morahan, P. S., P. C. Klykken, S. H. Smith, L. S. Harris, and A. E. Munson 1979. Effects of cannabinoids on host resistance to *Listeria monocytogenes* and herpes simplex virus. *Inf Immun* 23:670-4.

Munro, S., K. L. Thomas, and M. Abu-Shaar. 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365:61-5.

Munson, A. E., J. A. Levy, L. S. Harris, and W. L. Dewey. 1976. Effects of delta 9-tetrahydrocannabinol on the immune system. *The Pharmacology of Marijuana*. Braude, M. C., and S. Szara. New York: Raven Press.

Munson, A. E., and K. O. Fehr.1983. Immunological Effects of Cannabis. *Cannabis and Health Hazards*. Fehr, K. O. and H. Kalant. Toronto: Alcoholism and Drug Addiction Research Foundation.

Nahas, G. G., A. Morishima, and B. Desoize. 1977. Effects of cannabinoids on macromolecular synthesis and replication of cultured lymphocytes. *Fed Proc* 36:1748-52.

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

Nahas, G. G., N. Suciu-Foca, J. P. Armand, and A. Morishima 1974. Inhibition of cellular mediated immunity in marihuana smokers. *Science* 183:419-20.

Netzeband, J. G., S. M. Conroy, K. L. Parsons, and D. L. Gruol. 1999. Cannabinoids enhance NMDA-elicited Ca^{2+} signals in cerebellar granule neurons in culture. *J Neurosci* 19:8765-77.

Newton, C., T. Klein, and H. Friedman. 1998. The role of macrophages in THC-induced alteration of the cytokine network. *Adv Exp Med Biol* 437:207-14.

Newton, C. A., T. W. Klein, and H. Friedman. 1994. Secondary immunity to *Legionella pneumophila* and Th1 activity are suppressed by delta-9-tetrahydrocannabinol injection. *Inf Immun* 62:4015-20.

Noe, S. N., S. B. Nyland, K. Ugen, H. Friedman, and T. W. Klein. 1998. Cannabinoid receptor agonists enhance syncytia formation in MT-2 cells infected with cell free HIV-1MN. *Adv Exp Med Biol* 437:223-9.

Patel, V., M. Borysenko, M. S. A. Kumar, and W. J. Millard. 1985. Effects of acute and subchronic delta-9-tetrahydrocannabinol administration on the plasma catecholamine, beta-endorphin, and corticosterone levels and splenic natural killer cell activity in rats. *Proc Soc Exp Biol Med* 180:400-4.

Persaud, N. E., W. Klaskala, T. Tewari, J. Schultz, and M. Baum. 1999. Drug use and syphilis. Co-factors for HIV transmission among commercial sex workers in Guyana. *West Indian Med J* 48(2):52-6.

Peterson, B. H., J. Graham, and L. Lemberger. 1976. Marihuana, tetrahydrocannabinol and T-cell function. *Life Sci* 19:395-400.

Pross, S. H., Y. Nakano, R. Widen, S. McHugh, C. A. Newton, T. W. Klein, and H. Friedman. 1992. Differing effects of delta-9-tetrahydrocannabinol (THC) on murine spleen cell populations dependent upon stimulators. *Int J Immunopharmacol* 14: 1019-27.

Ramarathinam, L., S. Pross, O. Plescia, C. Newton, R. Widen, and H. Friedman. 1997. Differential immunologic modulatory effects of tetrahydrocannabinol as a function of age. *Mech Ageing Dev* 96(1-3):117-26.

Rinaldi-Carmona, M., F. Barth, M. Héaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Néliat, D. Caput, P. Ferrara, P. Soubrié, J. C. Breliére, and G. Le Fur. 1994. Sr141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Letters* 350:240-4.

Rinaldi-Carmona, M., F. Barth, J. Millan, J. M. Derocq, P. Casellas, C. Congy, D. Oustric, M. Sarran, M. Bouaboula, B. Calandra, M. Portier, D. Shire, J. C. Breliére, and G. Le Fur. 1998. SR144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther* 284:644-50.

Shohami, E., R. Gallily, R. Mechoulam, R. Bass, and T. Ben-Hur. 1997. Cytokine production in the brain following closed head injury: dexanabinol (HU-211) is a novel TNF-alpha inhibitor and an effective neuroprotectant. *J Neuroimmunol* 72(2): 169-77.

Simeon, D. T., B. Bain, G. E. Wyatt, E. LeFranc, H. Ricketts, C. C. Chambers, and M. B. Tucker. 1996. Characteristics of Jamaicans who smoke marijuana before sex and their risk status for sexually transmitted diseases. *West Indian Med J* 45(1):9-13.

Smith, S. R., C. Terminelli, and G. Denhardt. 2000. Effects of cannabinoid receptor agonist and antagonist ligands on production of inflammatory cytokines and anti-inflammatory interleukin-10 in endotoxemic mice. *J Pharmacol Exp Ther* 293(1): 136-50.

Smith, S. H., L. S. Harris, I. M. Uwaydah, and A. E. Munson. 1978. Structure-activity relationships of natural and synthetic cannabinoids in suppression of humoral and cell-mediated immunity. *J Pharmacol Exp Ther* 207:165-70.

Specter, S. C., T. W. Klein, C. Newton, M. Mondragon, R. Widen, and H. Friedman. 1986. Marijuana effects on immunity:suppression of human natural killer cell activity by delta-9-tetrahydrocannabinol. *Int J Immunopharmacol* 8:741-5.

Specter, S., G. Lancz, G. Westrich, and H. Friedman. 1991. Delta-9-tetrahydrocannabinol augments murine retroviral induced immunosuppression and infection. *Int J Immunopharmacol* 13:411-7.

Srivastava, M. D., B. I. Srivastava, and B. Brouhard. 1998. Delta-9-tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. *Immunopharmacology* 40(3):179-85.

Stefano, G. B., M. Salzet, and T. V. Bilfinger. 1998. Long-term exposure of human blood vessels to HIV gp120, morphine, and anandamide increases endothelial adhesion of monocytes: uncoupling of nitric oxide release. *J Cardiovasc Pharmacol* 31(6):862-8.

Stefano, G. B., Y. Liu, and M. S. Goligorsky. 1996. Cannabinoid receptors are coupled to nitric oxide release in invertebrate immunocytes, microglia, and human monocytes. *J Biol Chem* 271: 19238-42.

Timpone, J. G., D. J. Wright, N. Li, M. J. Egorin, M. E. Enama, J. Mayers, and J. Galetto. 1997. The safety and pharmokinetics of single-agent and combination therapy with Megestrol acetate and Dronabinol for the treatment of HIV wasting syndrome. *AIDS Res Hum Retroviruses* 13:305-15.

Tindall, B., D. A. Cooper, B. Donovan, T. Barnes, C. R. Philpot, J. Gold, and R. Penny. 1988. The Sydney AIDS Project: development of acquired immunodeficiency syndrome in a group of HIV seropositive homosexual men. *Aust N Z J Med* 18(1):8-15.

Waksman, Y., J. M. Olson, S. J. Carlisle, and G. A. Cabral. 1999. The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *J Pharmacol Exp Ther* 288:1357-66.

Wallace, J. M., R. Lim, B. L. Browdy, P. C. Hopewell, J. Glassroth, M. J. Rosen, L. B. Reichman, and P. A. Kvale.1998. Risk factors and outcomes associated with identification of *Aspergillus* in respiratory specimens from persons with HIV disease. Pulmonary complications of HIV infection study group. *Chest* 114(1):131-7.

Whitfield, R. M., L. M. Bechtel, and G. H. Starich. 1997. The impact of ethanol and Marinol/marijuana usage on HIV+/AIDS patients undergoing azidothymidine, azidothymidine/dideoxycytidine, or dideoxyinosine therapy. *Alcohol Clin Exp Res* 21(1): 122-7.

Wing, D. R., J. T. A. Leuschner, G. A. Brent, D. J. Harvey, and W. D. M. Paton. 1985. Quantification of *in vivo* membrane associated delta-9-tetrahydrocannabinol and its effects on membrane fluidity. *Proceedings of the 9th International Congress of Pharmacology 3rd satellite symposium on cannabis*. Harvey, D. J. Oxford: IRL Press.

Zhu, L. X., S. Sharma, M. Stolina, B. Gardner, M. D. Roth, D. P. Tashkin, and S. M. Dubinett. 2000. Delta-9-tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. *J Immunol* 165(1):373-80.

Zimmerman, S., A. M. Zimmerman, I. L. Cameron, and H. L. Lawrence. 1977. Delta1-tetrahydrocannabinol, cannabidiol and cannabinol effects on the immune response of mice. *Pharmacology* 15:10-23.