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Δ^9-Tetrahydrocannabinol Enhances the Secretion of Interleukin 1 from Endotoxin-Stimulated Macrophages[1]

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ABSTRACT

Interleukin (IL) 1 is a pleiotropic cytokine and an important mediator of various physiological responses including the acute phase response, inflammation, lymphocyte function and certain central nervous system responses. Because δ^9-tetrahydrocannabinol (THC) treatment also has been reported to affect these physiological responses, we tested the drug effect on IL1 production and secretion. Addition of THC to endotoxin (ETX)-treated murine, resident peritoneal macrophage cultures increased, in a dose-dependent manner, supernatant IL1 activity over ETX only treatment. Treatment with THC alone had no effect. Enzyme-linked immunosorbent assay studies and specific antibody neutralization studies demonstrated both IL1α and IL1β were increased by drug treatment. The steady-state levels of cellular IL1α and IL1β mRNAs, determined by Northern blotting and reverse transcription-polymerase chain reaction, were unchanged, suggesting the possibility THC was not increasing IL1 production. To examine this possibility further, ETX-activated macrophages, pulsed-labeled with ^{35}S-methionine, were chased for 2, 4 and 6 hr in the presence of THC and the levels of the various IL1 bioforms determined by immunoprecipitation. These results showed THC treatment had no effect on the level of ETX-induced intracellular promature IL1α and IL1β proteins; however, a THC-induced increase and prolongation of release of promature IL1α and mature IL1β were observed. The immunoprecipitation results were confirmed by studies examining supernatant bioactivity. These results suggest THC augments the ETX-induced processing of IL1β and release of IL1α rather than increasing the cellular production of IL1 protein. The molecular mechanisms responsible for this change and the biological consequences of drug-induced increases in IL1 release are currently under investigation.

THC has been reported to modify various physiological functions such as the acute phase response (Klein *et al.*, 1993), immunity (Martin, 1986; Klein and Friedman, 1990; Specter *et al.*, 1990) and central nervous system function (Martin *et al.*, 1992). Furthermore, an involvement of altered arachidonic acid metabolism (Burstein *et al.*, 1984) and phosphoinositide breakdown (Chaudhry *et al.*, 1988) accounting for THC effects has been suggested. IL1 is a pleiotropic cytokine with a variety of biological activities including those listed above associated with THC (Di Giovine and Duff, 1990). In addition, IL1 production is regulated by arachidonic acid metabolites (Kunkel *et al.*, 1986). It seems reasonable to suspect, therefore, that IL1 is involved in some of the biological effects of THC and, in fact, a preliminary report has appeared suggesting the drug increases macrophage IL1 (Klein and Friedman, 1990).

IL1 exists as two distinct forms, IL1α and IL1β, which are encoded by different genes, share biological activities, but little sequence homology (Rubartelli *et al.*, 1993). Both forms are translated as precursor molecules (31–33 kDa) which undergo post-translational processing into mature forms of 17 kDa (Giri *et al.*, 1985; March *et al.*, 1985). The bulk of biological activity is associated with the precursor form of IL1α and the mature form of IL1β. IL1 proteins lack a signal sequence and are released through a secretory pathway bypassing translocation to the endoplasmic reticulum (Rubartelli *et al.*, 1990). Processing and secretion appear to be linked and dependent upon mechanisms involving PKC activation (Bakouche *et al.*, 1992), exocytosis, protease activation and apoptosis (Dinarello and Wolff, 1993). Production is regulated by arachidonic acid metabolites (Kunkel *et al.*, 1986). In the current report we describe studies which establish THC as a facilitator of IL1 release rather than an enhancer of IL1 production by using a macrophage culture system stimulated with the microbial inflammatory substance, ETX.

Materials and Methods

Animals. Female BALB/c mice, 8- to 12-weeks old, and female C^{3}H/HeJ mice, 4- to 8-weeks old (Jackson Laboratory, Bar Harbor,

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ABBREVIATIONS: THC, Δ^9-tetrahydrocannabinol; IL, interleukin; PKC, protein kinase C; ETX, endotoxin; DMSO, dimethylsulfoxide; PBS, phosphate-buffered saline; RPMI, Rosswell Park Memorial Institute; ELISA, enzyme-linked immunosorbant assay; SDS, sodium dodeyl sulfate; RT-PCR, reverse transcription-polymerase chain reaction; PAGE, polyacrylamide gels; cAMP, cyclic AMP.

ME), were used for these studies and kept under pathogen-free conditions.

Marijuana components. THC was obtained from the National Institute on Drug Abuse (Rockville, MD) in absolute ethanol. For use, the alcohol was evaporated with nitrogen gas and THC was resuspended in DMSO (Sigma Chemical Co., St. Louis, MO). It was further diluted to the desired concentrations in warm tissue culture medium.

Preparation of peritoneal macrophages and generation of IL1. Resident peritoneal macrophages were obtained from BALB/c mice by peritoneal lavage with 5 ml of Dulbecco's PBS (Sigma). After washing 3 times in Hank's balanced salt solution, the cell concentration was adjusted to 1×10^6/ml in RPMI 1640 medium (Gibco, Grand Island, NY) containing 10% fetal calf serum (Hyclone Laboratories, Logan, UT), L-glutamine, antibiotics and 2-mercaptoethanol (complete RPMI 1640 medium). The cell suspension (1 ml) was added to individual wells of a 24-well tissue culture plate (Costar, Cambridge, MA). After a 2-hr incubation, nonadherent cells were removed by rinsing the plate and the adherent cell cultures were treated with 10 μg/ml of ETX (from *E. Coli*; Sigma) and the other test compounds (THC and DMSO). Cells were incubated at 37°C in 5% CO_2 atmosphere for various times. In some experiments, after the 2-hr adherence step, the cells were treated with ETX (10 μg/ml) for 2 hr followed by washing the cells with warm medium and incubation with THC for various times. In other experiments, after 2 hr of adherence, THC was incubated with macrophages for 24 hr followed by washing of the cells and incubation with ETX for additional 24 hr.

IL1 bioassay. Portions (0.1 ml) of macrophage culture supernatants were added to individual wells of 96-well flat bottom plates along with 1.5×10^6 thymocytes (0.1 ml) from C^3H/HeJ mice plus 0.2 μg/ml of Con A (Sigma). The cultures were incubated for 48 hr, pulsed for 18 hr with 0.5 μCi of [^{3}H]thymidine (2.0 Ci/mmol; ICN, Irving, CA), harvested on glass fiber filters and the incorporated radioactivity was determined by liquid scintillation counting. In some experiments, anti-IL1α and anti-IL1β antibodies (hamster monoclonals, Genzyme, Cambridge, MA) were diluted (1:100 final concentration) and added to supernatant aliquots for cytokine neutralization studies. These mixtures were incubated for 1 hr at 4°C before testing in the bioassay.

IL1α ELISA. Microtiter plates (Costar) were coated with 50 μl of hamster anti-IL1α antibody, 10 μg/ml (Genzyme), in PBS for 2 hr at 37°C. Plates were washed with PBS and blocked with PBS + 0.05% Tween 20 + 0.5% bovine serum albumin for 30 min at 37°C. The samples (50 μl) and the serial dilution of rIL1α (Genzyme) were incubated for 1 hr at 37°C. Rabbit anti-IL1α (50 μl, Genzyme) antibody was added and incubated for 1 hr at 37°C, and 50 μl of goat antirabbit immunoglobulin antibody conjugated to alkaline phosphatase was then added for 30 min at 37°C. Each of these steps was followed with three washes with doubled distilled water. Finally, the plates were developed (1 mg/ml of *p*-nitrophenyl phosphate in diethanolamine buffer) and read in a Microplate reader (Molecular Devices, Menlo Park, CA).

mRNA isolation and Northern blot analysis. For mRNA isolation, 2.5×10^6 resident macrophages were incubated with ETX and THC or DMSO for 2, 4, 6 and 24 hr. mRNA was isolated by using the Micro-Fast Tract mRNA Isolation Kit (Invitrogen, San Diego, CA), size fractionated in 1% agarose gel after denaturation with glyoxal and DMSO and blotted onto Nytran membranes (Schleicher & Schuell, Keene, NH). The filters were baked and hybridized at 55°C for 2 hr by using rapid hybridization buffer (Amersham, Arlington Heights, IL). The murine oligonucleotide IL1α and IL1β probes (Clontech Laboratories, Inc., Palo Alto, CA) were labeled by random-priming labeling system (Boehringer Mannheim, Indianapolis, IN). After hybridization, filters were washed 3 times at room temperature in 30 mM sodium chloride and 3.0 mM sodium citrate containing 0.1% SDS followed by two washes at 55°C in 1.5 mM sodium chloride and 0.15 mM sodium citrate with 0.1% SDS. All membranes were stripped and rehybridized with γ-actin as internal control for sample loading.

cDNA synthesis and PCR analysis. mRNA was reverse transcribed followed by PCR amplification. Briefly, cDNA was synthesized by using Mo-MuLV RT (Promega, Madison, WI) in the presence of RNasin and random primers. The cDNA was amplified by adding the following components: deoxyribonucleotide triphosphate mix (0.2 mM); 5 μl of 10 × PCR buffer (500 mM KCl, 200 mM Tris-HCl, pH 8.4, 25 mM $MgCl_2$ and 1 mg/ml of nuclease free bovine serum albumin); 0.1 ng of each primer and 1 U of *Thermus aquaticus* DNA polymerase (Promega). Amplification was accomplished by using 30 cycles (MJ Research, Watertown, MA) consisting of 40-sec denaturation at 92°C, 40-sec annealing at 60°C and 90-sec extension at 75°C. The primer sequences were as follows: for-IL1α, sense: 5′-AACTTTGTCATGAATGATTCCCTC-3′ and antisense: 5′-GTCTCACTACCTGTGATGAGT-3′; for IL1β, sense: 5′-CAGGATGAGGACATGAGCACC-3′ and antisense: 5′-CTCTGCAGACTCAAACTCCAC-3′; for β-actin, sense: 5′-GTGGGCCGCTCTAGGCACCA-3′ and antisense: 5′-CGGTTGGCCTTAGGGTTCAGGGGGG-3′. PCR products were analyzed on a 1.2% agarose gel, stained with ethidium bromide and visualized under UV light.

Pulse-chase labeling. Monolayers of adherent cells (3×10^6) were stimulated for 1 hr with ETX (10 μg/ml) in a 6-well plate (Costar). The monolayers were washed and overlaid with methionine-free RPMI 1640 medium containing 10% dialyzed FCS, L-glutamine, antibiotics, 2-mercaptoethanol and ETX (10 μg/ml). Twenty minutes later, ^{35}S-methionine (specific activity > 1000 Ci/ml; ICN) was added at 150 μCi/ml and the cells were incubated for 1 hr. After labeling, the pulse medium was discarded and replaced with 1 ml of complete RPMI 1640 medium containing THC (7 μg/ml) or DMSO. To harvest cells and supernatants for immunoprecipitation analysis, the chase medium was removed and saved and the cells were rinsed with cold PBS (0.15 M NaCL/10 mM phosphate, pH 7.3). The cells were then solubilized in 1 ml of PBS containing 1% Nonidet P-40 and protease inhibitors (10 mM iodoacetamide, 1 μg/ml of pepstatin, 1 mM EDTA, 1 μg/ml of leupeptin and 0.4 mM phenylmethylsulfonylfluoride; all from Sigma). Residual cells were removed from the culture supernatants by centrifugation and were added to the cell fractions. Protease inhibitors (as above) were added to the supernatants. Both cell lysates and supernatants were clarified by centrifugation at 16,000 × *g* for 20 min and stored at −70°C.

Immunoprecipitation of IL1α and IL1β. Hamster antimouse IL1α and IL1β monoclonal antibodies (Genzyme) were used in these studies. Both antibodies recognize the promature and mature forms of IL1. The clarified cell lysates and supernatants were preincubated with 20-μl suspension of protein A (*S. aureus*; Sigma) at 4°C for 30 min. Bacteria were discarded after centrifugation, anti-IL1α and anti-IL1β antibodies (both at 5 μg/ml) were added to each supernatant and incubated at 4°C for 2 hr. Next, 20 μl of protein A was added and the samples were mixed for 1 hr at 4°C. The bacteria were recovered by centrifugation and washed 3 times with PBS containing 1% Nonidet P-40 and 0.2% SDS. Labeled proteins in the immunoprecipitates were analyzed in 12% SDS-PAGE gels. The gels were fixed, dried and then exposed to Kodak X-omat AR film (Eastman Kodak, Rochester, NY). Autoradiographic films were analyzed by laser densitometric scanning on an LKB Ultroscan XL-500.

Statistics. Two-way analysis of variance was used to compare control and drug-treated groups. Paired *t* tests then were used to determine significant changes in response to drug treatment. Statistical analyses were done using Sigma Stat 1.0 (Jandel Scientific, San Rafael, CA) and Excel 4.0 (Microsoft Corp., Redmond, WA).

Results

THC increases supernatant IL1. Murine resident peritoneal macrophages were incubated with ETX and THC for 24 hr, culture supernatants recovered and IL1 bioactivity and IL1α protein measured by the thymocyte comitogen assay and ELISA, respectively. Figure 1A shows that treatment with THC and ETX significantly ($P < .05$) increased the IL1 bioactivity over ETX only treatment. The drug effect was dose-dependent, beginning at a concentration of 3 μg/ml. The observation (fig.

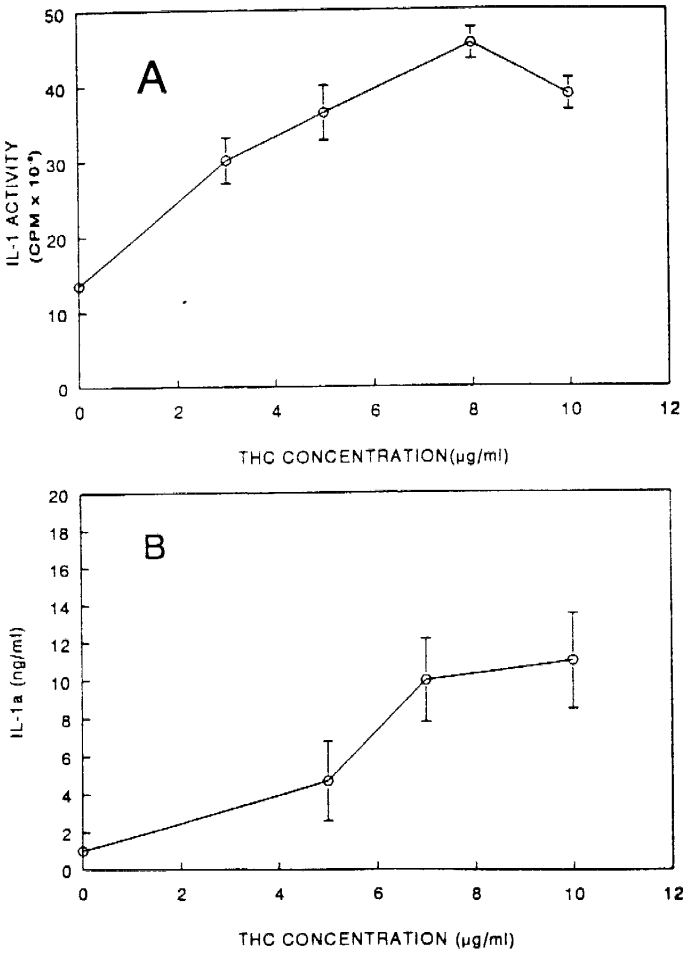

Fig. 1. THC treatment increases IL1 bioactivity and IL1 protein in culture supernatants. Resident macrophages from BALB/c mice were cultured for 24 hr with either ETX (10 μg/ml) alone (THC 0) or with ETX plus various concentrations of THC. After incubation, culture supernatants were harvested and tested for IL1 bioactivity by the comitogenic assay (A) and IL1α protein by ELISA (B). Each number represents the mean ± S.E.M. of four experiments. All drug-treated groups are significantly ($P < .05$) different from control (THC, 0).

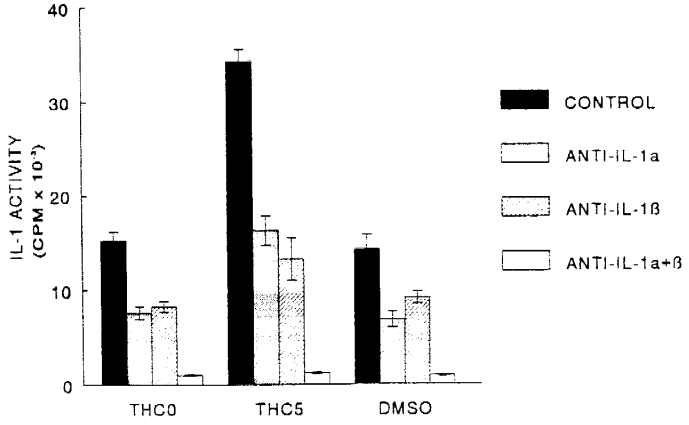

Fig. 2. THC-induced increase in bioactivity is neutralized by anti-IL1α and anti-IL1β antibodies. Macrophages were incubated with ETX only (10 μg/ml; THC 0), ETX plus THC (5 μg/ml; THC 5) or ETX plus DMSO (0.05%; DMSO) for 24 hr. Anti-IL1α, anti-IL1β, anti-IL1α and IL1β or saline (control) were added to supernatant aliquots and incubated at 4°C for 1 hr before IL1 bioassay. Each number represents the mean ± S.E.M. of three experiments. All antibody groups are significantly ($P < .05$) different from control.

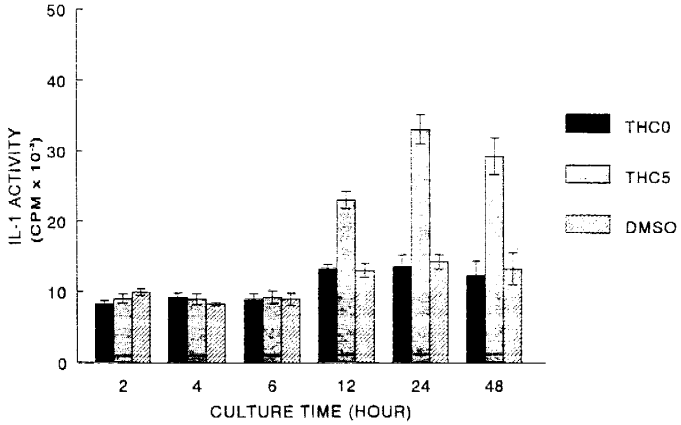

Fig. 3. THC treatment increases IL1 bioactivity by 12 hr. Macrophages were cultured with ETX only (10 μg/ml; THC 0), ETX plus THC (5 μg/ml; THC 5) or ETX plus DMSO (0.05%; DMSO) for various times up to 48 hr. IL1 activity in culture supernatants was measured by bioassay. Each number represents the mean ± S.E.M. of three experiments. The THC 5 group is significantly ($P < .05$) different from either THC 0 or DMSO groups at 12, 24 and 48 hr.

1B) that IL1α ELISA protein also was increased by drug treatment suggested that the increase in bioactivity was at least caused in part by IL1. It has been reported that cytokines other than IL1 contribute to the comitogen proliferation of thymocytes; therefore, we wanted to see what extent the increased bioactivity was due to IL1α and IL1β. Accordingly, supernatant preparations were treated with anti-IL1 antibodies and retested for proliferation activity. Figure 2 shows the proliferation was neutralized by antibodies to either IL1α or IL1β, with most of the activity neutralized by a combination of both antibodies. This verified THC treatment increased the supernatant concentration of IL1α and IL1β.

Supernatants obtained from macrophage cultures treated for various times with THC in the absence of ETX had no detectable IL1 activity (data not shown). Furthermore, pretreatment with THC for 24 hr followed by a 24-hr treatment with ETX resulted in IL1 levels comparable to cultures treated with ETX only (data not shown). This suggested that THC only exerted it's effect in combination with ETX. To explore this further, and to learn more about the kinetics of the THC effect, supernatants were collected at various times after the addition of THC and ETX, and the IL1 bioactivity was determined. Figure 3 shows the drug effect was observed at 12 hr of culture but not at 6 hr, suggesting a drug effect on cytokine release rather than cellular production (see below).

THC has no effect on the steady-state level of IL1 mRNA. The supernatant levels of IL1α and IL1β depend upon the extent of both the net cellular production of the proteins and the release of proteins from the cell. To test the hypothesis that cotreatment with THC and ETX was increasing cellular production, we examined the steady-state level of IL1α and IL1β mRNA by Northern blotting. Macrophage cultures were incubated with ETX and THC for 2, 4 and 6 hr and the poly A RNA prepared from cellular lysates and analyzed by Northern blotting. Figure 4 shows cellular mRNA for both IL1α and IL1β was detectable by 2 hr after ETX treatment, reached a peak at 4 hr and declined by 6 hr. This kinetic pattern has been reported by others (Yu *et al.*, 1990). Figure 4 also shows

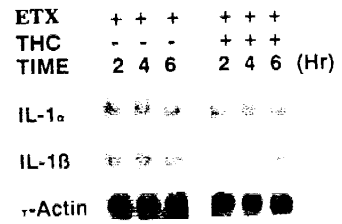

Fig. 4. THC has no effect on the ETX-induced steady-state level of IL1 mRNA at 2, 4 and 6 hr after stimulation. Macrophages were incubated with ETX only (10 μg/ml) or ETX plus THC (5 μg/ml) for 2, 4 and 6 hr. mRNA was prepared and analyzed by Northern blotting by using probes for IL1α, IL1β and γ-actin (control for RNA loading).

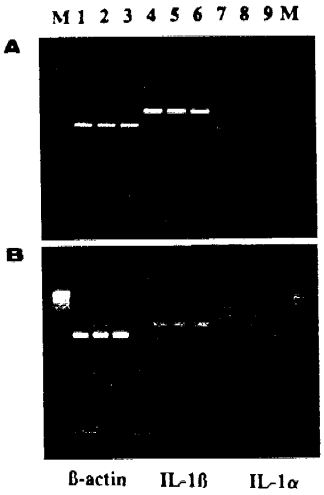

Fig. 5. THC has no effect on the ETX-induced steady-state level of IL1 mRNA at 12 and 24 hr after stimulation. Macrophages were incubated with ETX only (10 μg/ml; lanes 1, 4 and 7), ETX plus THC (5 μg/ml; lanes 2, 5 and 8) or ETX plus DMSO (lanes 3, 6 and 9) for either 12 (A) or 24 hr (B). mRNA was prepared and analyzed by RT-PCR by using probes for IL1α and IL1β and β-actin. The figure is an ethidium bromide stained gel and is representative of four different experiments.

THC treatment did not alter this pattern, suggesting message levels and possibly cellular production of IL1 was not altered through 6 hr. Beyond 12 hr after ETX treatment, mRNA for IL1α and IL1β was not detectable by Northern blotting (data not shown). We therefore switched to analyzing by the more sensitive method of RT-PCR in order to evaluate the level of mRNA 12 to 24 hr after cell stimulation. Figure 5 shows cellular mRNA levels for both IL1α and IL1β in ETX-treated cultures (fig. 5, lanes 4 and 7) is detectable by this method; furthermore, the levels decline from 12 hr (fig. 5A) to 24 hr (fig. 5B) post-treatment. Figure 5 also shows the steady-state levels in the ETX plus THC groups (fig. 5, lanes 5 and 8) and the ETX plus DMSO groups (fig. 5, lanes 6 and 9) were equivalent to the ETX only groups; thus suggesting mRNA levels were not altered by drug treatment through 24 hr after stimulation.

THC increases IL1 release and processing. The above results raised the possibility that THC was increasing cytokine release from cells rather than protein production. To test this directly, we pretreated macrophage cultures with ETX for 1 hr, incubated for an additional hr with ETX and ^{35}S-methionine to label cellular proteins and "chased" in unlabeled medium in the absence of ETX but in the presence of THC. Cell lysates and supernatants were prepared, and labeled IL1 proteins were immunoprecipitated, separated on SDS-PAGE and visualized by autoradiography. Figure 6A shows ETX-stimulated macrophages produced substantial amounts of intracellular (*i.e.*, lysate) immunoreactive IL1α and IL1β protein in the promature forms of 31 and 33 kDa, respectively (Hogquist *et al.*, 1991b; Gunther *et al.*, 1991). These protein levels reached a maximum after 2 hr of chase, which was 4 hr after ETX stimulation (fig. 6A). THC treatment had a slight lowering effect on the levels of intracellular (lysate) IL1α or IL1β.

The patterns of immunoreactive proteins were different, however, in the culture supernatants. Figure 6A shows maximum levels occurred at 4-hr chase and furthermore the majority of IL1β protein released was the processed (17 kDa) form, whereas only the promature form (31 kDa) of IL1α was released. THC treatment increased the amount of both cytokines at all time points with the exception of the 4-hr IL1α (fig. 6A). This was verified by densitometric analysis of films from three separate experiments (fig. 6B). These results suggest THC increases and facilitates the release of IL1α and IL1β from ETX-stimulated cells and also increases the processing of IL1β to the mature form. Similar results were obtained by using the bioassay. Macrophages were pretreated with ETX for 2 hr as before, washed to remove ETX and treated with THC for various times up to 24 hr. The culture supernatants were collected and IL1 bioactivity measured by thymocyte comitogen assay (fig. 7). As shown in figure 7A, supernatants from macrophages pulsed for only 2 hr with ETX contained low but detectable levels of IL1 activity for up to 24 hr later. Treatment of the cells with THC for either 2, 4, 6 or 24 hr, however, caused the activity to increase significantly ($P < .05$). This activity was composed of both IL1α and IL1β because antibodies to each cytokine neutralized in part the proliferation response (fig. 7B).

Discussion

The results reported here extend our preliminary findings (Klein and Friedman, 1990) that THC treatment increases IL1 bioactivity in the supernatants of ETX-stimulated macrophage cultures. THC treatment alone was shown not to induce detectable IL1 activity, but rather to increase the activity induced by ETX treatment. We also established in this report that increased bioactivity correlated with an increase in ELISA IL1 protein and was neutralized by antibodies to IL1α and IL1β, suggesting both types were involved in the drug effect. Because promature IL1α and mature IL1β are the main biologically active forms (Mosley *et al.*, 1987; Black *et al.*, 1988), these results suggested the drug was increasing the culture supernatant concentrations of these two IL1 proteins.

The supernatant concentrations of the different types of IL1 depend upon the degree of net transcriptional, translational and post-translational activity of the relevant genes and gene products. Therefore, we initially tested the hypothesis that THC was increasing the steady-state levels of IL1α and IL1β mRNA. Macrophage cultures were treated simultaneously with THC and ETX followed by extraction of cultures at 2, 4 and 6 hr and the preparation of poly A RNA. Northern blotting showed no differences induced by THC in the cellular levels of IL1α and IL1β mRNA which increased to a maximum level at 4 hr and declined thereafter. Because increased levels of IL1 accumulated in the supernatant at 12 and 24 hr after stimulation (fig. 3), and because mRNA for IL1α and IL1β was not detectable by Northern blotting at these time points, we analyzed mRNA levels by the more sensitive method of RT-PCR.

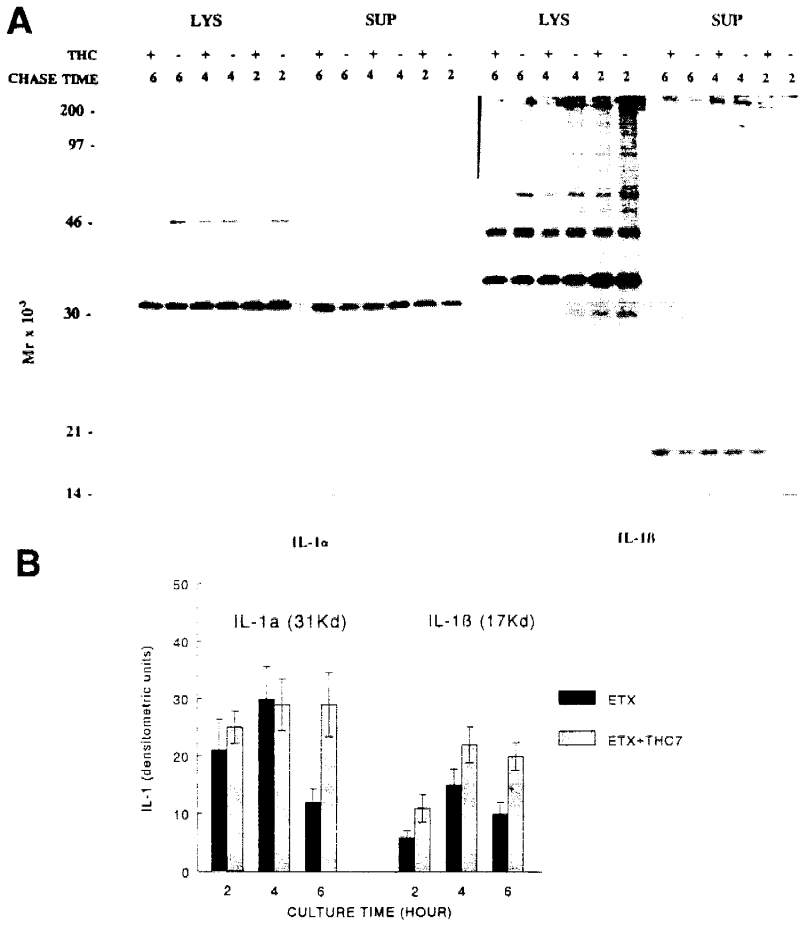

Fig. 6. A, THC enhances IL1 release and processing. Macrophage cultures were preincubated (1 hr) with ETX (10 μg/ml) and pulse-labeled (1 hr) with ^{35}S-methionine. The cells were chased in complete RPMI 1640 medium for 2, 4 and 6 hr in either the presence or absence of THC (7 μg/ml). Cells and supernatant were collected and IL1 proteins were isolated by immunoprecipitation from cell lysates (LYS) and culture supernatants (SUP). The precipitates were analyzed by SDS-PAGE and autoradiography. The films were exposed for 1 week for cell lysates and 3 weeks for supernatants at −70°C. The figure is an autoradiograph representative of three experiments. B, THC increases the supernatant levels of promature IL1α and mature IL1β. The supernatant films from A were analyzed by laser densitometry. Bars are the mean ± S.E.M. of three experiments. The THC groups (IL1α, 6 hr; and IL1β, 2, 4 and 6 hr) were significantly ($P < .05$) different from the ETX only groups.

IL1 message levels at 12 and 24 hr also were not affected by drug treatment. These results suggested that drug treatment was increasing supernatant IL1 protein by mechanisms other than increasing the steady-state level of IL1 message.

We next studied THC effects on the cellular and supernatant levels of IL1α and Il1β proteins by immunoprecipitation of radiolabeled proteins. To study this, ETX-activated macrophages, pulsed-labeled with ^{35}S-methionine, were chased for 2, 4 and 6 hr in the presence of THC, and the intracellular and supernatant levels of the various IL1 proteins were determined by immunoprecipitation. We observed ETX treatment of resident, mouse peritoneal macrophages induced an IL1 production and secretion profile which differed from that reported for mouse, exudate macrophages, but similar to fresh cultures of human blood monocytes (Hogquist *et al.*, 1991b; Rubartelli, 1993). That is, detectable cellular IL1α and IL1β were exclusively in the precursor forms, whereas the supernatant forms consisted primarily of the precursor IL1α and mature IL1β. The reason for the difference in the IL1 profile among the various cell types is not clear, but possibly involves either changes in the extent of cell differentiation or the relative viability of the cells in culture (Hogquist *et al.*, 1991b). THC treatment did not increase the cellular levels of IL1 precursor proteins induced by ETX or change the release profile. However, the drug did quantitatively enhance the release of both precursor IL1α and mature IL1β. These results support the above findings showing THC quantitatively increased both biologically active IL1α and IL1β in the supernatant (figs. 1 and 2), but had no effect on the steady-state level of both mRNAs (figs. 4 and 5). We conclude, therefore, THC treatment of ETX-stimulated macrophages increases the processing and release of IL1β and the release of IL1α rather than increasing the cellular production of these cytokinins.

The mechanism of the THC effect is not clear at this time. Enhanced release of IL1 has been reported following cell injury due to either treatment with toxic substances such as hydrogen peroxide and saponin or exposure to heat (Hogquist *et al.*, 1991a). The types of IL1 released under these conditions were mature and precursor IL1α and precursor IL1β. The authors concluded that, although cell injury released IL1, the injury failed to destroy processing enzymes for IL1α but did destroy enzymes for processing IL1β. This release profile associated with cell injury is different from that observed in the present study in response to ETX and THC. Here, we observed a preferential increase in mature IL1β and little in the way of mature IL1α. Furthermore, THC treatment for up to 24 hr did not decrease cell viability as determined by trypan blue exclusion (data not shown). The THC profile, however, is similar to that observed after treatment with ATP and induction of apoptosis (Hogquist *et al.*, 1991a), suggesting THC may be acting through a similar mechanism.

Processing and release of IL1 is reported to also be regulated by prostaglandins (Kunkel *et al.*, 1986) and cAMP production (Knudsen *et al.*, 1986; Viherluoto *et al.*, 1991). Both mediators suppressed the level of supernatant IL1, but had no effect on cellular IL1 protein or mRNA. The THC receptor is a G-protein coupled receptor that when activated inhibits cellular adenylate cyclase and cAMP accumulation (Howlett and Flem-

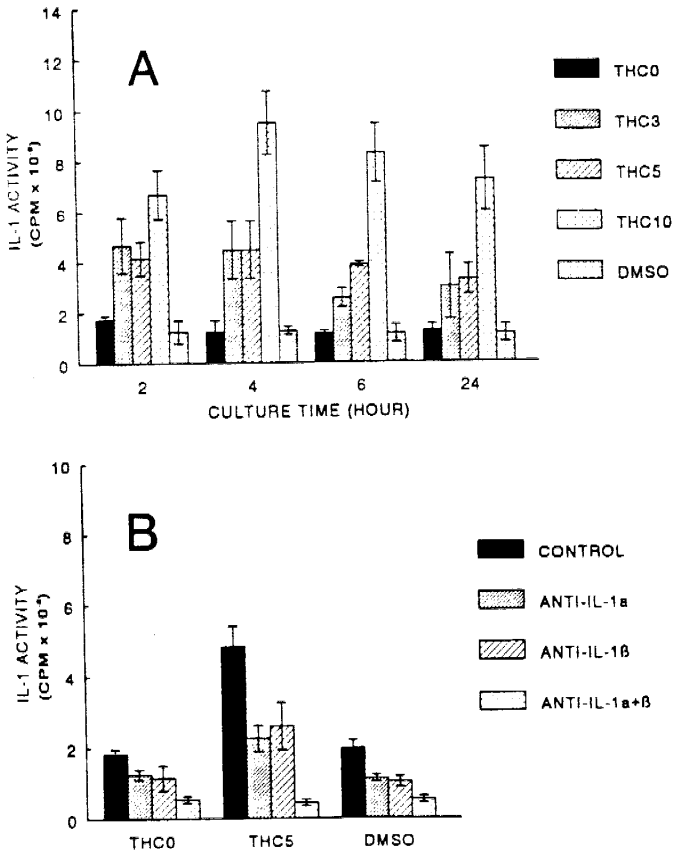

Fig. 7. THC increases the release of IL1α and IL1β from ETX-activated macrophages. Macrophages were preincubated with ETX (10 μg/ml) for 2 hr, washed to remove ETX and then THC was added for various times up to 24 hr. Culture supernatants were isolated and tested for IL1 bioactivity (A). Supernatant aliquots were also treated (see fig. 2) with anti-IL1 antibodies (B) for neutralization studies. Bars represent the mean ± S.E.M. of three experiments. The drug-treated (A) and antibody-treated (B) groups are significantly ($P < .05$) different from controls.

ing, 1984; Matsuda *et al.*, 1990). We have observed that resident macrophages express THC receptor mRNA (Y. Daaka and T. W. Klein, unpublished observations). Therefore, it is speculated that THC combining with the macrophage receptor might enhance IL1 secretion by inhibiting the accumulation of intracellular cAMP. Alternatively, THC has been shown to suppress cyclooxygenase activity and prostaglandin E2 synthesis and release (Evans *et al.*, 1987; Reichman *et al.*, 1987; Rettori *et al.*, 1990). A decrease in the cellular expression of this metabolite might also account for the increase of IL1 release (Kunkel *et al.*, 1986; Knudsen *et al.*, 1986; Viherluoto *et al.*, 1991). The activity of cellular PKC appears to be inversely correlated with IL1 secretion (Bakouche *et al.*, 1992). Although, we are not aware of any reports showing an effect of THC on this enzyme, it has been reported that THC decreases the cellular levels of inositol 4,5-diphosphate and inositol 1,4,5-triphosphate (Chaudhry *et al.*, 1988), which might in turn effect cellular PKC activity. Clearly, more work is required to define the molecular pathways responsible for the drug effect on IL1 release. Experiments are currently in progress to determine the role of the macrophage THC receptor.

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