

Cannabinoid Inhibition of the Processing of Intact Lysozyme by Macrophages: Evidence for CB2 Receptor Participation¹

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ABSTRACT

Delta⁹-tetrahydrocannabinol (THC) impairs multiple immunological functions. The ability of a macrophage hybridoma to function as an antigen-presenting cell was examined by the stimulation of a soluble protein antigen-specific helper T cell hybridoma to secrete interleukin-2. THC exposure significantly reduced the T cell response to the native form of the antigen after a 24-h pretreatment of the macrophages with nanomolar drug concentrations. However, THC did not affect interleukin-2 production when the macrophages presented a synthetic peptide of the antigen to the T cells, suggesting that the drug may interfere with antigen processing, not peptide presentation. Cannabinoid inhibition of the T cell response to the native antigen was stereoselective consistent with the involvement of

a cannabinoid (CB) receptor. Bioactive CP-55,940 diminished T cell activation, whereas the inactive stereoisomer CP-56,667 did not. The macrophage hybridoma expressed mRNA for the CB2 but not the CB1 receptor whereas the T cells expressed an extremely low level of mRNA for the CB2 receptor. The CB1-selective antagonist SR141716A did not reverse the suppression caused by THC, demonstrating that the CB1 receptor was not responsible for the drug's inhibitory effect. In contrast, the CB2-selective antagonist SR144528 completely blocked THC's suppression of the T cell response, implicating the participation of the CB2 receptor. These findings suggest that the CB2 receptor may be involved in CB inhibition of antigen processing by macrophages in this system.

Before the discovery of cannabinoid (CB) receptors, diverse physiological consequences of delta⁹-tetrahydrocannabinol (THC) exposure were attributed to nonspecific membrane perturbations due to the compound's highly lipophilic nature (Pertwee, 1988; Felder et al., 1992). Many neurological and immunological effects of THC are now known to be receptor-mediated (Howlett, 1995; Matsuda, 1997). Two receptor subtypes have been described and are called cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). Both receptors belong to the G protein-coupled receptor superfamily and bind G_{i/o} proteins that inhibit adenylate cyclase (Childers and Deadwyler, 1996; Schatz et al., 1997). The CB1 receptor is primarily expressed in the central nervous system (Herkenham et al., 1990; Matsuda et al., 1990; Thomas et al., 1992) whereas the CB2 receptor is prevalent in lymphoid organs (Bouaboula et al., 1993; Munro et al., 1993; Galiègue et al., 1995). Before the cloning of cDNA-encoding CB1 and CB2 receptors, stereoselectivity of physiological changes was the basis for identifying receptor participation (Devane et al., 1988; Thomas et al., 1990; Martin et al., 1991). Several THC

analogs have been synthesized to elucidate receptor-mediated mechanisms. For example, the active agonist, CP-55,940, and its inactive enantiomer, CP-56,667, bind both CB receptors (D'Ambra et al., 1992; Compton et al., 1993; Felder et al., 1995), but only CP-55,940 elicits a biological response via the receptors. In addition, reversal of the drug's effect by the selective antagonist SR141716A is confirmation of CB1 receptor involvement (Rinaldi-Carmona et al., 1994). Very recently, a potent and selective antagonist SR144528 of the CB2 receptor has been characterized (Rinaldi-Carmona et al., 1998). These and other cannabimimetic compounds in various combinations provide a response profile indicative of CB receptor participation (Howlett, 1995; Matsuda, 1997).

THC inhibits various macrophage functions at both biochemical and molecular levels. For example, the drug depresses induction of newly synthesized proteins during priming and activation of macrophages (Cabral and Fischer-Stenger, 1994). Maturation and secretion (Watzl et al., 1991; Nakano et al., 1992; Berdyshev et al., 1997) of cytokines by activated macrophages also is altered (Fischer-Stenger et al., 1993; Zheng and Specter, 1996). Furthermore, THC suppresses nitric oxide production by macrophages (Coffey et al., 1996; Jeon et al., 1996).

Another important function of macrophages is to act as

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ABBREVIATIONS: CB, cannabinoid; CB1, cannabinoid receptor 1; CB2 cannabinoid receptor 2; HEL, hen egg lysozyme; IL-2, interleukin-2; MHC, major histocompatibility complex; MRT-PCR, mutagenic reverse transcription-polymerase chain reaction; PCR, polymerase chain reaction; RT, reverse transcription; THC, delta⁹-tetrahydrocannabinol.

antigen-presenting cells for helper/inducer CD4⁺ T cell responses. Once activated, helper T cells produce numerous regulatory cytokines critical in orchestrating various immune functions. CD4⁺ T cell activation depends on occupancy of the T cell receptor (Chien and Davis, 1993; Germain, 1994). Its ligand, which is expressed on the surface of antigen-presenting cells, consists of antigenic peptides bound to major histocompatibility complex (MHC) class II molecules (Chien and Davis, 1993; Germain, 1994). A helper CD4⁺ T cell response occurs only when a sufficient number of the appropriate peptide-MHC class II is expressed on the surface of antigen-presenting cells. Production and expression of such complexes are known as antigen processing and involve many intricate steps (Singer and Linderman, 1990; Germain, 1994; Xu and Pierce, 1995). Any one of these steps, if altered, can impair CD4⁺ T cell activation and, therefore, depress T cell responsiveness. Hence, antigen processing is not merely protein degradation. For example, several mutant antigen-presenting cell lines are defective in antigen processing, yet their overall proteolytic activity is normal (Xu and Pierce, 1995). In fact, greater than 98% of internalized antigen has been estimated to be cleaved to nonstimulatory peptides and amino acids (Singer and Linderman, 1990). Thus, the major constraint on productive antigen processing is helper CD4⁺ T cell activation.

We previously reported that THC interferes with the ability of a murine macrophage hybridoma to process the antigen, hen egg lysozyme (HEL), resulting in decreased cytokine production by a murine T cell hybridoma (McCoy et al., 1995). However, THC does not influence peptide presentation by macrophages, and, thus, the T cell response to a HEL peptide is normal (McCoy et al., 1995). Our current study utilized THC agonists and CB-selective antagonists to examine a possible functional role of CB receptors in this immunosuppression. In addition, macrophages expressed high levels of mRNA encoding the CB2 receptor, whereas CB2 mRNA was barely detected in the T cells. Neither cell type expressed CB1 mRNA. Our findings suggest that the CB2 receptor may mediate the drug's inhibitory effect on the processing of HEL.

Materials and Methods

Cells and Cell Culture. Murine T cell hybridoma 930.B2 is specific for HEL and MHC I-A^d class II molecules, and was provided by Dr. Eli Sercarz (University of California, Los Angeles, CA). Murine macrophage hybridoma clone 63 constitutively expresses MHC I-A^d class II molecules and was provided by Dr. Betty Diamond (Albert Einstein School of Medicine, New York, NY). Murine macrophages P388D₁ and RAW 264.7 were obtained from the American Type Culture Collection (Rockville, MD). Cell lines were cultured in DMEM (Biofluids, Rockville, MD) with 4.5% glucose, 2 mM glutamine, 5% heat-inactivated fetal calf serum and antibiotics.

Drug Exposure. THC was obtained from the National Institute on Drug Abuse (Rockville, MD). CP-55,940 and CP-56,667 were supplied by Pfizer, Inc. (Groton, CT). SR141716A and SR144528 were supplied by Sanofi Recherche (Montpellier, France). All drugs were prepared in ethanol as described (Fischer-Stenger et al., 1993) and used from 0.1 to 1000 nM diluted in complete medium. Vehicle consisted of 0.1% ethanol and did not affect cell viability. Cells were exposed to CBs as described (McCoy et al., 1995). Briefly, clone 63 cells were preincubated with vehicle or agonists for 24 h. T cells and antigen were added, and the concentrations of the agonists and vehicle were maintained. For antagonist experiments, clone 63 cells were preincubated with 1 μ M SR141716A, SR144528, or vehicle for

4 h, and then with 10 nM THC or vehicle for an additional 24 h. T cell stimulation assays were conducted as described below.

T Cell Stimulation. T cells 930.B2 at 3×10^4 per well and 5×10^4 clone 63 per well in complete medium were cultured in replicates with and without 100 μ M HEL (Boehringer Mannheim, Indianapolis, IN) or 50 μ M synthetic peptide 11–25 of HEL (Genosys, Woodlands, TX) for 24 h at 37°C in 96-well microtiter plates. Cell-free culture supernatants were assayed for interleukin-2 (IL-2). IL-2 was quantified using Quantikine M Elisa from R & D Systems (Minneapolis, MN) according to the company's directions. Absorbance at 450 nm was measured with a SpectraMax 250 microplate reader (Molecular Devices, Sunnyvale, CA). The quantity of IL-2 in the culture supernatants was calculated from standard curves.

Preparation of CB1 and CB2 Primers. Oligonucleotide sequences were designed using the GAP and BESTFIT programs on the Genetics Computer Group software (University of Wisconsin, Madison, WI). The following CB1 reverse transcription (RT) primer was synthesized: 5'-GGCCTGTGAA TGGATATGTA CCTGTCGATG GCTGTGAGGA ACCGGCTGCC CAC3', which corresponds to bases 613 to 665 of rat CB1. The C is a single base mismatch that introduces a unique *Msp*I site into the cDNA. For polymerase chain reaction (PCR) amplification of CB1, 5'-ATGAA GTCGA TCCTA GATGG3' (forward primer; bases 1–20) and 5'-GGCCT GTGAA TG-GAT ATGTA3' (reverse primer; bases 646–665) were used. The CB2 RT primer was 5'-GCAGCAGGCT GCCACAG AGGCTGTGAA GGTCATGGTCAC ACTGCAGATC TTCAGCAGG3' (bases 318–376 of murine CB2), and the A is a single base mismatch that introduces a unique *Bgl*II site into the cDNA. For PCR amplification of CB2, 5'-AGCGA ATTCA TGGAG GGATG CCGGG AGACAG3' (forward primer; bases –9 to +22), and 5'-GCAGC AGGCT GCCCA CAGAGGC3' (reverse primer; bases 354–376) were utilized.

Mutagenic Reverse Transcription-Polymerase Chain Reaction (MRT-PCR). An MRT-PCR assay, which discriminates between cDNA amplified from mRNA and product amplified from genomic DNA (Taniguchi et al., 1994), was modified to identify CB receptor mRNA (Dove-Pettit et al., 1996). Total RNA was isolated using Trizol reagent (GIBCO Life Technologies, Grand Island, NY). The appropriate RT primer at 10 fmol and 3 μ g total RNA in 1 mM Tris-HCl, pH 8.3, 50 mM KCl, 4 mM MgCl₂, 1 mM dithiothreitol, and 1 mM each dNTP were heated to 75°C for 5 min and cooled to 42°C. Murine leukemia virus reverse transcriptase at 10 U and 5 U Rnase inhibitor were added, and the reaction mixtures were incubated at 42°C for 1 h. For PCR amplification, 100 ng of each appropriate PCR primer and 2.5 U of AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT) in PCR buffer were added. Samples were heated to 94°C for 1 min and subjected to 35 cycles of 1-min denaturation at 94°C, 1.5-min annealing at 50°C for CB1 or 72°C for CB2, and 2-min extension at 72°C. PCR products were digested with 20 units *Msp*I or *Bgl*II for CB1 or CB2, respectively, for 12 h at 37°C. The 665-bp PCR product derived from CB1 mRNA is digested with *Msp*I to generate 623- and 42-bp fragments. The 385-bp PCR product from CB2 mRNA is digested with *Bgl*II to generate 343- and 42-bp fragments. PCR products from genomic DNA remain undigested. Digestion products were separated by electrophoresis through a 1.5% agarose gel and transferred onto Qiabran Nylon-Plus membranes (Qiagen, Chatsworth, CA) with 0.4 M NaOH transfer buffer. The Southern blots were hybridized with [³²P]-labeled (3000 Ci/mM dCTP; DuPont-NEN, Boston, MA) random-primed CB1 or CB2 fragments (10⁹ dpm/ μ g, specific activity) as probes (Rediprime, Amersham Life Sciences, Arlington Heights, IL) in 0.5 M phosphate buffer, pH 7.0, 7% SDS, and 1 mM EDTA for 4 h at 65°C. CB1 and CB2 fragments were generated by amplification from a pCD-SKR6 template (Matsuda et al., 1990) and a murine CB2 cDNA, which was a gift from Dr. Thomas I. Bonner (National Institute of Mental Health, Bethesda, MD). Blots were washed four times with 40 mM phosphate buffer, 0.5% SDS and 1 mM EDTA for 15 min at 65°C, air dried, and subjected to phosphorimager analysis (445 SI, Molecular Dynamics, Sunnyvale, CA).

Statistical Analysis. Parametric ANOVA was performed by a two-tailed Student's *t* test for unmatched pairs. All results within an experiment were found to have comparable S.D. by Barlett's test. *P* values $\leq .05$ were considered significant for treatment groups compared with the vehicle control.

Results

THC Suppresses T Cell Response to Native Antigen.

We investigated the consequence of THC exposure on the ability of a macrophage hybridoma clone 63 to activate HEL-specific helper CD4⁺ T cell hybridoma 930.B2. Clone 63 cells were preincubated with vehicle or various concentrations of THC for 24 h before T cell stimulation with native HEL. The selected HEL concentration was determined by an antigen dose-response curve in the absence of THC and elicited a submaximal response along the linear portion of the curve (data not shown). T cell activation was assessed by IL-2 secretion. THC significantly decreased IL-2 production by the T cells compared with the level in vehicle control cultures (Fig. 1A). Maximal suppression of approximately 45% was observed at 10 nM THC, whereas higher drug concentrations were less inhibitory. Similar results were also observed for interferon- γ secretion (data not shown). A loss of cell viability did not account for the diminished T cell response.

THC Does Not Interfere with Peptide Presentation.

To distinguish between a problem in antigen processing or peptide presentation, T cells were stimulated with the synthetic peptide 11–25 of HEL. Peptides do not require process-

ing to activate CD4⁺ T cells (Chien and Davis, 1993; Germain, 1994). Analogous to native HEL, the selected peptide concentration induced a submaximal response along the linear portion of a peptide-dose-response curve in the absence of THC (data not shown). Unlike the results with native HEL, THC did not influence the amount of IL-2 produced by the T cells stimulated with the peptide (Fig. 1B), suggesting that THC may impair processing, but not presentation.

CB Inhibition of T Cell Activation Is Stereoselective.

Stereoselectivity of functional responses to CBs indicates a receptor-mediated mode of action. To determine whether the drug's effect on the T cell response to native HEL was stereoselective, we utilized the paired enantiomers CP-55,940 and CP-56,667, which are active and inactive THC analogs, respectively. Again, clone 63 cells were preincubated with either enantiomer or vehicle for 24 h before the addition of T cells and HEL. Biologically active CP-55,940 significantly decreased the quantity of secreted IL-2 (Fig. 2A). The analog's dose profile was similar to that for THC (see Fig. 1A). Analogous to THC, 10 nM CP-55,940 maximally diminished the T cell response to HEL by approximately 42%, whereas higher concentrations were less suppressive. In contrast, inactive CP-56,667 did not significantly decrease IL-2 production at any concentration examined (Fig. 2B). At low concentrations, CP-56,667 had no effect on T cell activation with HEL, whereas higher enantiomer concentrations increased the quantity of secreted IL-2. Thus, CB interference with antigen-specific T cell stimulation was stereospecific.

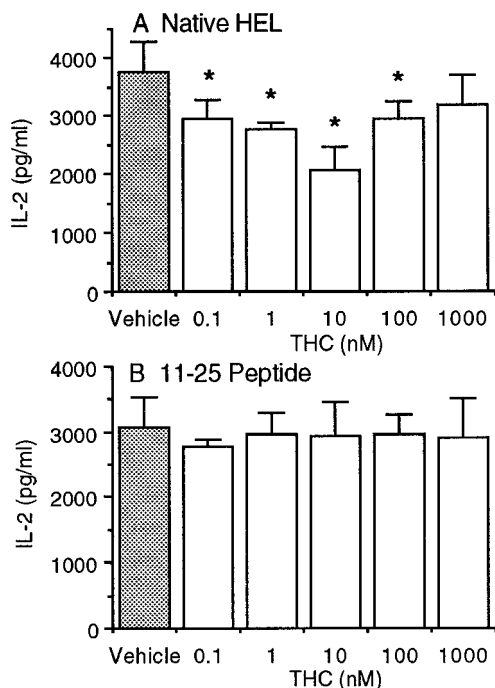


Fig. 1. THC decreases T cell activation with native HEL, but not with peptide. Clone 63 cells as antigen-presenting cells were preincubated with 0.1% ethanol (vehicle) or various THC concentrations for 24 h, followed by the addition of T cells 930. B2 and antigen. After another 24 h, IL-2 secreted into culture supernatants was measured by ELISA. Medium controls were below the level of detection. A, T cell response to 100 μ M native HEL. Values are the mean IL-2 $\pm$ S.D. from quadruplicate cultures. The experiment is representative of four. *denotes significantly different from vehicle control, *P* < .05. B, T cell response to 50 μ M synthetic peptide 11–25 of HEL. Values are the mean IL-2 $\pm$ S.D. from triplicate cultures. The experiment is representative of three.

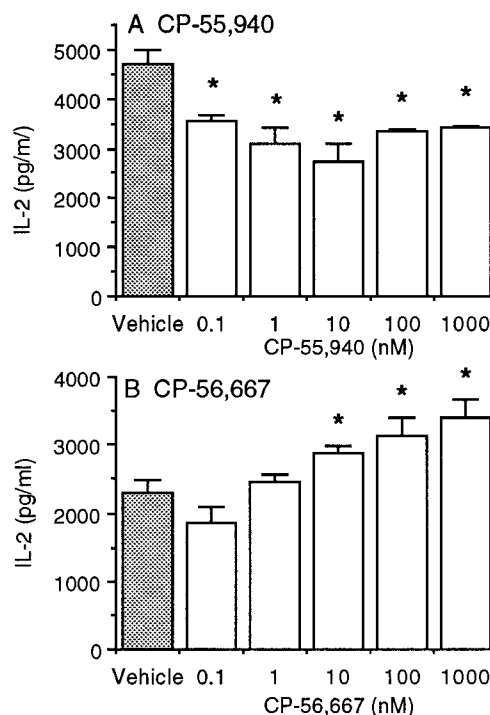


Fig. 2. Active THC analog inhibits IL-2 secretion. Clone 63 cells were preincubated with vehicle or various concentrations of the THC enantiomers, and T cell stimulation assays were performed with 100 μ M HEL (see Fig. 1 legend). Values are the mean IL-2 $\pm$ S.D. from triplicate cultures. A, CP-55,940, the active enantiomer. The experiment is representative of four. *denotes significantly different from vehicle control, *P* < .01. B, CP-56,667, the inactive enantiomer. The experiment is representative of five. *denotes significantly different from vehicle control, *P* < .02.

Hematopoietic Cells Express CB2 mRNA. Although the inhibition was stereoselective, THC and its analogs bind both CB1 and CB2 receptors. To determine whether clone 63 cells expressed mRNA encoding a CB receptor, MRT-PCR was applied to total RNA extracts. PCR products were visualized by Southern blot hybridization using radiolabeled murine CB1 or CB2 cDNA probes (Fig. 3). CB1 mRNA was detected only in RNA extracted from mouse brain, a positive control, as demonstrated by the presence of the smaller 623-bp band after *MspI* digestion. CB1 mRNA was not detected in any of the hematopoietic cell lines examined. All macrophage cell lines, including clone 63 cells, expressed CB2 mRNA at various levels, as evidenced by the appearance of the smaller 343-bp band after *Bgl/II* digestion (Fig. 3). P388D₁ and RAW 264.7 cells, murine macrophage cell lines, served as positive controls for CB2 mRNA expression. In contrast, mouse brain did not contain detectable CB2 mRNA. T cell hybridoma 930.B2 expressed a very low level of CB2 transcripts.

CB2 Antagonist Reverses Suppression. To examine whether a CB receptor may participate in the inhibition, CB-selective antagonists were utilized. We assessed whether the antagonists would compete with THC to reverse the drug's inhibition of the T cell response to native HEL. SR141716A is a selective antagonist for the CB1 receptor, whereas SR144528 is a selective antagonist for the CB2 receptor. The chosen concentration of 10 nM THC maximally inhibited IL-2 secretion (see Fig. 1A), and a 100-fold higher concentration of the antagonists was used for competition. Clone 63 cells were incubated with or without either antagonist for 4 h and then THC for another 24 h. The T cell stimulation assay was conducted as above. Neither antagonist alone significantly influenced the amount of IL-2 produced compared with the vehicle control (Fig. 4). As before, THC alone significantly diminished IL-2 secretion by approximately 40%. In combination, the inhibitory effect of THC was not abrogated by SR141716A, suggesting that the CB1

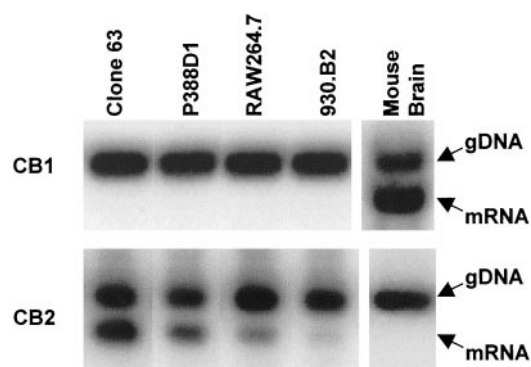


Fig. 3. Murine hematopoietic cell lines express CB2 mRNA. Southern blot analysis of MRT-PCR products of total RNA from murine cell lines and brain amplified with CB1 (top) or CB2 (bottom) primers. Total RNA was reverse transcribed using the appropriate anti-sense primer with a single base mismatch to introduce a unique restriction site. After PCR amplification, the products were digested with *MspI* or *Bgl/II* for CB1 or CB2, respectively, and electrophoresed. After transfer, the blots were hybridized with radiolabeled CB1 or CB2 cDNA probes, and the products were visualized by phosphorimager analysis. The expected cDNA fragments derived from mRNA are 623 bp for CB1 and 343 bp for CB2 after digestion. The larger undigested fragments are derived from genomic DNA. The labels on the right indicate the DNA templates: genomic (gDNA) or mRNA. P388D₁ and RAW 264.7 cells are murine macrophage cell lines.

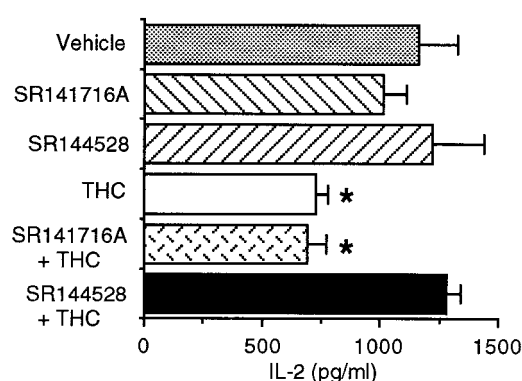


Fig. 4. CB2 antagonist abolishes CB inhibition. Clone 63 cells were incubated with vehicle or 1.0 μ M SR141716A or SR144528 for 4 h, and then with vehicle or 10 nM THC for an additional 24 h. T cell stimulation assays were performed with 100 μ M HEL (see Fig. 1 legend). Values are the mean IL-2 $\pm$ S.D. from triplicate cultures. The experiment is representative of three. *denotes significantly different from vehicle control, $P < .02$.

receptor does not participate. However, SR144528 completely reversed the suppression caused by THC, suggesting that the CB2 receptor may be involved.

Discussion

Macrophage clone 63 cells exposed to THC had a decreased ability to activate HEL-specific CD4⁺ T cells with the native form of the antigen. The level of secreted IL-2 was the indicator of T cell stimulation and was deficient in cultures containing THC, in agreement with our previous report (McCoy et al., 1995). The diminished cytokine secretion caused by THC exposure was stereoselective. When the active enantiomer CP-55,940 was present, T cell activation with native HEL decreased, whereas the inactive enantiomer CP-56,667 did not impair the T cell response. The stereoselectivity of CB inhibition suggests the participation of a CB receptor. SR141716A, a CB1-selective antagonist, at a 100-fold higher concentration was unable to reverse the suppressive effect of THC. Conversely, SR144528, a CB2-selective antagonist, completely eliminated the drug's inhibition. These results are consistent with the postulate that the CB2, but not the CB1, receptor may be involved in this immunosuppression.

Expression of CB receptors in various tissues has been studied extensively. The CB1 receptor is predominantly found in the central nervous system (Herkenham et al., 1990; Matsuda et al., 1990), whereas the CB2 receptor primarily occurs in the peripheral immune tissues. The CB2 receptor is expressed on B cells, natural killer cells, macrophages, and other hematopoietic cells (Bouaboula et al., 1993; Munro et al., 1993; Galiègue et al., 1995), although leukocytes also may express the CB1 receptor (Bouaboula et al., 1993; Galiègue et al., 1995; Schatz et al., 1997). The presence of mRNA encoding the CB2 receptor and the lack of CB1 mRNA in clone 63 cells confirmed our functional data with the THC analogs and the CB-selective antagonists.

The T cells in our study expressed a very low level of CB2 mRNA. This finding raises the possibility that CBs may directly affect these T cells. Contrary to this alternative, the T cell response was not inhibited by THC, when clone 63 cells presented the synthetic HEL peptide, which does not require processing, in agreement with our previous study (McCoy et

al., 1995). When macrophages are pulsed with native HEL in the presence of THC, fixed, and cultured with the T cells in the absence of THC, the level of T cell stimulation is still decreased (McCoy et al., 1995). Furthermore, THC does not diminish T cell receptor expression on these T cells (McCoy et al., 1995). Finally, T cell activation induced by cross-linking the T cell receptor complex with immobilized anti-CD3 antibody in cultures lacking macrophages is normal in the presence of THC (Clements et al., 1996). Thus, the impaired T cell response caused by CBs is most likely due to their interference with the processing of HEL by macrophages.

Antigen processing is a complex multi-step pathway involving internalization of antigen, cleavage of the antigen, formation of a peptide-MHC class II, and transport of the complex to the cell surface (Singer and Linderman, 1990; Germain, 1994; Xu and Pierce, 1995). Engagement of the G protein-coupled CB2 receptor inhibits adenylate cyclase activity (Howlett, 1995; Childers and Deadwyler, 1996) and decreases the level of intracellular cyclic AMP (Matsuda, 1997; Schatz et al., 1997). Treatment of B cells with dibutyryl cyclic AMP enhances their capacity to process antigens (Faassen and Pierce, 1995). Perhaps, the intracellular level of cyclic AMP may also regulate the efficiency of antigen processing by macrophages. A remaining question is what antigen processing step may be impaired by the potential signal transduced through the CB2 receptor.

CBs induce physiological changes in cells that may (Howlett, 1995; Matsuda, 1997) or may not be mediated by CB receptors (Pertwee, 1988; Felder et al., 1992; Derocq et al., 1998). Although the inactive enantiomer was not suppressive, CP-56,667 at the highest concentrations examined enhanced the level of secreted IL-2, which would not involve a CB receptor. A stimulatory effect of CBs that is receptor-independent is not a unique finding. For example, CP-55,940 can increase free arachidonic acid level without stereoselectivity (Felder et al., 1992). Furthermore, anandamide, which is an endogenous ligand of CB receptors, augments the replication of hematopoietic cells and activates mitogen-activated protein kinase by a mechanism that is independent of CB receptors (Derocq et al., 1998). In contrast, the active cannabinoids progressively diminished the T cell response reaching a maximum at 10 nM, but were less inhibitory at higher concentrations. Similarly, 3 nM THC maximally inhibits, whereas 3 μ M THC enhances, proinflammatory cytokine production by human monocytes (Berdyshev et al., 1997). Other investigators have also reported biphasic effects of THC on lymphocyte proliferation (Luo et al., 1992; Pross et al., 1992). Perhaps, at the higher active CB concentrations in our study, inhibition via the CB receptor may be partially negated by CB stimulation that is receptor-independent leading to less suppression. The final outcome at high active CB concentrations may be the combined effects of both receptor-mediated and receptor-independent mechanisms.

Identification of CB receptors has been achieved through the use of radiolabeled ligand binding assay to detect the proteins, and RT-PCR and Rnase protection for their mRNA. Radiolabeled ligand binding assays have limitations in detecting low levels of subtype-specific receptor proteins. On the other hand, RT-PCR has a potential difficulty when used to study intron-less genes such as those encoding CB1 and CB2 receptors, whereby genomic DNA amplification products would be the same size as products derived from cDNA. Thus,

the generated products could be derived from possible residual genomic DNA rather than from reverse transcribed mRNA. MRT-PCR involves a cDNA synthesis step using a long anti-sense RT primer that is specific for the mRNA of interest. The RT primer includes a single nucleotide substitution to generate a unique restriction site; this circumvents potential problems in distinguishing the products. In the present study, MRT-PCR indicated that clone 63 cells exclusively expressed mRNA for the CB2 receptor.

In summary, the macrophage hybridoma clone 63 cells expressed message for the CB2 but not CB1 receptor. The bioactive enantiomer CP-55,940 exerted differential suppression of cytokine secretion by the T cells when compared with its inactive stereoisomer CP-56,667. Finally, the CB2, but not CB1, selective antagonist reversed the decreased IL-2 secretion caused by THC. Collectively, the data indicate that the CB2 receptor may mediate inhibition of macrophage processing of HEL leading to a diminished helper T cell antigen response.

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