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# $\Delta^9$-Tetrahydrocannabinol (THC) Causes the Variable Expression of IL2 Receptor Subunits[1]

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## ABSTRACT
Previously, we reported that the cannabinoid $\Delta^9$-tetrahydrocannabinol (THC) suppressed interleukin 2 (IL2)-induced proliferation of a cloned, natural killer-like cell line (NKB61A2) and decreased the number of high- and intermediate-affinity IL2 binding sites. However, the surface expression of interleukin 2 receptor *alpha* (IL2R$\alpha$) chain, as measured by flow cytometry, was increased rather than decreased by THC treatment. This suggested that the drug-induced deficiency in IL2 binding and cell activation involved a defect in the cell-surface expression of IL2R subunits other than the $\alpha$ chain. Because the IL2 receptor complex is composed of $\alpha$, $\beta$ and $\gamma$ chains, we examined the effect of THC treatment on the expression of these chains. In a result consistent with our previous findings, we observed that treatment of NKB61A2 cells with THC increased the cellular immunoprecipitable IL2R$\alpha$ protein (p55) and mRNA. Furthermore, the cellular production of IL2R$\beta$ chain protein (p75) and mRNA, determined by immunoprecipitation and Northern blotting, respectively, was also increased. The mRNA stability assay showed that THC increased the stability of IL2$\beta$ mRNA, and nuclear run-on experiments suggested that the increase in subunit production was not due to a drug effect on gene transcription. The IL2R$\gamma$ chain was also affected by THC treatment in that Northern blotting studies showed a drug-induced decrease in the cellular level of $\gamma$ chain mRNA. In addition, THC treatment decreased the $^{125}$I-labeled IL2 internalization under high-affinity binding conditions. These results show that THC treatment of NKB61A2 cells modulates the expression of IL2R$\alpha$, $\beta$ and $\gamma$ chains and suggests that these effects may account at least in part for drug-induced suppression of high- and intermediate-affinity IL2 binding as well as IL2-dependent cell activation.

THC, the major psychoactive cannabinoid in marijuana, has been shown to suppress immune responses (Klein and Friedman, 1990; Specter *et al.*, 1990). Previously, we demonstrated that THC suppressed IL2-dependent proliferation and lytic activation of freshly isolated lymphocytes and the cloned, NK-like cell line NKB61A2 (Kawakami *et al.*, 1988a, b). We also showed that the suppression of IL2-induced proliferation was due to a decrease in high- and intermediate-affinity IL2 binding. However, the surface expression of the IL2R$\alpha$ chain was increased by drug treatment (Zhu *et al.*, 1993). These results suggested that the THC effect was not due to a deficiency in the production of the IL2R$\alpha$ chain.

IL2 is a glycoprotein produced by activated lymphocytes that binds to and activates other cells through the IL2R. The IL2R is a complex of at least three subunit proteins termed $\alpha$ (p55), $\beta$ (p75) and $\gamma$ (p64). These proteins may exist on the cell surface as $\alpha$ chain only with low affinity of $K_d$ $10^{-8}$ M, as a complex of $\beta$ and $\gamma$ chains with intermediate affinity of $K_d$ $10^{-10}$ M, or as a complex of all three proteins with high affinity of $K_d$ $10^{-11}$ M. Both high- and intermediate-affinity receptors are involved in IL2 internalization and signal transduction for the activation of receptor positive immune cells (Taniguchi and Minami, 1993; Takeshita *et al.*, 1992; Kumaki *et al.*, 1993). In the present study, we demonstrate that THC enhances the cellular level of IL2R$\alpha$ and IL2R$\beta$ mRNA and the production of the corresponding proteins. Furthermore, these increased levels are probably due to a stabilization of message rather than to an increase in transcription. However, THC treatment suppresses IL2 internalization under high-affinity binding conditions and the cellular level of IL2R$\gamma$ chain mRNA. These data suggest that the THC-induced decreased in IL2 high and intermediate affinity binding and cell activation is at least partly due to a modulation of the production and expression of all three IL2R proteins.

## Materials and Methods

**THC.** THC was obtained from the National Institute on Drug Abuse (Rockville, MD) as a 98.6% tar. The drug was initially dis-

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**ABBREVIATIONS:** THC, $\Delta^9$-tetrahydrocannabinol; DMSO, dimethylsulfoxide; IL2, interleukin 2; IL2R$\alpha$, interleukin 2 receptor *alpha*; IL2R$\beta$, interleukin 2 receptor *beta*; IL2R$\gamma$, interleukin 2 receptor *gamma*; NK cell, natural killer cell.



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solved in DMSO (Sigma Chemical, St. Louis, MO) to a concentration of 150 mg/ml and stored under nitrogen gas at $-20°C$. We periodically analyzed the drug using reverse-phase HPLC and verified that it contained only one peak with a retention time identical to that of a THC standard (Sigma). At the time of experiments, the stock drug was diluted (50 mg/ml) in DMSO and further diluted in warm tissue culture medium.

**Cell culture.** The cloned NK-like, murine cell line NKB61A2, which is IL2-dependent, was a kind gift of Dr. Gunther Dennert (Warner and Dennert, 1982). Cells were grown in RPMI 1640 medium (Gibco, Grand Island, NY) containing 10% fetal calf serum (Hyclone Lab., Logan, UT), L-glutamine, antibiotics, 2-mercaptoethanol ($5 \times 10^{-5}$ M), and 20 units/ml human recombinant IL2 kindly provided by Hoffman La Roche, Inc. (Nutley, NJ). Cells were harvested and reestablished in fresh growth medium 24 hr before each experiment.

**Internalization of $^{125}$I-labeled IL2.** The IL2 internalization study was performed as described by Robb and Greene (1987). Briefly, NKB61A2 cells were treated with THC (10 $\mu$g/ml) or DMSO for 2 and 4 hr and then incubated with $^{125}$I-IL2 (NEN, Boston, MA) under high-affinity conditions (100 pM) at 4° for 1 hr. The cells were washed twice, resuspended in prewarmed medium and incubated at 37°C. At selected times, 100-$\mu$l aliquots of the cell suspension were centrifuged, and the supernatant radioactivity was counted to determine the level of free $^{125}$I IL2. The cell pellets were resuspended in 200 $\mu$l of 10 mM citrate (pH4.0) containing 0.14M NaCl and 0.1% BSA. After 15 sec at 23°C, the cells were centrifuged through a 400-$\mu$l oil mixture of 20% olive oil and 80% di-n-butylphthalate. The radioactivity of the resultant supernatant and cell pellet was counted to determine the levels of surface-bound (acid-sensitive) and internalized (acid-resistant) IL2, respectively.

**Northern blotting and mRNA stability.** Total RNA was isolated from NKB61A2 cells using Tri-reagent (Molecular Research Center, Inc., Cincinnati, OH) and was size-fractionated in 1% agarose gels after denaturation with glyoxal and dimethyl sulfoxide. The gels were then blotted onto Nytran membranes (Schleicher & Schuell, Keene, NH), which were then baked and hybridized at 55°C for 2 hr using rapid hybridization buffer (Amersham, Arlington Heights, IL). The murine IL2 receptor $\alpha$ chain cDNA (gift from Dr. T. Honjo, Kyoto University, Japan), $\beta$ chain cDNA (gift from Dr. T. Taniguchi, Osaka University, Osaka, Japan) and $\gamma$ chain cDNA (gift from Dr. K. Sugamura, Tohoku University, Sendai, Japan) were labeled by the random-priming labeling system (Boehringer Mannheim, Indianapolis, IN). After hybridization, membranes were washed three times at room temperature in $2 \times$ SSC (15 mM sodium chloride, 1.5 mM sodium citrate) containing 0.1% SDS followed by two washes at 55°C in $0.1 \times$ SSC with 0.1% SDS. All membranes were stripped and rehybridized with $\gamma$ actin as internal control. The filters were exposed to Kodak AR X-Omat films (Eastman Kodak, Rochester, NY) at $-70°C$ and analyzed by laser densitometry scanning on an LKB Ultroscan XL-500. The densities of the blot areas were determined for the IL2R and $\gamma$ actin messages and reported as a percentage of the actin density. In some experiments, transcription was inhibited by adding actinomycin D (10 $\mu$g/ml, Sigma) at the start of incubation. The cultures were also treated at this time with THC, DMSO, or tissue culture medium. Cultures were then harvested at various times, and the RNA was analyzed by Northern blotting.

**Nuclear run-on analysis.** Isolation of nuclei and nuclear run-on analysis were performed using the method described by Greenberg and Ziff (1984). In brief, $7 \times 10^6$ NKB61A2 cells were treated with NP-40 lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$ and 0.5% Nonidet P-40). The nuclear pellet was obtained by centrifugation ($500 \times g$ for 5 min at 4°C) and stored in 200 $\mu$l glycerol storage buffer (50 mM Tris-HCl, pH 8.3, 40% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA) in liquid nitrogen. Nuclei were mixed with 200 $\mu$l reaction mixture of 10 mM Tris-HCl, pH 8, 5 mM $MgCl_2$, 0.3 M KCl, 5 mM dithiothreitol and 10 mM each of ATP, CTP, GTP and 100 $\mu$Ci$^{32}$P-UTP (760 Ci/mmol, 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 with proteinase K (Boehringer Mannheim) for 30 min at 42°C. The $^{32}$P-labeled RNA was isolated by isopropanol ammonium acetate precipitation. IL2R$\beta$ and $\gamma$ actin cDNA (3 $\mu$g) 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 RNA. The filters were washed, treated with RNase A and then exposed to Kodak AR X-Omat films at $-70°C$.

**Metabolic labeling and immunoprecipitation.** A modification of the procedure described by Hogquist *et al.* (1991) was used. Briefly, NKB61A2 cells ($5 \times 10^6$) were preincubated for 20 min in 6-well plates (Costar, Cambridge, MA) in methionine-free RPMI 1640 medium (Gibco) containing 10% FCS, L-glutamine, antibiotics and 2-ME. Next, 150 $\mu$Ci/ml of $^{35}$S-methionine (specific activity > 1000 Ci/ml; ICN) was added to the drug-treated cultures, which were then incubated for 4 hr. The cells were washed with ice-cold Dulbecco's phosphate-buffered saline (Sigma) and solubilized in 1 ml phosphate-buffered saline (0.15 M NaCl/10 mM phosphate, pH 7.3) containing 1% Nonidet P-40 and protease inhibitors (iodoacetamide 10 mM, pepstatin 1 $\mu$g/ml, EDTA 1 mM, leupeptin 1 $\mu$g/ml and phenylmethylsulfonylfluoride 0.4 mM; all from Sigma). The cell lysates were clarified by centrifugation at $16{,}000 \times g$ for 20 min and preadsorbed with a 20-$\mu$l suspension of protein-G *S. aureus* (Sigma) at 4°C for 30 min. Bacteria were discarded after centrifugation, and monoclonal, anti-mouse IL2R$\alpha$ chain (5 $\mu$g/ml; Boehringer Mannheim) and IL2R$\beta$ chain (5 $\mu$g/ml; Seikagaku Corp., Tokyo, Japan) antibody were added to each supernatant for 2 hr at 4°C. Next, 20 $\mu$l protein-G *S. aureus* was added, and the samples were mixed for 1 hr at 4°C. The bacteria were recovered by centrifugation and washed three times with phosphate-buffered saline containing 1% Nonidet P-40 and 0.2% SDS. Labeled proteins in the immunoprecipitates were analyzed using 10% SDS-PAGE. The gels were fixed, dried and then exposed to Kodak X-omat AR film. The films were analyzed by laser densitometry scanning, and the density areas were determined for each lane.

## Results

**THC increases IL2R$\alpha$ cellular protein and mRNA.** Our previous data showed that THC decreased the number of high- and intermediate-affinity IL2 binding sites but increased the cell-surface expression of IL2R$\alpha$ (Zhu *et al.*, 1993). The latter finding was determined by flow cytometry. To confirm these results with another test, we studied IL2R$\alpha$ cellular protein levels using immunoprecipitation. NKB61A2 cells were cultured in methionine-free medium in the presence of $^{35}$S-methionine and THC (10 $\mu$g/ml) or DMSO for 4 hr. Labeled IL2R$\alpha$ proteins were immunoprecipitated and analyzed by SDS-PAGE. Figure 1A shows that THC treatment (lane 2) increased the total cellular IL2R$\alpha$ protein (p55) relative to DMSO treatment (lane 1). Treatment of the cells with tissue culture medium only gave results similar to those obtained with DMSO (data not shown). These results supported our previous observation and confirmed that THC treatment enhances both surface expression and cellular accumulation of IL2R$\alpha$ protein.

To determine whether the elevated level of IL2R$\alpha$ protein is due to a THC-induced increase in production, we measured the cellular level of IL2R$\alpha$ mRNA by Northern blotting. NKB61A2 cells were incubated with IL2 and either THC or DMSO for 4 and 8 hr. After incubation, total RNA was prepared, separated by agarose gel electrophoresis, blotted onto Nytran membranes and hybridized with $^{32}$P-labeled

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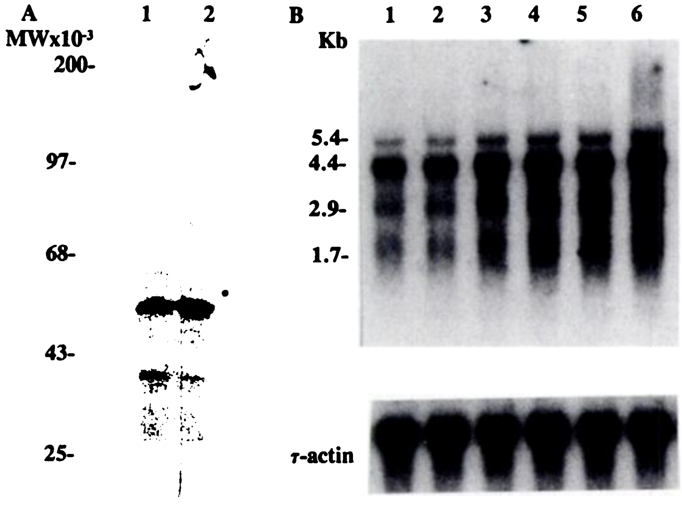


**Fig. 1.** THC treatment augments the cellular protein and mRNA expression of IL2Rα. A) NKB61A2 cells were labeled with $^{35}$S-methionine in the presence of IL2 and either THC (lane 2, THC 10 μg/ml) or DMSO (lane 1) for 4 hr. Cellular IL2Rα protein was isolated by immunoprecipitation and the precipitates analyzed by SDS-PAGE. Densitometry units are as follows: lane 1, 44; lane 2, 72. B) NKB61A2 cells were incubated with either THC or DMSO and IL2 for 4 hr (lane 1, DMSO; lane 2, THC 5 μg/ml; lane 3, THC 10 μg/ml) and 8 hr (lane 4, DMSO; lane 5, THC 5 μg/ml; lane 6; THC 10 μg/ml). Total RNA was prepared and analyzed by Northern blotting assay. Densitometry units are as follows: lane 1, 80; lane 2, 85; lane 3, 135; lane 4, 150; lane 5, 175; lane 6, 230. NKB61A2 cells incubated for 4 hr in tissue culture medium only plus IL2 yielded results similar to those obtained with cells incubated with DMSO. The results presented are representative of five experiments.

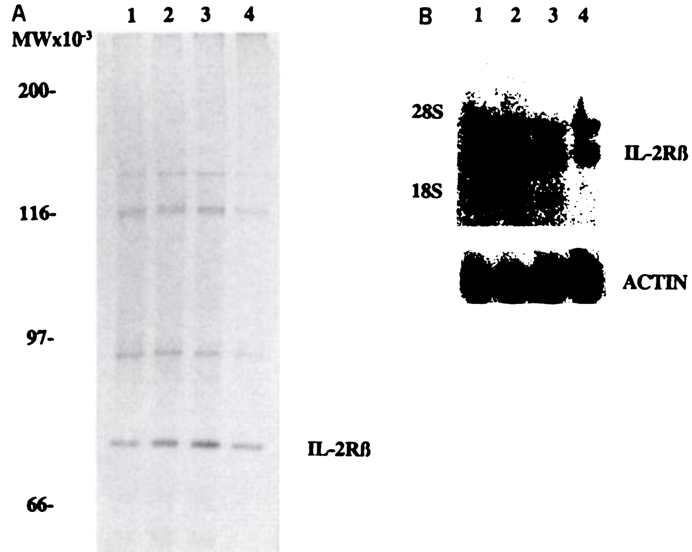


**Fig. 2.** THC treatment augments the cellular protein and mRNA expression of IL2Rβ. NKB61A2 cells were incubated with IL2 and either THC or DMSO for 4 hr (lane 1, medium only; lane 2, THC 5 μg/ml; lane 3, THC 10 μg/ml; lane 4, DMSO). Cellular IL2Rβ protein (A) and mRNA (B) were analyzed by immunoprecipitation and Northern blotting, respectively, as described in "Materials and Methods." Densitometry units are as follows: (A) lane 1, 18; lane 2, 28; lane 3, 42; lane 4, 17; and (B) lane 1, 17; lane 2, 13; lane 3, 30; lane 4, 15. The results presented are representative of three experiments.

IL2Rα cDNA. As shown in figure 1B, four species of IL2Rα mRNA with different sizes (1.7 Kb, 2.9 Kb, 4.4 Kb and 5.4 Kb) were detected. These data are consistent with those reported by others (Shimuzu and Honjo, 1985). THC treatment (lanes 2, 3, 5 and 6) increased the level of IL2Rα mRNA relative to DMSO control (lanes 1 and 4), especially at a drug concentration of 10 μg/ml.

**THC increases IL2Rβ cellular protein and mRNA.** The foregoing results indicated that the IL2Rα chain was increased after drug treatment. Next we tested the effects of THC on IL2Rβ expression. As before, NKB61A2 cells were treated with IL2 and with THC (5 and 10 μg/ml), DMSO, or tissue culture medium only for 4 hr, and the cellular IL2Rβ protein (p75) and mRNA were analyzed by immunoprecipitation and Northern blotting, respectively. Figure 2A shows that the cells expressed increased protein in a dose-dependent manner after THC treatment with 5 (lane 2) and 10 (lane 3) μg/ml relative to either medium only (lane 1) or DMSO (lane 4). Increased surface expression of IL2Rβ was also confirmed by flow cytometry (data not shown). Drug treatment also increased the cellular level of IL2Rβ mRNA. Figure 2B shows that two species of IL2Rβ transcripts were detected, as reported by others (Kono *et al.*, 1990; Nakanishi *et al.*, 1992). Furthermore, an increase in the IL2Rβ mRNA was observed after the 4-hr incubation, especially at a concentration of 10 μg/ml THC (lane 3).

**THC increases IL2Rβ message stability.** The level of IL2Rβ mRNA is probably regulated by transcriptional and post-transcriptional mechanisms. To evaluate which mechanism might be involved in the THC-induced increase in cellular IL2Rβ mRNA, we first examined the THC effect on gene transcription rate using the nuclear run-on assay. Cell cultures were incubated with IL2 and either THC or DMSO for 1, 2, 3 and 4 hr, and intact nuclei were isolated. Radiolabeled nuclear RNA was then prepared and hybridized to slot-blotted IL2Rβ and γ-actin cDNAs. Figure 3 shows that the transcriptional activity of IL2Rβ was the same for both THC- and DMSO-treated groups at from 1 to 4 hr after drug treatment. This suggested that the level of transcription for the IL2Rβ gene was the same in both vehicle- and drug-treated cells. This lack of drug effect on transcription suggested that THC increased IL2Rβ mRNA at the post-transcriptional level. To test this, we incubated cell cultures with IL2 and THC or DMSO in the presence of actinomycin D to block transcription. At varying time points, total RNA was prepared and the amount of specific mRNA for IL2Rβ and γ actin was determined by Northern blotting. As shown in figure 4, the level of IL2Rβ message declined gradually, with a half-life of about 2 hr, after actinomycin D and DMSO treatment. NKB61A2 cells incubated in tissue culture medium only plus IL2 yielded results similar to those obtained with cells incubated with DMSO (data not shown). However, THC treatment increased the half-life to >4 hr, which indicated that THC in some way stabilized or inhibited the degradation of IL2Rβ mRNA.

**THC decreases IL2Rγ cellular mRNA.** The foregoing results showed that alterations in ILR2α and ILR2β chains were induced by THC treatment. We next examined drug effects on the levels of IL2Rγ chain mRNA. NKB61A2 cells were treated with IL2 and with either THC (5 and 10 μg/ml) or DMSO for 2, 4 and 6 hr. Total cellular RNA was extracted, and the levels of IL2Rγ mRNA were determined by Northern blotting. Figure 5 shows that the NKB61A2 cell line ex-

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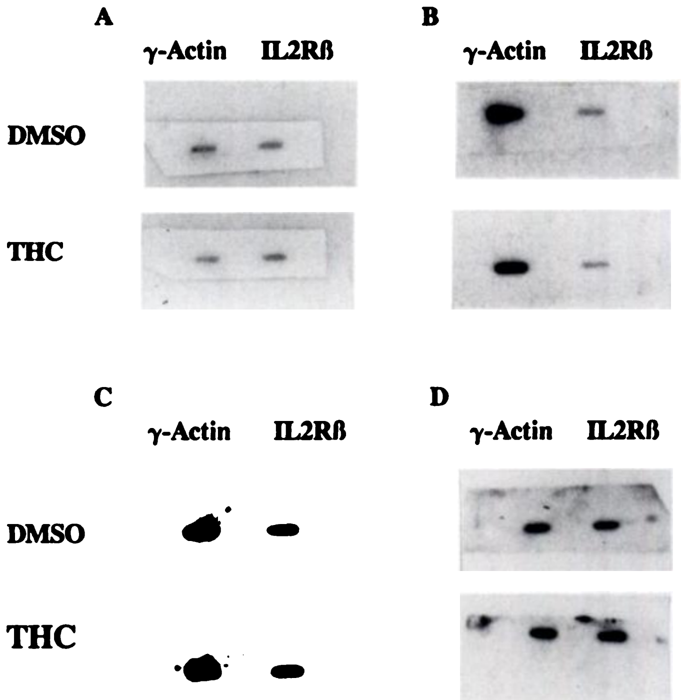


**Fig. 3.** THC has no effect on transcriptional activity of the IL2R$\beta$ gene. NKB61A2 cells were incubated with IL2 and either THC or DMSO for 1 (A), 2 (B), 3 (C) or 4 (D) hr. Nuclei were isolated, and radiolabeled RNA was purified and then hybridized to the slot-blotted cDNAs for IL2R$\beta$ and $\gamma$ actin. Blots were exposed to X-ray film for various times up to 1 week. NKB61A2 cells incubated in tissue culture medium only plus IL2 yielded results similar to those obtained with cells incubated with DMSO. The results presented are representative of four experiments.

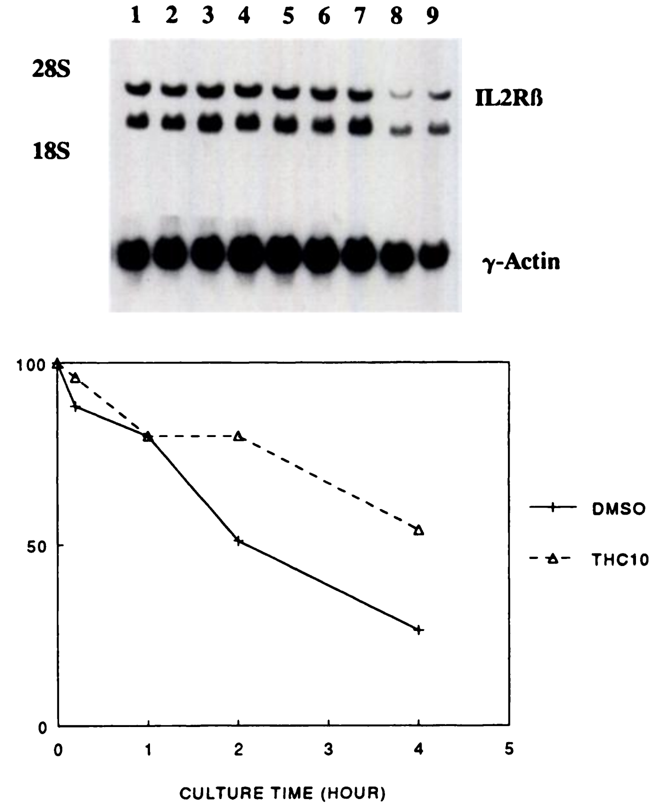


**Fig. 4.** THC increases the half-life of IL2R$\beta$ mRNA. NKB61A2 cells were incubated with IL2 and either THC (10 $\mu$g/ml) or DMSO in the presence of actinomycin D (10 $\mu$g/ml) for various times or untreated (0 time). Total RNA was prepared from control cells (lane 1) or cells treated with actinomycin D plus drugs for 30 min (lane 2, DMSO; lane 3, THC 10 $\mu$g/ml), 1 hr (lane 4, DMSO; lane 5, THC 10 $\mu$g/ml), 2 hr (lane 6, DMSO; lane 7, THC 10 $\mu$g/ml) and 4 hr (lane 8, DMSO; lane 9, THC 10 $\mu$g/ml). The RNAs were probed with IL2R$\beta$ and $\gamma$ actin probes by Northern blotting, and the band intensity was determined by densitometric scanning. The IL2R$\beta$ densities are expressed as a percentage of the corresponding $\gamma$ actin density with the control value set at 100%. The values for the other samples are plotted relative to control. NKB61A2 cells incubated in tissue culture medium only plus IL2 yielded results similar to those obtained with cells incubated with DMSO. The results presented are representative of four experiments.

presses the IL2R$\gamma$ mRNA at relatively lower levels than for the $\alpha$ and $\beta$ chains (Kumaki *et al.*, 1993). Control (DMSO) message decreased slightly from 2 hr to 6 hr, and THC treatment had the greatest effect on the message levels at 2 and 6 hr, with a lesser effect at 4 hr. These results showed that although THC treatment increased IL2R$\alpha$ and IL2R$\beta$ chain production, the drug under identical conditions suppressed the message level of IL2R$\gamma$ chain.

**THC decreases the internalization of IL2.** A functional $\gamma$ chain is required for fully functional receptor activity. Because the internalization of IL2 is a measure of receptor function, we studied this event as a means of indirectly assessing $\gamma$ chain expression and function in drug-treated cells. NKB61A2 cells were incubated with either THC or DMSO for 2 or 4 hr, followed by incubation with $^{125}$I-labeled IL2 under high-affinity conditions. Internalized and surface-bound IL2 were evaluated after acid treatment. As shown in figure 6, panels A and B, $^{125}$I IL2 binding and internalization occurred rapidly after DMSO treatment; most of the label was internalized by 5 min. The results for 2-hr and 4-hr pretreatment were almost identical (panels A and B), and pretreatment with medium only gave similar results (data not shown). Treatment with THC, however, for 2 and 4 hr (panels C and D), decreased the total amount of cell-associated IL2, appeared to delay the kinetics of total binding and decreased the percentage of internalized label. Drug treatment, therefore, inhibited IL2 binding and internalization under high-affinity binding conditions.

## Discussion

The activation and proliferation of immune cells such as lymphocytes and NK cells are central to activation of the immune response. IL2 binding to high- and intermediate-affinity receptors provides critical signals for immune activation (Taniguchi and Minami, 1993). Our previous studies demonstrated that THC suppressed lymphocyte and NK cell proliferation in response to mitogens and IL2 (Kawakami *et al.*, 1988a,b). In addition, we showed that the drug also suppressed the functional expression of high- and intermediate-affinity IL2R, which suggested that the IL2/IL2R system might be a critical target of drug suppression of immune activation (Zhu *et al.*, 1993; Friedman *et al.*, 1994).

It is well documented that the IL2R consists of at least three component proteins termed $\alpha$, $\beta$ and $\gamma$. All three of these chains are required for optimal receptor function. Because our previous results suggested an increase rather than a decrease in $\alpha$ chain expression on cells treated with THC (Zhu *et al.*, 1993), we wanted to confirm this finding using immunoprecipitation with anti-$\alpha$ chain-specific antibodies.

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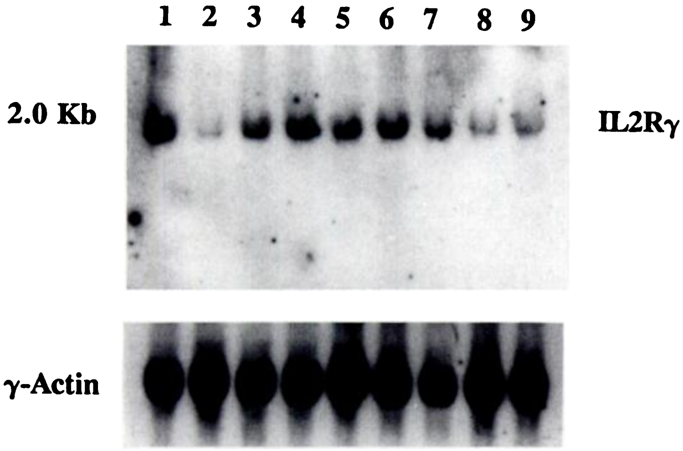


**Fig. 5.** THC treatment suppresses the expression of IL2Rγ mRNA. NKB61A2 cells were incubated with IL2 and either THC or DMSO for 2 hr (lane 1, DMSO; lane 2, THC 5 μg/ml; lane 3, THC 10 μg/ml), 4 hr (lane 4, DMSO; lane 5, THC 5 μg/ml; lane 6, THC 10 μg/ml) and 6 hr (lane 7, DMSO; lane 8, THC 5 μg/ml; lane 9, THC 10 μg/ml). Total RNA was prepared and analyzed by Northern blotting. Densitometry units are as follows: lane 1, 20; lane 2, 4; lane 3, 12; lane 4, 16; lane 5, 12; lane 6, 15; lane 7, 13; lane 8, 3; lane 9, 4. NKB61A2 cells incubated in tissue culture medium only plus IL2 yielded results similar to those obtained with cells incubated with DMSO. The results presented are representative of four experiments.

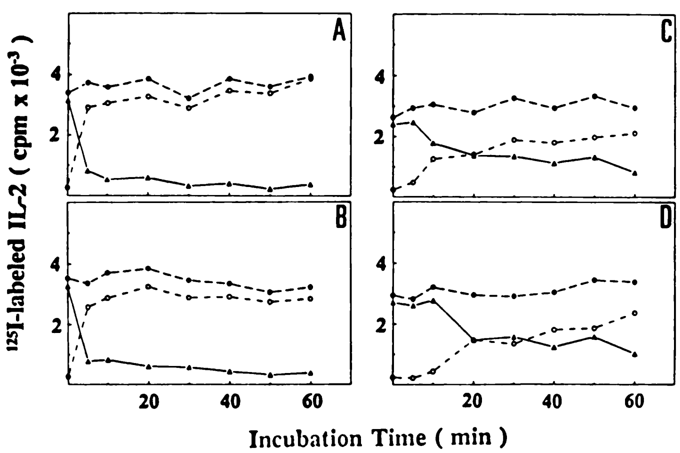


**Fig. 6.** THC decreases the internalization of $^{125}$I-labeled IL2 into NKB61A2 cells. NKB61A2 cells were cultured with either THC or DMSO for 2 hr (panel A, DMSO; panel C, THC 10 μg/ml) or 4 hr (panel B, DMSO; panel D, THC 10 μg/ml). The cells were washed and incubated with $^{125}$I-labeled IL2 under high-affinity conditions (100 pM). The acid-resistant, internalized IL2 (○), and the acid-sensitive, membrane-bound IL2 (△), were determined as described in "Materials and Methods." The sum of the two fractions (●) is also graphed. The results presented are representative of three experiments.

Figure 1A showed that THC treatment of NKB61A2 cells for 4 hr caused an increase in the cellular level of immunoprecipitable IL2Rα protein, which confirmed our previous finding using flow cytometry (Zhu *et al.*, 1993). Figure 1 also showed that drug treatment increased the cellular level of IL2Rα mRNA, which suggested that the THC either increased gene transcription or decreased message turnover (fig. 1B).

Because IL2Rα chain protein was not suppressed by THC treatment, and because the β chain is needed to form both high- and intermediate-affinity IL2R, we next measured the drug effect on IL2Rβ chain expression. Figure 2 showed that THC treatment increased the cellular level of both IL2Rβ protein and mRNA. Nuclear run-on experiments (fig. 3) showed no effect of THC on the transcription rate of the IL2Rβ gene; however, mRNA stability assays showed that THC increased the half-life of IL2Rβ mRNA, which suggested that message turnover was decreased by drug treatment (fig. 4).

At least one other study has shown an increase in the level of IL2Rβ message. This study involved retinoic acid treatment of human thymocyte blasts. However, it was not determined whether this result was due to an effect on transcription or stability of message (Sidell *et al.*, 1993). In addition to the level of IL2Rβ message, the level of IL2Rα message has also been reported to be increased after treatment with exogenous agents. IL1 and IL6 treatment of a murine, myeloid leukemia cell line increased both message transcription and stability over treatment with IL1 only (Ruhl *et al.*, 1992). Again, however, no clue to the molecular mechanism was presented. The stability of mRNAs of a variety of growth factors appears to be at least partially regulated by 3′ untranslated region AU-rich elements (Kruys *et al.*, 1989). We have run a computer analysis of this region of the IL2Rβ cDNA and confirmed the existence of this AU-rich sequence with interspersed repeats of the AUUUA motif. This structure has been linked to the stability of other messages (Schuler and Cole, 1988), and it seems reasonable to speculate that it plays the same role for the IL2Rβ message. The AUUUA motifs are subject to binding by proteins termed AU binding factors (AUBF), and the resulting complex is RNase-resistant (Stephens *et al.*, 1992). AUBF proteins are believed to be activated by phosphorylation, so a THC-induced increase in phosphorylation might increase the AUBF activity and the message stability, which is what we observed for IL2Rβ message. Although the mechanism of this increase in phosphorylation is unclear at this time, it probably does not involve cannabinoid receptors on the cells, because these are known to inhibit the level of cAMP, which would lead to a decrease in PKA-induced phosphorylation.

It seems paradoxical that there is an overexpression of both IL2Rα and β proteins but an underexpression of surface receptor binding sites. This might be explained by a THC-induced deficiency in the production or function of the γ chain subunit, and indeed we found that drug treatment decreased the cellular level of γ chain mRNA (fig. 5). Other results also suggested that γ chain function is suppressed. The γ chain is a crucial component for high (α/β/γ)- and intermediate (β/γ)-affinity receptor binding and internalization of IL2. Internalization will not occur without γ chain participation, although the cell does display high-affinity binding sites (Kumaki *et al.*, 1992; Arima *et al.*, 1992; Takeshita *et al.*, 1992). Figure 6 showed that bound IL2 internalized rapidly after treatment with DMSO at kinetics similar to those reported in other systems (Kumaki *et al.*, 1993). THC treatment, however, decreased the extent of IL2 internalization, which suggested a deficiency in γ chain function. Although these data suggest a deficiency in the expression of the γ chain gene, studies are planned to analyze γ chain cellular protein and mRNA stability to clarify this point.

Other possible mechanisms might account for THC's effects on the IL2/IL2R system. For example, THC treatment might result in the overexpression of structurally immature

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or otherwise defective $\alpha$ and $\beta$ chains that lack IL2 binding affinity. Cannabinoid receptors (Matsuda *et al.*, 1990; Munro *et al.*, 1993) might also be involved. Receptor mRNA has been reported in various immune cells, including T cells, B cells and macrophages (Bouaboula *et al.*, 1993; Kaminski *et al.*, 1992), and we have preliminary data (unpublished) showing that NKB61A2 cells also express the receptor message. Furthermore, it has been demonstrated that receptor activation decreases cellular cAMP (Matsuda *et al.*, 1990; Felder *et al.*, 1992), and because this second messenger has been shown to decrease the production and expression of IL2R$\alpha$ chain and IL2R $\beta$ chain (Krause and Deutsch, 1991; Anastassiou *et al.*, 1992), it is possible that THC binding to the receptor on NKB61A2 cells enhances IL2R$\alpha$ and IL2R$\beta$ protein production through a cAMP-mediated mechanism. However, as we have already discussed, no readily discernible molecular mechanism involving cAMP accounts for the increase in IL2R$\beta$ message stability. In addition to receptor mechanisms, nonreceptor mechanisms might also be involved. For example, the drug acting at the level of the cell membrane might affect the molecular association of the IL2R subunits that is necessary for functional high- and intermediate-affinity receptors. Subunit association on the cell surface can be tested using cross-linking studies, and the role of the cannabinoid receptors in the drug effect on suppression of the IL2 system can be tested using antisense oligodeoxynucleotide technology.

Finally, the THC-induced effects on IL2R subunits could account for other drug effects noted in immune responses. The genes for both IL2R$\beta$ chain and IL2R$\gamma$ chain belong to a cytokine superfamily of genes and play critical roles not only in the IL2/IL2R system but also in other immune functions. For example, X-linked, severe combined immunodeficiency (XSCID), which is characterized by defects of early T cell development, is associated with the mutation of IL2R$\gamma$, which suggests that IL2R$\gamma$ is involved in the thymic maturation of T cells (Noguchi *et al.*, 1993a). Regarding THC and thymic effects, it is interesting to note a report showing the suppression of thymus cell maturation and proliferation (Pross *et al.*, 1987) after THC treatment. IL2R$\gamma$ also is shared by IL4 and IL7 receptors, and IL2$\beta$ is shared by IL3, IL5 and CM-CSF receptors, which indicates the importance of these subunits in the cytokine network (Kondo *et al.*, 1993; Noguchi *et al.*, 1993b; Russell *et al.*, 1993; Kondo *et al.*, 1994; Kitamura *et al.*, 1991; Miyajima *et al.*, 1992). Again, it is interesting that we recently found that THC increased the supernatant level of IL4 in stimulated, murine splenocyte cultures (Newton *et al.*, 1994), which suggests the possibility that a decrease in IL4 receptors, because of underexpression of IL2R$\gamma$, could result in an apparent increase of free, supernatant IL4.

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