DNA AND CELL BIOLOGY
Volume 16, Number 3, 1997
Mary Ann Liebert, Inc.
Pp. 301–309

Induction of Interleukin-2 Receptor α Gene by Δ^9-Tetrahydrocannabinol Is Mediated by Nuclear Factor κB and CB1 Cannabinoid Receptor

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ABSTRACT

Previously, we reported that the cannabinoid Δ^9-tetrahydrocannabinol (THC) increased the expression of interleukin-2 (IL-2) receptor (R) α and β proteins and mRNAs in NKB61A2 cells, but decreased the level of the γ-chain message. The drug increased β-chain message stability rather than increased transcription. In the present study, we examined the mechanism responsible for the drug-induced increase in α-chain message in NKB61A2 cells. Nuclear run-on and mRNA stability studies showed THC increased the level of α gene transcription but had no effect on mRNA stability. Because expression of this gene is regulated by nuclear factor (NF)-κB, we next tested the drug effect on the nuclear level of this protein using the electromobility shift assay. These studies showed a drug-induced increase in NF-κB activity. To link the increased nuclear factor activity with the THC-induced increase in IL-2Rα message, antisense oligodeoxynucleotides were used to inhibit expression of the RelA component of NF-κB. These results showed anti-RelA antisense eliminated the cannabinoid-induced upregulation of both α mRNA and RelA protein. Furthermore, inhibition of the cannabinoid receptor type 1 with antisense oligomers also eliminated the drug effect on the α message. These results suggest that THC treatment of NKB61A2 cells increases IL-2Rα gene transcription by increasing the nuclear level of NF-κB through a mechanism involving cannabinoid receptor type 1 expression.

INTRODUCTION

EXPOSURE OF LYMPHOCYTES to cannabinoids results in modulation of immune responses (Specter *et al.*, 1990; Friedman *et al.*, 1994) but little is known regarding the molecular mechanisms involved. Several years ago we reported that proliferation of NKB61A2 cells in response to interleukin-2 (IL-2) was inhibited by Δ^9-tetrahydrocannabinol (THC) (Kawakami *et al.*, 1988a,b). At the molecular level, this suppression was subsequently shown to be due to a decrease in the number of high- and intermediate-affinity IL-2 binding sites (Zhu *et al.*, 1993). Interestingly, however, THC increased the levels of IL-2 receptor (R) α and β mRNAs but decreased the IL-2Rγ chain message (Zhu *et al.*, 1995).

The IL-2Rα gene encodes a highly glycosylated 55-kD protein (Leonard *et al.*, 1984) that is expressed in lymphoid (Waldman, 1993) and nonlymphoid cells (Plaisance *et al.*, 1992). Expression of the α-chain, and its subsequent association with the $\beta\gamma$ complex, is critical for the formation of high- (10^{-11} *M*) affinity IL-2 receptors (Kumaki *et al.*, 1993; Takeshita *et al.*, 1992; Taniguchi and Minami, 1993). IL-2Rα mRNA expression in resting T lymphocytes is undetectable but is efficiently induced upon cell activation. Expression of this message is dependent on the activity of multiple regulatory elements 5′ to the coding region, including the CArG box (bases −253 to −244) (Ballard *et al.*, 1988; Toledano *et al.*, 1990), the GC box (bases −245 to −240) (Ballard *et al.*, 1988; Pomerantz *et al.*, 1989; Roman *et al.*, 1990), and the κB enhancer element (bases −267 to −258) (Leung and Nabel, 1988; Ballard *et al.*, 1989). The κB enhancer element is engaged by a variety of inducers such as tumor necrosis factor-α (TNF-α) and the human T-cell leukemia virus type I (HTLV-I) (Ballard *et al.*, 1988; Leung and Nabel, 1988; Ruben et al., 1988) and contains the 11-bp sequence, 5′-GGGGAATCTCC-3′, which has striking homology to the κB

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enhancer of human immunodeficiency virus (HIV) long terminal repeat (LTR) and immunoglobulin κ chain. Transcriptional activation, mediated by the κB enhancer element, is initiated upon the binding of the protein nuclear factor, κB, or NF-κB. The NF-κB activity is very low or undetectable in most cells, except B lymphocytes, but can be induced by a variety of agents or stress conditions (Rice *et al.*, 1992; Devary *et al.*, 1993; Weih *et al.*, 1994). Also, NF-κB activation and translocation to the nucleus is regulated by IκB proteins. Upon activation, IκB is phosphorylated and dissociated from the NF-κB heterodimer. The transcriptionally active complex, p50/RelA heterodimer, then translocates to the nucleus and initiates transcription of the target gene (Baeuerle and Henkel, 1994).

The mechanism involved in THC effects on IL-2Rα gene activation is not clear. Moreover, it is not clear whether cannabinoid receptors (Matsuda *et al.*, 1990; Munro *et al.*, 1993; Shire *et al.*, 1995) play a role in this effect, although, recently, ligation of the CB1 receptor has been linked to the expression of immediate-early gene products (Bouaboula *et al.*, 1995a) and the activation of mitogen-activated protein kinases (Bouaboula *et al.*, 1995b). Here, we present evidence to support a signaling pathway composed of CB1, NF-κB, and IL-2Rα gene. Activation of the CB1 by THC increases transcriptionally active NF-κB that, in turn, mediates a drug-induced increase in the IL-2Rα mRNA. Inhibiting the expression of either CB1 or NF-κB results in a substantial decrease in the THC effect.

MATERIALS AND METHODS

Cell culture

NKB61A2 is a cloned, NK-like, IL-2 dependent, murine cell line (gift from Dr. G. Dennert, University of Southern California, Los Angeles, CA) (Warner and Dennert, 1982). Cells were grown in RPMI-1640 medium (GIBCO, Grand Island, NY) containing 10% fetal calf serum (FCS; Hyclone, Logan, UT), L-glutamine, antibiotics, β-mercaptoethanol, and 20 units/ml of human recombinant IL-2 (kindly provided by Hoffman La Roche, Nutley, NJ). Cells were harvested and reestablished in fresh medium 24 hr before each experiment (Zhu *et al.*, 1995).

Cannabinoid

THC was provided by the Research Technology Branch, National Institute on Drug Abuse (Rockville, MD) as a 98.6% tar. For use, the cannabinoid was initially dissolved in dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO) to a concentration of 150 mg/ml and stored under nitrogen gas at −20°C (Zhu *et al.*, 1995). At the time of experiments, the stock was further diluted in DMSO and finally in warm tissue culture medium.

Northern blotting and mRNA stability analysis

Ribonucleic acid samples were isolated from NKB61A2 cells using the Tri-reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions. Samples were denatured with glyoxal and DMSO (Sigma) followed by heating for 1 hr at 50°C. The RNA was fractionated in 1% agarose in 10 m*M* phosphate buffer, transferred to a Nytran membrane (Schleicher & Schuell, Keene, NH), air dried, and baked for 2 hr under vacuum. Filters were prehybridized at 55°C for 2 hr using Rapid-hyb buffer (Amersham Life Sciences, Arlington Heights, IL) followed by hybridization with an IL-2Rα cDNA (gift from Dr. T. Honjo, Kyoto University, Japan) and γ-actin cDNA (gift from Dr. P. Lai, Tampa Bay Research Institute, St. Petersburg, FL) probes labeled with [α-^{32}P]dCTP (DuPont NEN, Boston, MA) by random priming (Boehringer Mannheim, Indianapolis, IN). After hybridization, filters were thoroughly washed, exposed to Kodak X-Omat AR film (Eastman Kodak, Rochester, NY), and autoradiographed. For message stability assays, transcription was inhibited by adding actinomycin D (10 μg/ml, Sigma) at the start of incubation. Cell cultures were also treated at this time with either THC, DMSO, or tissue culture medium. At the end of each time point, cultures were harvested and the RNA analyzed by Northern blotting.

Nuclear run-on analysis

Cultures were incubated with IL-2 and either THC (5 μg/ml) or DMSO (0.05%) for 1 and 2 hr at 37°C, washed in PBS (Sigma), and nuclear extracts obtained and assayed as previously described (Greenberg and Ziff, 1984; Zhu *et al.*, 1995). Briefly, 7×10^6 NKB61A2 cells were treated with NP-40 lysis buffer (10 m*M* Tris-HCl pH 7.4, 10 m*M* NaCl, 3 m*M* $MgCl_2$, and 0.5% NP-40). The nuclear pellet was obtained by centrifugation (500 × *g*, 5 min at 4°C) and stored in 200 μl of glycerol storage buffer (50 m*M* Tris-HCl pH 8.3, 40% glycerol, 5 m*M* $MgCl_2$, 0.1 m*M* EDTA) in liquid nitrogen. Nuclei were mixed with 200 μl of reaction mixture of 10 m*M* Tris-HCl pH 8, 5 m*M* $MgCl_2$, 0.3 *M* KCl, 5 m*M* dithiothreitol (DTT), and 10 m*M* each of ATP, CTP, GTP, and 100 μCi [^{32}P]UTP (760 Ci/mmole, ICN, Irving, CA). After incubation at 30°C for 30 min, the material was treated with RNase-free DNase for 5 min at 30°C and proteinase K (Boehringer Mannheim) for 30 min at 42°C. The ^{32}P-labeled RNA was isolated by isopropanol ammonium acetate precipitation. IL-2Rα and γ-actin cDNAs (3 μg) were denatured and immobilized on Nytran membranes (Schleicher & Schuell) using a slot-blot apparatus (BRL, Gaithersburg, MD). The membranes were baked and hybridized with equal amounts (normalized by cpm) of the purified ^{32}P-labeled RNAs. The filters were washed, treated with RNase A, and then exposed to Kodak AR X-Omat films at −70°C.

Electromobility shift assay

The sequence-specific, DNA-binding proteins in crude extracts of NKB61A2 cell nuclei were determined by the mobility-shift assay (Ausubel, 1987; Fried, 1981). An oligonucleotide containing the NF-κB binding site (underlined)-5′-GTAGGGGACTTTCCGAGCTCGAGATCCTATG-3′ was made double stranded following incubation with the complementary oligonucleotide 5′-AGGATCTCGAGCTCGGAAAGTCCCCTAC-3′ in 10 m*M* ammonium acetate. The double-stranded DNA (dsDNA) was labeled with [α-^{32}P]dCTP and [α-^{32}P]dATP (DuPont NEN) by filling the protruding ends using Klenow fragment of DNA polymerase (Boehringer Mannheim) and used as the specific binding site for NF-κB. Nuclear extracts were prepared according to previously described methods (Dignam *et al.*, 1983; Levy *et al.*, 1988). Cel-

lular pellets were resuspended in five volumes of Buffer A [10 m*M* HEPES pH 7.9, 1.5 m*M* $MgCl_2$, 10 m*M* KCl, 0.5 m*M* DTT, and 0.5 m*M* phenylmethylsulfonyl fluoride (PMSF)] and lysed on ice using NP-40 (Sigma) at 0.5% final concentration. The resulting crude nuclei fraction was separated from cytoplasmic extract by centrifugation and the supernatant was mixed with 0.1 volume of Buffer B (0.3 *M* HEPES pH 7.9, 1.4 *M* KCl, 30 m*M* $MgCl_2$) and was considered the cytoplasmic fraction. The nuclei were washed in Buffer A and resuspended in Buffer C [20 m*M* HEPES pH 7.9, 20% glycerol (vol/vol), 0.42 *M* NaCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* EDTA, 0.5 m*M* PMSF, and 0.5 m*M* DTT] for 30 min. The preparation was centrifuged and the supernatant fluids dialyzed against Buffer D (20 m*M* HEPES pH 7.9, 20% glycerol, 0.1 *M* KCl, 0.2 m*M* EDTA, 0.5 m*M* PMSF, and 0.5 m*M* DTT) overnight at 4°C. The nuclear extracts (10 μg) and labeled probe (1 ng; 25,000 cpm) were mixed for 15 min at ambient temperature. Protein–DNA complexes were separated from free probe on a 4% nondenaturing, polyacrylamide gel run in 25 m*M* Tris-borate and 0.25 m*M* EDTA followed by autoradiography. Competitive binding studies were performed in the presence of 100-fold excess unlabeled, NF-κB oligomers.

Antisense oligodeoxynucleotide treatment

Cultures of NKB61A2 cells were established and preincubated for 24–30 hr with either antisense or sense oligodeoxynucleotides (ODNs) for the RelA protein or CB1 receptor. In the case of RelA, cells were treated with 10 μ*M* of either RelA

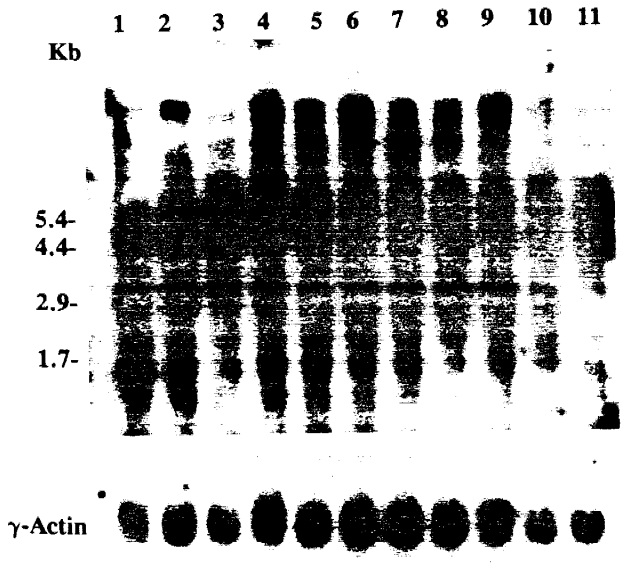

FIG. 1. THC has no effect on IL-2Rα message stability. NKB61A2 cells were incubated with IL-2 and either THC (10 μg/ml in DMSO) or DMSO in the presence of actinomycin D (10 μg/ml) for up to 5 hr or untreated (0 time). Total RNA was isolated from control cells (lane 1) or cells treated with actinomycin D plus drugs for: 30 min (lane 2, DMSO, lane 3, THC); 1 hr (lane 4, DMSO, lane 5, THC); 1.5 hrs (lane 6, DMSO, lane 7, THC); 3 hr (lane 8, DMSO, lane 9, THC); 5 hr (lane 10, DMSO, lane 11, THC), and Northern blotted. Filters were probed with IL-2Rα and γ-actin probes.

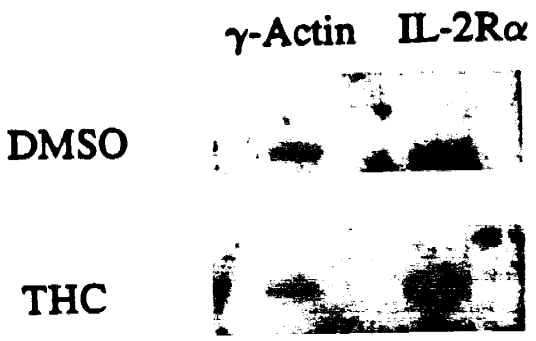

FIG. 2. THC increases the transcriptional activity of the IL-2Rα gene. NKB61A2 cells were incubated with IL-2 and either THC (5 μg/ml) or DMSO for 2 hr. Nuclei were isolated, message labeled *in vitro*, and radiolabeled RNA hybridized to filter-fixed cDNAs for IL-2Rα and γ-actin followed by autoradiography.

sense 5′-ACCATGGACGATCTGTTTCCCCTG-3′ as control, or RelA antisense 5′-CAGGGGAAACAGATCGTCCATGGT-3′ (Perez *et al.*, 1994). For CB1, 10 μ*M* ODN was used that was either complementary to the main AUG initiation codon 5′-CGACTTCATAACCTCCGTC-3′ or was the control sense strand 5′-GACGGAGGTTATGAAGTCG-3′ (Chakrabarti *et al.*, 1995; Shire *et al.*, 1995). In each experiment, cells were treated twice with fresh ODNs because of their short half-life of approximately 4–6 hr (Daaka and Wickstrom, 1990).

Metabolic labeling and immunoprecipitation

NKB61A2 cultures were preincubated with ODNs as described above. The cultures were then washed with warm PBS and incubated for 30 min with RPMI-1640 medium lacking methionine and cysteine but supplemented with 10% FCS and ODNs and then incubated for 2 hr with ^{35}S-labeled methionine and cysteine, THC, and ODNs (Zhu *et al.*, 1995). The cells were washed 3× with ice-cold PBS and solubilized in PBS containing 1% NP-40 and protease inhibitors (Zhu *et al.*, 1995). The cell lysates were clarified by centrifugation at 16,000 × *g* for 20 min and preadsorbed with a suspension of protein G *Staphylococcus aureus* at 4°C for 30 min. Bacteria were discarded after centrifugation and anti-RelA (5 μg/ml) antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was added to the supernatants and incubated at 4°C for 2 hr. Following incubation, protein G *S. aureus* was added and incubated for 1 hr at 4°C and the precipitates containing bacteria were washed and analyzed on 12% $NaDodSO_4$-PAGE. The gels were dried and exposed to X-Omat film, and the densities were analyzed by laser densitometry.

RESULTS

THC causes an increase in IL-2Rα gene transcription

Previously, we showed that exposure of the NKB61A2 cells to THC increased expression of the IL-2Rα protein (Zhu *et al.*, 1993) and message (Zhu *et al.*, 1995), suggesting that the

cannabinoid either increased gene transcription or decreased the rate of mRNA turnover. To investigate which mechanism was involved, we examined the turnover rate in THC-treated cells transcriptionally blocked by actinomycin D. Blocked cells were treated with IL-2 and either THC or DMSO for various times up to 5 hr, and RNA was extracted for Northern blotting with IL-2Rα cDNA. Figure 1 shows the characteristic four species of IL-2Rα message (Shimuzu *et al.*, 1985; Zhu *et al.*, 1995) and also shows that the amount of detected IL-2Rα mRNA remained steady in both drug- and vehicle-treated cells through 5 hr of testing. These stability studies suggested that THC treatment did not increase the level of mRNA by increasing its stability but by increasing transcription. To test transcription directly, we examined the drug effect in the nuclear run-on assay. NKB61A2 cells were treated with IL-2 and either THC or DMSO for 1 and 2 hr followed by isolation of intact nuclei. The rate of newly synthesized RNA was monitored by hybridizing radiolabeled nuclear RNA to slot-blotted IL-2Rα and γ-actin cDNAs. As shown in Fig. 2, THC increased the level of IL-2Rα message synthesis at 2 hr compared to the DMSO control, whereas the γ-actin control remained relatively constant. This increase in IL-2Rα transcription occurred also at 1 hr after drug addition and was observed upon exposure of cells to 10 μg/ml THC (data not shown).

THC increases NF-κB activity

The above results indicated that THC increased transcription of the IL-2Rα gene. Several reports in the literature suggest that increased transcription of this gene by extracellular stimuli is mediated by activation of the NF-κB complex (Baeuerle and Henkel, 1994). Therefore, we investigated the effect of THC

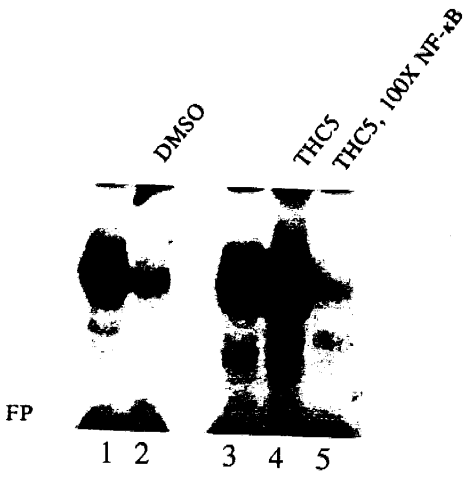

FIG. 3. THC treatment augments NF-κB activity. NKB61A2 cells were treated with IL-2, and either THC (5 μg/ml), DMSO, or tissue culture medium for 2 hr followed by isolation and extraction of cell nuclei. Nuclear proteins were incubated with radiolabeled dsDNA probe containing the κB binding site, and the protein–DNA complexes were separated from free probe by electrophoresis and autoradiographed. Lanes 1 and 3, IL-2 and culture medium (control); lane 2, IL-2 plus DMSO; lane 4, IL-2 plus THC 5 μg/ml; lane 5, same as lane 4 except treated with 100-fold excess unlabeled probe. The results are representative of five experiments.

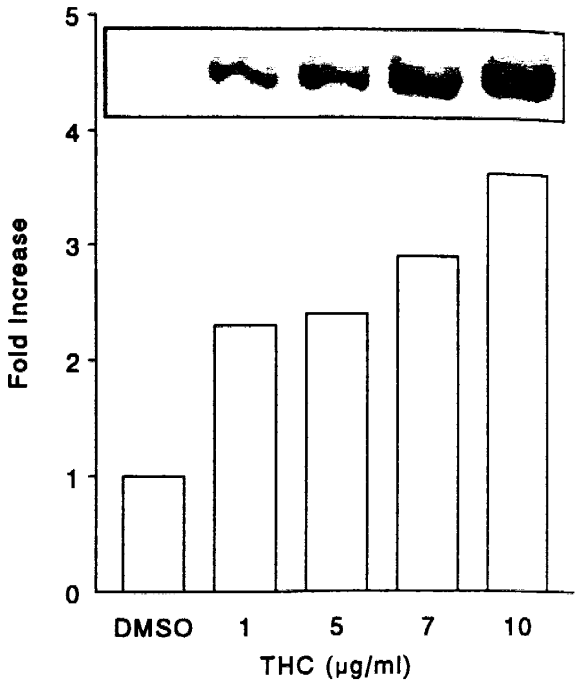

FIG. 4. Augmentation of the NF-κB activity by THC is dose dependent. NKB61A2 cells were treated with DMSO or varying amounts of THC for 2 hr at 37°C. Nuclear proteins were isolated and analyzed as in Fig. 3, and the blot densities of the DNA–protein complexes were measured by densitometry. The fold increase in density relative to the DMSO sample was recorded. The results are representative of four independent experiments.

treatment on NF-κB activity as measured by the electromobility shift assay (EMSA). NKB61A2 cells were treated with IL-2 and either THC (5 μg/ml), DMSO, or tissue culture medium for 2 hr followed by isolation and extraction of cell nuclei. Nuclear extracts and the labeled NF-κB probe were incubated together, resolved on native polyacrylamide gels, and visualized by autoradiography. Figure 3 shows the autoradiograph of several gel-shift samples. Lanes 1 and 3 are extracts from cells incubated in tissue culture medium only and show roughly equivalent amounts of retarded DNA–protein complex. The free probe (FP) is seen at the bottom of the gel. Treatment of cells with the drug vehicle (DMSO, lane 2) greatly reduced the amount of the labeled complex; however, addition of THC (5 μg/ml; lane 4) not only restored NF-κB activity to basal levels, but also increased it relative to cells incubated with culture medium alone.

Specificity of retarded DNA–protein complexes was examined by addition of excess unlabeled probe as a competitor. Results show reduced intensity of the retarded complex, indicating the sequence specificity of the complex (lane 5). In EMSA, the band intensity can be diminished by either limiting amounts of DNA-binding proteins or DNA probe. However, the intensity of our complexes was due to limiting proteins and not probe, as evident by the presence of labeled free probe at the ends of the lanes. To study the drug effect on NF-κB in greater detail, NKB61A2 cells were incubated with increasing concentrations of THC (1–10 μg/ml) for 2 hr and the extracts applied to EMSA. The blot densities of the DNA–protein complexes were analyzed by densitometry and the fold increase

relative to the DMSO sample was recorded. Figure 4 shows that 1 μg/ml of THC doubled the intensity of the retarded DNA–protein complex relative to DMSO and that the intensity doubled again following 10 μg/ml. These results show that THC treatment increases the nuclear level of NF-κB binding factors in a dose-dependent manner.

Inhibition of RelA expression eliminates THC increase of IL-2Rα mRNA

The above results suggested THC treatment increased IL-2Rα mRNA by increasing transcriptionally active NF-κB. To examine this relationship further, antisense ODNs to the RelA (p65) component of NF-κB were used. Cells were preincubated for 24 hr with either culture medium only or sense and antisense ODNs for RelA and then incubated for 3 hr with THC or DMSO in the presence of RelA ODNs. At the end of the incubation period, cells were washed, and total RNA was extracted and analyzed for IL-2Rα mRNA by Northern blotting. Figure 5 shows the results of these studies. Lanes 1 and 2 are from cells preincubated with culture medium only for 24 hr and show the characteristic four mRNA species for IL-2Rα (Shimuzu *et al.*, 1985; Zhu *et al.*, 1995) as well as the increase in message level in response to THC treatment (lane 2). The densitometry reading for lane 2 was approximately twice that of lane 1 when adjusted for the γ-actin densities. Preincubation with the sense ODN for RelA (lanes 3 and 4) followed by THC treatment also resulted in an approximate two-fold increase in IL-2Rα message; however, antisense preincubation attenuated the THC-induced increase in IL-2Rα (lanes 5 and 6). The reason for the increase in IL-2Rα message level following antisense treatment

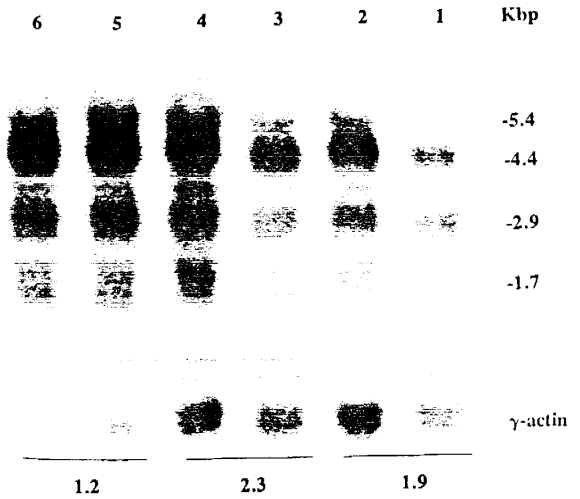

FIG. 5. Inhibition of RelA expression suppresses the THC effect on IL-2Rα. NKB61A2 cells were cultured as follows: lane 1, DMSO; lane 2, THC (10 μg/ml); lane 3, DMSO and RelA sense ODN; lane 4, THC and RelA sense ODN; lane 5, DMSO and RelA antisense ODN; lane 6, THC and RelA antisense ODN for 3 hr at 37°C. Total RNA was prepared and analyzed for IL-2Rα mRNA by Northern blotting. Band intensities were measured by densitometry and adjusted for γ-actin. The +THC/−THC ratios are indicated below the line for the three different groups. The sizes of IL-2Rα transcripts are indicated in kbp. The results are representative of three experiments.

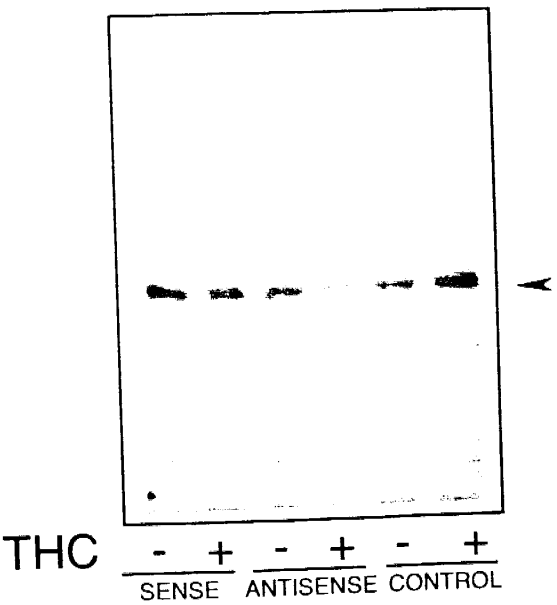

FIG. 6. RelA antisense attenuates the THC-induced increase in RelA protein. NKB61A2 cells were preincubated with RelA antisense, RelA sense, or medium only (control) and then radiolabeled and stimulated for 2 hr with THC or DMSO. Cell lysates were immunoprecipitated using anti-RelA antibody and analyzed by autoradiography and densitometry. The two left lanes are from cells treated with the sense ODN either with (+) or without (−) THC. The densitometry readings were 1.1 (−) and 1.4 (+). The middle two lanes are from antisense-treated cells with densitometry readings of 0.9 (−) and 0.6 (+). The last two lanes are from medium only pretreated cells with densitometry 1.0 (−) and 1.7 (+). The results are representative of two experiments.

(compare lane 5 with lanes 1 and 3) is not clear at this time. Others have reported a similar effect of nucleic acid preparations in other leukocyte populations; however, no mechanism was provided (Krieg *et al.*, 1995; Klinman *et al.*, 1996).

What is clear from these studies is that THC treatment in the presence of RelA antisense does not increase the level of IL-2Rα message relative to DMSO treatment as it does in the presence of sense ODN (lanes 3 and 4) or culture medium (lanes 1 and 2). This suggests that the THC effect on IL-2Rα involves RelA and the NF-κB system. Additional support for the linkage between RelA and the THC effect comes from immunoprecipitation studies with RelA antibody. In these studies, NKB61A2 cultures were preincubated with RelA ODN, metabolically labeled with ^{35}S amino acids, and stimulated with THC prior to immunoprecipitation for RelA protein and analysis by autoradiography. Figure 6 shows THC treatment increased the total cellular RelA protein in both culture medium (control) and sense ODN-treated cells; however, this increase was not observed in RelA antisense-treated cultures, suggesting that THC treatment increased the level of RelA protein and that RelA antisense inhibits this drug effect.

Inhibition of CB1 suppresses the THC-induced increase of IL-2Rα message

Because of the hydrophobic nature of THC, the drug could be affecting the NF-κB pathway by intercalating into the mem-

brane and modulating the function of a variety of membrane proteins. An alternative explanation is that the drug is working through cannabinoid receptors, and, in fact, we have preliminary data that NKB61A2 cells express CB1 protein (unpublished). Therefore, we wanted to establish a link between the THC effect and CB1 receptor function by knocking out receptor expression. To knock out CB1 receptor expression, cells were preincubated with CB1 antisense ODNs. The ODN sequences targeted the AUG start codon for CB1 (Chakrabarti *et al.*, 1995; Shire *et al.*, 1995). The NKB61A2 cells pretreated with ODNs were next incubated 3 hr with THC or DMSO in the presence of fresh antisense or sense DNA sequences. Cells were washed, and RNA was extracted and analyzed for IL-2Rα and γ-actin mRNA content by Northern blotting. Figure 7 shows densitometry ratios of the IL-2Rα to γ-actin blot densities for the various culture groups. It is evident that in both the control (culture medium preincubation) and sense ODN-treated groups, THC induced the typical two-fold increase of IL-2Rα message relative to DMSO control (−THC group). However, in the presence of specific antisense ODNs against CB1, the drug did not induce this increase over DMSO. These results suggest CB1 receptor expression is linked to the THC-induced increase in cellular IL-2Rα mRNA.

DISCUSSION

Cannabinoids and related agonists modulate a variety of molecular pathways in different cell types. For example, in cells

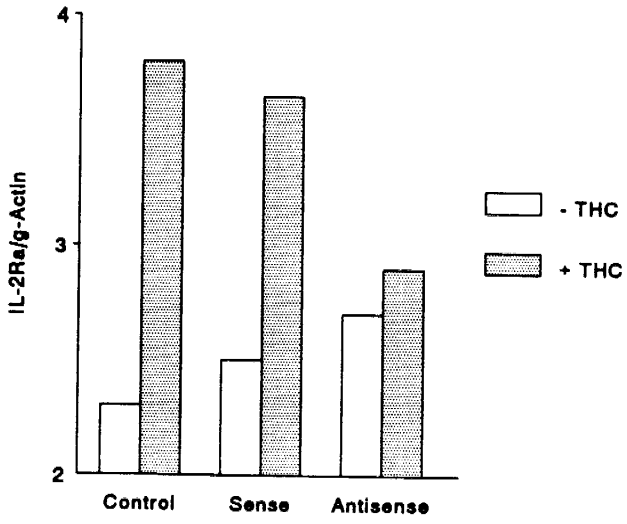

FIG. 7. Inhibition of CB1 suppresses the THC-induced increase in IL-2Rα message. NKB61A2 cells were cultured in either culture medium alone (control), in medium containing either DMSO (−THC) or THC, in CB1 sense ODNs (sense group), or in medium containing DMSO or THC and CB1 antisense ODNs (antisense group). Cells were then washed and total RNA was extracted and analyzed for IL-2Rα and γ-actin messages by Northern blotting. Band intensities were measured and adjusted for γ-actin. The IL-2Rα/γ-actin ratios are plotted for the various groups. The results are representative of three experiments.

of neuronal origin they reduce the forskolin-stimulated rise in cAMP through a G_i mechanism (Howlett, 1985; Howlett *et al.*, 1986) and inhibit N-type calcium channels (Mackie and Hille, 1992). In other cell types such as immune cells, effects on cAMP and calcium levels have been reported (Yebra *et al.*, 1992; Kaminski *et al.*, 1994). Cannabinoid agonists have also been shown to increase in various cell types the release of arachidonic acid (Burstein *et al.*, 1994), activation of MAP kinases (Bouaboula *et al.*, 1995b; Wartmann *et al.*, 1995), and expression of the lactoferrin gene (Das *et al.*, 1995), but the mechanism of these effects is unclear. Cannabinoid receptors are most certainly involved in some of these changes, although nonreceptor, membrane effects cannot be excluded (Charalambous *et al.*, 1992). Cannabinoid receptors are expressed in brain and in the periphery, especially in the immune system (Bouaboula *et al.*, 1993; Daaka *et al.*, 1995; Galieque *et al.*, 1995; Kaminski *et al.*, 1992), and transfection studies with the receptor genes have linked them to cAMP metabolism (Felder *et al.*, 1992; Slipetz *et al.*, 1995) and the expression of immediate-early genes such as *krox*-24 (Bouaboula *et al.*, 1995a).

Cannabinoid receptors are members of the G-protein-coupled receptor superfamily of proteins (Matsuda *et al.*, 1990; Munro *et al.*, 1993; Savarese and Fraser, 1992). These receptors regulate cell signaling by interacting with a membrane, heterotrimeric G-protein complex and causing an exchange of GTP for bound GDP at a site within the α-subunit of the G-protein. The separated α- and $\beta\gamma$-subunits regulate other proteins in the signaling cascades (Clapham, 1996). The spectrum of such proteins affected by cannabinoid receptor ligation is not currently known; however, several signaling pathways have been reported to be affected by cannabinoids (Howlett, 1985; Mackie and Hille, 1992; Burstein *et al.*, 1994; Bouaboula *et al.*, 1995a; Das *et al.*, 1995). Recently, we reported that THC affected the expression of IL-2R genes (Zhu *et al.*, 1995), which suggested to us that cannabinoids might be modulating the signaling pathways regulating these genes. Activation of the IL-2Rα gene as well as activation of the TNF-α gene and induction of apoptosis are all controlled in part by the transcription factor NF-κB (Leung and Nabel, 1988; Ballard *et al.*, 1989; Bessho *et al.*, 1994; Ito *et al.*, 1994). This has special significance in that cannabinoids, besides inducing IL-2Rα (Zhu *et al.*, 1995), also induce TNF-α (Shivers *et al.*, 1994), and apoptosis (Schwarz *et al.*, 1994). Moreover, NF-κB activity has been linked to cellular cAMP levels (Chen and Rothenberg, 1994) and a G-protein-coupled receptor (Kravchenko *et al.*, 1995). Therefore, we decided to study the THC effect on IL-2Rα pathway in greater detail to test for a link between cannabinoid receptor function and IL-2Rα gene expression.

Previously, we showed enhanced expression of IL-2Rα mRNA and protein in NKB61A2 cells following THC treatment, but did not establish if this was due to message stabilization or increased transcription (Zhu *et al.*, 1995). Therefore, we initially performed these studies, and the results presented in Figs. 1 and 2 demonstrate that the drug caused increased transcription rather than increased stability. These results differ from those obtained with IL-2Rβ wherein THC was found to increase message stability rather than transcription (Zhu *et al.*, 1995). Recently, it has also been shown that cannabinoids, including THC, can increase the message and protein levels of the immediate-early genes, *jun*B, *krox*-20, and *krox*-24; how-

ever, it was not determined whether or not this was due to an effect on gene transcription (Bouaboula *et al.*, 1995a). These increases were linked, however, to phosphorylation and activation of MAP kinases (Bouaboula *et al.*, 1995b).

The IL-2Rα gene promoter contains multiple regulatory regions targeted by immediate-early gene products such as NF-κB. To elucidate a possible role for NF-κB in the drug-mediated increase of IL-2Rα mRNA, we looked for a drug-induced increase in NF-κB activity using EMSA. Figures 3 and 4 show that DMSO (drug vehicle) decreased the DNA-binding activity whereas THC treatment increased it in a dose-dependent manner. The reason for the DMSO effect is not clear at this time; however, this observation is consistent with results previously reported by Kelly *et al.* (1994). Our finding that THC increases the transcription factor NF-κB is similar to that reported for the transcription factor *krox*-24 (Bouaboula *et al.*, 1995). In those studies, astrocytoma cells treated with CP55,940 were shown to increase the *krox*-24 DNA-binding activity and *krox*-24 protein. The drug affect was linked to CB1 activity, and it was also concluded that the CB1 was linked to the activation of the transcription factor by a pertussis toxin-sensitive GTP-binding protein but was independent of the action of cAMP (Bouaboula *et al.*, 1995a). It is not known at this time if pertussis toxin attenuates the THC effect on NF-κB and IL-2Rα; however, it has been reported that increasing cAMP in T cells decreased NF-κB (Chen and Rothernberg, 1994) and also decreased the expression of IL-2Rα message (Krause and Deutsch, 1991). Thus, it is reasonable to speculate that a decrease in cAMP mediated by cannabinoids and CB1 (Howlett and Fleming, 1984; Howlett, 1985) might cause an increase in NF-κB and IL-2Rα. However, as stated above, a role for cAMP was not established in the CB1-mediated increase in *krox*-24 (Bouaboula *et al.*, 1995a)

To learn more about the mechanisms linking THC and CB1 to increases in NF-κB and IL-2Rα, antisense ODN methods were used to knock out expression of the RelA component of NF-κB and then reexamine the THC effect on IL-2Rα. Results presented in Figs. 5 and 6 clearly show that THC can increase RelA in the cells and that the THC effect on IL-2Rα mRNA is closely linked to the expression of RelA protein. Antisense studies were also used to examine the possible linkage between CB1 receptor and IL-2R expression. We had already observed that NKB61A2 cells expressed CB1 message (Daaka *et al.*, 1995), and, therefore, we wanted to see if suppressing the activity of this message with antisense ODNs would attenuate the THC effect on IL-2Rα message level. Figure 7 shows that CB1 antisense did attenuate the drug-induced increase in IL-2R. Taken together, these antisense studies suggest a signaling pathway in NKB61A2 cells consisting of CB1, NF-κB, and the IL-2Rα promoter.

Activation of NF-κB by THC could be initiated by mechanisms other than CB1. For example, it has been reported that alterations in cytoskeletal architecture activate NF-κB and induce NF-κB-specific genes (Rosette and Karin, 1995). Because THC is known to perturb cytoplasmic membranes (Charalambous *et al.*, 1992), the possibility exists that it may alter the cytoskeleton through this mechanism and therefore alter NF-κB activity. TNF-α might also be involved. This cytokine is known to increase NF-κB activity (Ito *et al.*, 1994) and, as already stated, we have observed that THC increases the production and release of this cytokine in immune cells (Shivers *et al.*, 1994). Furthermore, NKB61A2 cells contain TNF-α-type II receptors (Y.D., unpublished) and it is possible that they also produce TNF in response to THC. If this is the case, an autocrine mechanism can be envisioned in which THC causes an increase in TNF signaling that culminates in an increase of the NF-κB activity. Finally, several reports have demonstrated an increase in NF-κB activity mediated by ceramides (Pushkareva *et al.*, 1995). Because structural and functional similarities between THC, endotoxin, and ceramide can be proposed (Wright and Kolesnick, 1995), it is tempting to postulate a common, shared signaling pathway between THC, ceramide, and endotoxin that may explain the increase in NF-κB observed in our studies. A role for CB1, however, in this pathway is not clear.

The increase of NF-κB activity mediated by THC may have implications in the development of acquired immunodeficiency syndrome (AIDS). It has been suggested that drug abusers infected with the HIV virus develop AIDS faster than HIV-positive individuals not abusing drugs. The evidence supporting this is limited and the mechanism underlying such an effect remains unknown. From our results, however, it is possible to speculate that marijuana abuse might augment AIDS development due to an increase in NF-κB, which is known to activate the HIV genome and increase retroviral replication (Pierce *et al.*, 1988). The possibility that cannabinoids as well as other drugs of abuse can alter significantly the levels of various transcription factors suggests they might be involved as co-factors in disease and that this aspect of drug action should be given special attention in future studies.

ACKNOWLEDGMENTS

This work was supported by public health service grants DA03646 and DA07245 from the National Institute on Drug Abuse.

REFERENCES

BAEUERLE, P.A., and HENKEL, T. (1994). Function and activation of NF-κB in the immune system. Annu. Rev. Immunol. **12,** 141–179.

BALLARD, D.W., BOHNLEIN, E., LOWENTHAL, J.W., WANO, Y., FRANZA, B.R., and GREENE, W.C. (1988). HTLV-I tax induces cellular proteins that activate the κB element in the IL-2 receptor α gene. Science **241,** 1652–1655.

BALLARD, D.W., BOHNLEIN, E., HOFFMAN, J.A., BOGERD, H.P., DIXON, E.P., FRANZA, B.R., and GREENE, W.C. (1989). Activation of the-interleukin-2 receptor α gene: regulatory role for DNA-protein interactions flanking the κB enhancer. New Biol. **1,** 83–92.

BESSHO, R., MATSUBARA, K., KUBOTA, M., KUWAKADO, K., HIROTA, H., WAKAZONO, Y., LIN, Y., OKUDA, A., KAWAI, M., NISHIKOMORI, R., and HEIKE, T. (1994). Pyrrolidine dithiocarbamate, a potent inhibitor of nuclear factor kB (NF-kB) activation, prevents apoptosis in human promyelocytic leukemia HL-60 cells and thymocytes. Biochem. Pharm. **48,** 1883–1889.

BOUABOULA, M., BOURRIE, B., RINALDI-CARMONA, M., SHIRE, D., LEFUR, G., and CASELLAS, P. (1995a). Stimulation of cannabinoid receptor CB1 induces *knox-24* expression in human astrocytoma cells. J. Biol. Chem. **270,** 13973–13980.

BOUABOULA, M., POINOT-CHAZEL, C., BOURRIE, B., CANAT, X., CALANDRA, B., RINALDI-CARMONA, M., LEFUR, G., and CASELLAS, P. (1995b). Activation of mitogen-activated protein ki-

nases by stimulation of the central cannabinoid receptor CB1. Biochem. J. **312,** 637–641.

BOUABOULA, M., RINALDI, M., CARAYON, P., CARILLON, C., DELPECH, B., SHIRE, D., LEFUR, G., and CASELLAS, P. (1993). Cannabinoid-receptor expression in human leukocytes. Eur. J. Biochem. **214,** 173–180.

BURSTEIN, S., BUDROW, J., DEBATIS, M., HUNTER, S.A., and SUBRAMANIAN, A. (1994). Phopholipase participation in cannabinoid-induced release of free arachidonic acid. Biochem. Pharmacol. **48,** 1253–1264.

CHAKRABARTI, A., ONAIVI, E., and CHAUDHURI, G. (1995). Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. DNA Sequence **5,** 385–388.

CHARALAMBOUS, A., YAN, G., HOUSTON, D.B., HOWLETT, A.C., COMPTON, D.R., MARTIN, B.R., and MAKRIYANNIS, A. (1992). 5′Azido-Δ^8-THC:A novel photoaffinity label for the cannabinoid receptor. J. Med. Chem. **35,** 3076–3079.

CHEN, D., and ROTHENBERG, E.V. (1994). Interleukin 2 transcription factors as molecular targets of cAMP inhibition: Delayed inhibition kinetics and combinatorial transcription roles. J. Exp. Med. **179,** 931–942.

CLAPHAM, D.E. (1996). The G-protein nanomachine. Nature **379,** 297–299.

DAAKA, Y., and WICKSTROM, E. (1990). Target dependence of antisense oligodeoxynucleotide inhibition of c-Ha-ras p21 expression and focus formation in T24-transformed NIH3T3 cells. Oncogene Res. **5,** 267–275.

DAAKA, Y., KLEIN, T.W., and FRIEDMAN, H. (1995). Expression of cannabinoid receptor mRNA in murine and human leukocytes. In *Advances in Experimental Medicine and Biology*, B. Sharp, T.K. Eisenstein, J.J. Madden, and H. Friedman, eds. (Plenum Press, New York), pp. 91–96.

DAS, S.K., PARIA, B.C., CHAKRABORTY, I., and DEY, S.K. (1995). Cannabinoid ligand-receptor signaling in the mouse uterus. Proc. Natl. Acad. Sci. USA **92,** 4332–4336.

DEVARY, Y., ROSETTE, C., DIDONATO, J.A., and KARIN, M. (1993). NF-kappa B activation by ultraviolet light not dependent on nuclear signal. Science **261,** 1442–1445.

DIGNAM, J.D., LEBOVITZ, R.M., and ROEDER, R.G. (1983). Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11,** 1475–1489.

FELDER, C.C., VELUZ, J.S., WILLIAMS, H.L., BRILEY, E.M., and MATSUDA, L.A. (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,** 838–845.

FRIED, M. and CROTHERS, D.M. (1981). Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9,** 6505–6525.

FRIEDMAN, H., SHIVERS, S., and KLEIN, T.W. (1994). Drugs of abuse and the immune system. In *Immunotoxicology and Immunopharmacology*, 2nd ed. J.H. Dean, M.I. Luster, A.E. Munson, and I. Kimber, eds. (Raven Press, New York), pp. 303–322.

GALIEQUE, S., MARY, S., MARCHAND, J., DUSSOSSOY, D., CARRIERE, D., CARAYON, P., BOUABOULA, M., SHIRE, D., LEFUR, G., and CASELLAS, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur. J. Biochem. **232,** 54–61.

GREENBERG, M., and ZIFF, E. (1984). Stimulation of 3T3 cells induced transcription of the c-fos protooncogene. Nature **311,** 433–438.

HOWLETT, A.C. (1985). Cannabinoid inhibition of adenylate cyclase: Biochemistry of the response in neuroblastoma cell membranes. Mol. Pharmacol. **27,** 429–436.

HOWLETT, A.C., and FLEMING, R.M. (1984). Cannabinoid inhibition of adenylate cyclase: Pharmacology of the response in neuroblastoma cell membranes. Mol. Pharmacol. **26,** 532–538.

HOWLETT, A.C., QUALY, J.M., and KHACHATRIAN, L.L. (1986). Involvement of G_i in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol. Pharmacol. **29,** 307–313.

ITO, C.Y., KAZANTSEV, A.G., and BALDWIN, A.S. (1994). Three NF-κB sites in the Iκ B-α promoter are required for induction of gene expression by TNF. Nucleic Acids Res. **22,** 3787–3792.

KAMINSKI, N., KOH, W.S., YANG, K.H., LEE, M., and KESSLER, F.K. (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. Pharm. **48,** 1899–1908.

KAMINSKI, N.E., ABOOD, M.E., KESSLER, F.K., MARTIN, B.R., and SCHATZ, A.R. (1992). Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. Mol. Pharmacol. **42,** 736–742.

KAWAKAMI, Y., KLEIN, T.W., NEWTON, C., DJEU, J.Y., SPECTER, S., and FRIEDMAN, H. (1988a). Suppression by delta-9-tetrahydrocannabinol of interleukin 2-induced lymphocyte proliferation and lymphokine-activated killer cell activity. Int. J. Immunopharmacol. **10,** 485–488.

KAWAKAMI, Y., KLEIN, T.W., NEWTON, C., McCARTHY, C.A., DJEU, J., DENNERT, G., SPECTER, S., and FRIEDMAN, H. (1988b). Suppression by cannabinoids of a cloned cell line with natural killer cell activity. Proc. Soc. Exp. Biol. Med. **187,** 355–359.

KELLY, K.A., HILL, M.R., YOUKHANA, K., WANDER, F., and GIMBLE, J.M. (1994). Dimethyl sulfoxide modulates NF-κB and cytokine activation in lipopolysaccharide-treated murine macrophages. Infect. Immun. **62,** 3122–3128.

KLINMAN, D., YI, A.-K., BEAUCAGE, S.L., CONOVER, J., and KRIEG, A.M. (1996). CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ. Proc. Natl. Acad. Sci. USA **93,** 2869–2883.

KRIEG, A.M., YI, A-K, MATSON, S., WALDSCHMIDT, T.J., BISHOP, G.A., TEASDALE, R., KORETZKY, G.A., and KLINMAN, D. (1995). CpG motifs in bacterial DNA trigger direct B-cell activation. Nature **374,** 546–549.

KRAUSE, D.S., and DEUTSCH, C. (1991). Cyclic AMP directly inhibits IL-2 receptor expression in human T cells: Expression of both p55 and p75 subunits is affected. J. Immunol. **146,** 2285–2294.

KRAVCHENKO, V.V., PAN, Z., HAN, J., HERBERT, J.-M., ULEVITCH, R.J., and YE, R.D. (1995). Platelet-activating factor induces NF-κB activation through a G protein-coupled pathway. J. Biol. Chem. **270,** 14928–14934.

KUMAKI, S., KONDO, M., TAKESHITA, T., ASAO, H., NAKAMURA, M., and SUGAMURA, K. (1993). Cloning of the mouse interleukin 2 receptor chain: Demonstration of a functional difference between the mouse and human receptors. Biochem. Biophy. Res. Commun. **193,** 356–363.

LEONARD, W.J., DEPPER, J.M., CRABTREE, G.R., RUDIKOF, S., PUMPHREY, J., ROBB, R., KRONKE, M., SVETLIK, P.B., PEFFER, N.J., WALDMAN, T.A., and GREENE, W.C. (1984). Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. Nature **311,** 626–631.

LEUNG, K., and NABEL, G.J. (1988). HTLV-1 transactivator induces interleukin-2 receptor expression through an NF-κB-like factor. Nature **333,** 776–778.

LEVY, D.E., KESSLER, D.S., PINE, R., REICH, N., and DARNELL, J.E., Jr. (1988). Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. Genes & Dev. **2,** 383–393.

MACKIE, K., and HILLE, B. (1992). Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. Proc. Natl. Acad. Sci. USA **89,** 3825–3829.

MATSUDA, L.A., LOLAIT, S.J., BROWNSTEIN, M.J., YOUNG, A.C., and BONNER, T.I. (1990). Structure of cannabinoid receptor and functional expression of the cloned cDNA. Nature **346,** 561–564.

MUNRO, S., THOMAS, K.L., and ABU-SHAAR, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. Nature **365,** 61–65.

PEREZ, J.R., HIGGINS-SOCHASKI, K.A., MALTESE, J.-Y., and NARAYANAN, R. (1994). Regulation of adhesion and growth of fibrosarcoma cells by-NF-κ B RelA involves transforming growth factor β. Mol. Cell. Biol. **14,** 5326–5332.

PIERCE, J.W., LENARDO, M., and BALTIMORE, D. (1988). Oligonucleotide that binds nuclear factor NF-κB acts as a lymphoid-specific and inducible enhancer element. Proc. Natl. Acad. Sci. USA **85,** 1482–1486.

PLAISANCE, S., RUBINSTEIN, E., ALILECHE, A., SAHRAOUI, Y., KRIEF, P., AUGERY-BOURGET, Y., JASMIN, C., SUAREZ, H., and AZZARONE, B. (1992). Expression of interleukin-2 receptor on human fibroblasts and its biological significance. Int. Immunol. **4,** 739–746.

POMERANTZ, J.L., MAUXION, F., YOSHIDA, M., GREENE, W.C., and SEN, R. (1989). A second sequence element located 3′ to the NF-κB binding site regulates IL-2 receptor-α gene induction. J. Immunol. **143,** 4275–4281.

PUSHKAREVA, M., OBEID, L.M., and HANNUN, Y.A. (1995). Ceramide: An endogenous regulator of apoptosis and growth suppression. Immunol. Today **16,** 294–297.

RICE, N.R., MACKECHAN, M.L., and ISRAEL, A. (1992). The precursor of NF-κB p50 has Iκ B-like function. Cell **71,** 243–253.

ROMAN, D.G., TOLEDANO, M.B., and LEONARD, W.J. (1990). Sp1 represses IL-2 receptor α chain gene expression. New Biol. **2,** 642–647.

ROSETTE, C., and KARIN, M. (1995). Cytoskeletal control of gene expression: depolymerization of microtubules activates NK-κB. J. Cell Biol. **128,** 1111–1119.

RUBEN, S., POTEAT, H., TAN, T.H., KAWAKAMI, K., ROEDER, R., HASELTINE, W., and ROSEN, C.A. (1988). Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-I tax gene product. Science **241,** 89–92.

SAVARESE, T.M., and FRASER, C.M. (1992). *In vitro* mutagenesis and the search for structure-function relationships among G protein-coupled receptors. Biochem. J. **283,** 1–19.

SCHWARZ, H., BLANCO, F.J., and LOTZ, M. (1994). Anandamide, an endogenous cannabinoid receptor agonist inhibits lymphocyte proliferation and induces apoptosis. J. Neuroimmunol. **55,** 107–115.

SHIMUZU, A., KONDO, S., TAKEDA, S., YODOI, J., ISHIDA, N., SABE, H., OSAWA, H., DIAMANSTEIN, T., NIKAIDA, T., and HONJO, T. (1985). Nucleotide sequence of mouse IL-2 receptor cDNA and its comparison with the human IL-2 receptor sequence. Nucleic Acids Res. **13,** 1505–1516.

SHIRE, D., CARILLON, C., KAGHAD, M., CALANDRA, B., RINALDI-CARMONA, M., LEFUR, G., CAPUT, D., and FERRARA, P. (1995). An amino- terminal variant of the central cannabinoid receptor resulting from alternative splicing. J. Biol. Chem. **270,** 3726–3731.

SHIVERS, S.C., NEWTON, C., FRIEDMAN, H., and KLEIN, T.W. (1994). Δ^9-Tetrahydrocannabinol (THC) modulates IL-1 bioactivity in human monocyte/macrophage cell lines. Life Sci. **54,** 1281–1289.

SLIPETZ, D.M., O'NEILL, G.P., FAVREAU, L., DUFRESNE, C., GALLANT, M., GAREAU, Y., GUAY, D., LABELLE, M., and METTERS, K.M. (1995). Activation of the human peripheral cannabinoid receptor results in inhibition of adenylyl cyclase. Mol. Pharmacol. **48,** 352–361.

SPECTER, S., LANCZ, G., and FRIEDMAN, H. (1990). Marijuana and immunosuppression in man. In *Drugs of Abuse and Immune Function.* R.R. Watson, ed. (CRC Press, Boca Raton, FL), pp. 73–85.

TAKESHITA, T., ASAO, H., AHTANI, K., ISHII, N., KUMAKI, S., TANAKA, N., MUNAKATA, H., NAKAMURA, M., and SUGAMURA, K. (1992). Cloning of the γ chain of the human IL-2 receptor. Science **257,** 379–382.

TANIGUCHI, T., and MINAMI, Y. (1993). The IL-2/IL-2 receptorsystem: a current overview. Cell **73,** 5–8.

TOLEDANO, M.B., ROMAN, D.G., HALDEN, N.F., LIN, B.B., and LEONARD, W.J. (1990). The same target sequences are differentially important for activation of the interleukin 2 receptor α-chain gene in two distinct T-cell lines. Proc. Natl. Acad. Sci. USA **87,** 1830–1834.

WALDMAN, T.A. (1993). The IL-2/IL-2 receptor system: a target for rational immune intervention. Immunol. Today **14,** 264–269.

WARNER, J.F., and DENNERT, G. (1982). Effect of a clonal cell line with NK activity on bone marrow transplants, tumor development and metastasis *in vivo.* Nature **300,** 31–34.

WARTMANN, M., CAMPBELL, D., SUBRAMANIAN, A., BURSTEIN, S., and DAVIS, R.J. (1995). The MAP kinase signal transduction pathway is activated by the endogenous cannabinoid anandamide. FEBS Lett. **359,** 133–136.

WEIH, F., CARRASCO, D., and BRAVO, R. (1994). Constitutive and inducible Rel/NF-κB activities in mouse thymus and spleen. Oncogene **9,** 3289–3297.

WRIGHT, S.D., and KOLESNICK, R.N. (1995). Does endotoxin stimulate cells by mimicking ceramide? Immunol. Today **16,** 297–302.

YEBRA, M., KLEIN, T.W., and FRIEDMAN, H. (1992). Δ^9-Tetrahydrocannabinol suppresses concanavalin A induced increase in cytoplasmic free calcium in mouse thymocytes. Life Sci. **51,** 151–160.

ZHU, W., IGARASHI, T., QI, Z.-T., NEWTON, C., WIDEN, R.E., FRIEDMAN, H., and KLEIN, T.W. (1993). Delta-9-tetrahydrocannabinol (THC) decreases the number of high and intermediate affinity IL-2 receptors of the IL-2 dependent cell line NKB61A2. Int. J. Immunopharmacol. **15,** 401–408.

ZHU, W., IGARASHI, T., FRIEDMAN, H., and KLEIN, T.W. (1995). Δ^9-Tetrahydrocannabinol (THC) causes the variable expression of IL2 receptor subunits. J. Pharmacol. Exp. Therap. **274,** 1001–1007.

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Received for publication May 22, 1996; accepted October 11, 1996.