Biochemical Pharmacology, Vol. 51, pp. 967–973, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02434-4

# Delta-9-tetrahydrocannabinol: An Inhibitor of STAT1α Protein Tyrosine Phosphorylation

*Zhi-Ming Zheng* and Steven Specter*

DEPARTMENT OF MEDICAL MICROBIOLOGY AND IMMUNOLOGY, UNIVERSITY OF SOUTH FLORIDA COLLEGE OF MEDICINE, TAMPA, FL, U.S.A.

**ABSTRACT.** Tyrosine-phosphorylated signal transducer and activator of transcription 1 alpha (STAT1α) is a 91-kDa protein responsible for interferon-γ (IFN-γ)-dependent transcription. The present study demonstrates that activation by IFN-γ of murine macrophages resulted in tyrosine phosphorylation of STAT1α identified by immunoprecipitation. The tyrosine phosphorylation of STAT1α was found highly sensitive to treatment by delta-9-tetrahydrocannabinol (THC), a major marijuana component. Subsequently, the isoform formation of p91 due to tyrosine phosphorylation was reduced in THC-treated macrophages. Although inhibition by THC of the tyrosine phosphorylation of STAT1α induced by IFN-γ was in a THC concentration-related manner, the tyrosine phosphorylation of other proteins induced by lipopolysaccharide/IFN-γ treatment of macrophages appeared insensitive to THC treatment. Our data suggest that blockade by THC of tyrosine phosphorylation of STAT1α may be an important mechanism involved in the broad immunosuppressive effects of THC. BIOCHEM PHARMACOL 51;7: 967–973, 1996.

**KEY WORDS.** signal transduction; macrophages; immunosuppression; interferon-stimulated gene factor 3

STAT† (previously named ISGF-3) proteins are involved in the transcriptional activation of many cytokine- and growth factor-inducible genes [1–3]. IFN-α and IFN-β stimulate protein tyrosine phosphorylation of STAT1, STAT2, and STAT3 [4, 5], whereas IFN-γ only activates the protein tyrosine phosphorylation of STAT1 [6, 7]. After tyrosine phosphorylation by IFN induction, STAT1α (91 kDa), normally sequestered in the cytoplasm, is either assembled into a multimeric complex or remains as an unbound form and then directly translocates to the nucleus where it binds to *cis*-acting enhancer elements in the regulatory regions of IFN-stimulated genes for gene activation [1, 2, 8–10]. Recent studies have shown that STAT1α, but not STAT1β (an 84-kDa protein lacking the C-terminal 38 amino acids on STAT1α), is responsible for IFN-γ-dependent transcription [11, 12].

THC, a major marijuana component, has many suppressive effects on immune functions [13–17], including cytokine production [17–20]. However, the mechanism of the immunosuppressive effects of THC is not understood. We have proposed that disturbance by THC of cellular protein tyrosine phosphorylation may be involved in the suppressive mechanisms of THC and confirmed that this is actually one of the important steps that THC affects during macrophage activation. Our previous study noted that THC treatment of LPS/IFN-γ-stimulated macrophage blocks LPS/IFN-γ-induced protein tyrosine phosphorylation of p77 and p82 [21]. Further analysis in the present study demonstrated that the protein tyrosine phosphorylation of the p82 is actually induced by IFN-γ and is a 91-kDa STAT1α protein, whereas p77 is an 84-kDa STAT1β protein. In THC-treated murine macrophages, IFN-γ-induced tyrosine phosphorylation of STAT1α was inhibited dramatically, whereas tyrosine phosphorylation of other proteins seemed insensitive to THC treatment. Our results indicate that blockade by THC of protein tyrosine phosphorylation of STAT1α may be important in immunosuppression due to this cannabinoid.

## MATERIALS AND METHODS

### *Mouse Macrophage Preparation*

BALB/c mice (8–12 weeks old; Jackson Laboratories, Bar Harbor, ME) were used as a source of resident peritoneal macrophages. Cells were obtained by washing the peritoneal cavity with 5 mL of Hanks' balanced salt solution (Gibco, Grand Island, NY) buffered with 50 mM HEPES (pH 7.4). The cells were washed once by centrifugation (200 *g* for 10 min) and resuspended in RPMI 1640 medium (Gibco). Then macrophages were counted using a hemocytometer, adjusted to $2 \times 10^6$ cells/mL, and plated in 6-well plates at 3 mL/well (Costar, Cambridge, MA). After incubation for 2 hr at 37° in an atmosphere of 5% $CO_2$,

* Corresponding author (and present address): Dr. Zhi-Ming Zheng, Laboratory of Tumor Virus Biology, Bldg. 41, Rm. C-111, NCI/NIH, Bethesda, MD 20892. Tel. (301) 496-9489; FAX (301) 402-0055.

†*Abbreviations:* ECL, enhanced chemiluminescence; IFN, interferon; IOD, integrated optical density; ISGF-3, interferon-stimulated gene factor-3; LPS, lipopolysaccharide; STAT, signal transducer and activator of transcription; THC, delta-9-tetrahydrocannabinol; and TTBS, Tween 20 (0.1%)-containing tris-buffered saline.

Received 22 September 1995; accepted 16 November 1995.

95% air, nonadherent cells were removed by washing with RPMI 1640 medium. The adherent cells (macrophages) were used throughout the study.

### THC

THC stock at 20 mg/mL in DMSO was prepared as described [21].

### *Anti-phosphotyrosine and Anti-STAT1α Immunoblotting Assay*

Murine resident peritoneal macrophages ($6 \times 10^6$ cells/well) in 6-well plates were cultured for 3 days in standard RPMI 1640 medium (no serum). Following treatment with IFN-γ and/or other stimulators, cells were washed and solubilized in 1% Triton X-100 lysis buffer, and then the anti-phosphotyrosine immunoblotting assay was performed as described [21]. These procedures were also used for anti-STAT1α immunoblot assay. After blocking and washing as described [21, 22], the membranes were probed for 1 hr with monoclonal murine anti-STAT1α (1 μg/mL in TTBS; Transduction Laboratories, Lexington, KY), incubated with goat anti-mouse IgG-horseradish peroxidase (1:3000 in TTBS) (Bio-Rad, Richmond, CA), and visualized using an ECL western blotting detection system (Amersham, Arlington Heights, IL). If the membrane was first probed with monoclonal PY20 (anti-phosphotyrosine) antibody (ICN Biochemicals, Inc., Costa Mesa, CA) in the ECL western blotting assay, the same membrane was then stripped for 10 min at room temperature with stripping solution [7 M guanidine hydrochloride, 50 mM glycine (pH 10.8), 0.05 mM EDTA, 0.1 M KCl, 20 mM mercaptoethanol] [23], blocked with TTBS containing 3% BSA and 1% ovalbumin for 2 hr, and reprobed using another antibody for the second immunoblot assay. Blots were analyzed further in a Bio-image Scanner (Millipore, Bedford, MA). The IOD of each band in each lane on each blot was chosen for final analysis [21].

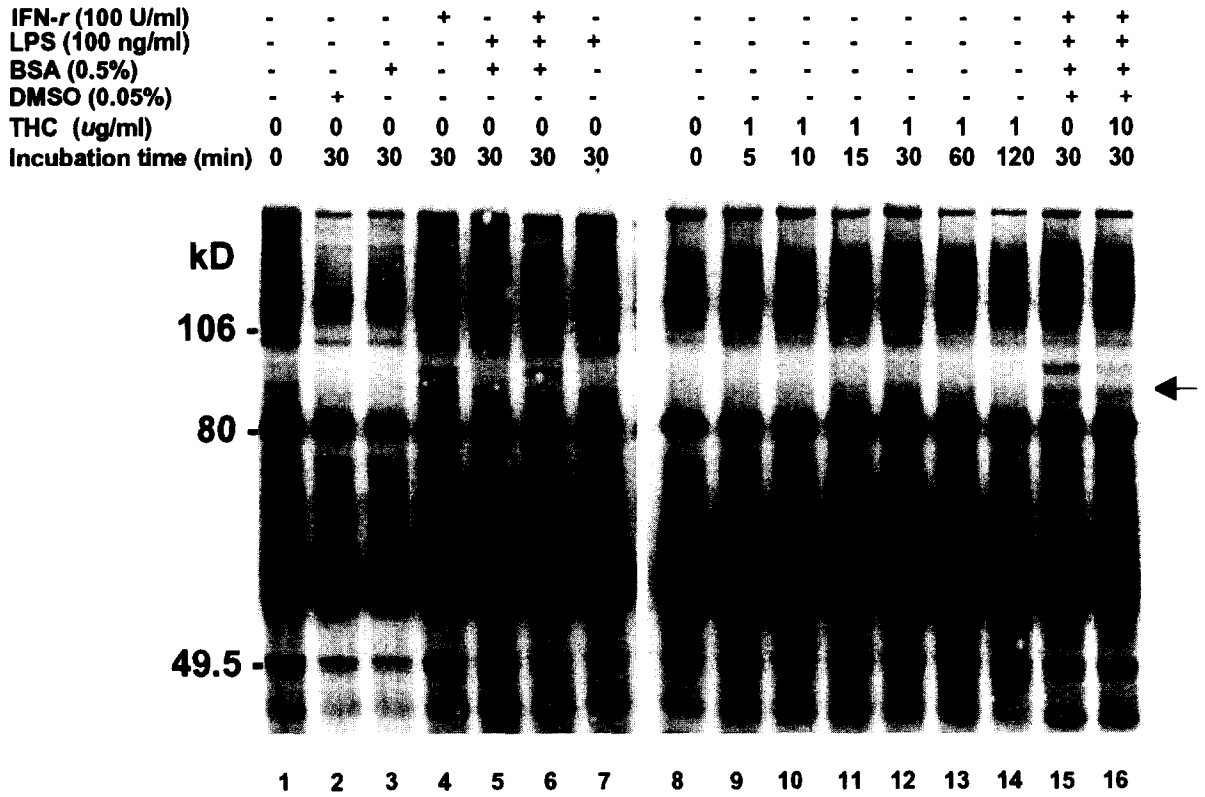



**FIG. 1. IFN-γ induction of protein tyrosine phosphorylation in murine resident peritoneal macrophages and its sensitivity to THC. Murine resident peritoneal macrophages ($6 \times 10^6$ cells/well) in 6-well plates were cultured for 3 days in standard RPMI 1640 medium (no serum) and then stimulated at 37° with RPMI 1640 medium containing stimulator(s) as indicated. The macrophages were washed with ice-cold PBS containing 1 mM $Na_3VO_4$ and lysed in 0.25 mL of 1% Triton X-100 lysis buffer [21]. The lysate was subjected to 12% SDS-PAGE and then electrotransferred to a pre-wet nitrocellulose membrane overnight at 20 V. The anti-phosphotyrosine immunoblot assay was performed using mouse monoclonal anti-phosphotyrosine antibody PY20 (1 μg/mL) (ICN) and then goat anti-mouse IgG-horseradish peroxidase (1:3000 in TTBS). An ECL detection system (Amersham) was used for visualization of specific bands on the blot. The arrow indicates IFN-γ-induced tyrosine-phosphorylated protein.**

### *Immunoprecipitation*

Following treatment with IFN-γ, macrophages ($6 \times 10^6$) were solubilized in 200 μL of 1% Triton X-100 lysis buffer as prepared for the anti-phosphotyrosine immunoblotting assay [21]. The macrophage lysate was immunoprecipitated overnight at 4° by mixing with 2 μg of murine monoclonal anti-STAT1α antibody. After addition of 50 μL of protein G-Sepharose (50% slurry; Pharmacia, Uppsala, Sweden), the mixture was incubated for 1 hr at 37° with gentle shaking. The suspension was centrifuged, and the pellet (beads) was washed three times in the lysis buffer. The precipitates on the beads were boiled for 5 min in 25 μL of 2× SDS sample buffer and separated on 12% SDS-PAGE. The gels were transferred overnight at room temperature to nitrocellulose membranes (Bio-Rad). Anti-phosphotyrosine and anti-STAT1α immunoblotting analysis was performed as described above.

## RESULTS

### *Protein Tyrosine Phosphorylation Induced by IFN-γ Treatment in Macrophages*

Since macrophages are prime target cells for IFN-γ, experiments were performed to determine whether IFN-γ induces protein tyrosine phosphorylation in these cells. Cytoplasmic extracts were prepared from mouse resident peritoneal macrophages incubated with IFN-γ or other stimuli and analyzed by an anti-phosphotyrosine immunoblotting assay using a monoclonal anti-phosphotyrosine antibody, PY20 (Fig. 1). The experiments demonstrated that the extracts prepared from macrophages treated with IFN-γ showed a newly phosphorylated protein with tyrosine phosphorylation (Fig. 1). This protein, with an apparent molecular mass of approximately 91 kDa (arrow), was found to be sensitive to THC treatment of the cells. Its tyrosine phosphorylation was inhibited approximately 50% by treatment with 10 μg THC/mL [lane 15 (IOD value 0.74) vs lane 16 (IOD value 0.38) in Fig. 1]. However, 1 μg THC/mL (instead of 10 μg/mL which is very toxic to the cells at 2–3 hr of incubation in the protein-free medium [17]) was unable to induce such a protein tyrosine phosphorylation, as indicated in Fig. 1 (lanes 9–14).

### *Identification of IFN-γ-Induced Tyrosine-Phosphorylated Protein as a STAT1α Protein Sensitive to THC*

Since the size of the protein is similar to the size of STAT1α, and IFN-γ is able to induce tyrosine phosphorylation of STAT1α, experiments were conducted to determine if the p91 sensitive to THC treatment is actually STAT1α. Cytoplasmic extracts were prepared from macrophages incubated with IFN-γ for 30 min at 37° and subjected to immunoprecipitation with monoclonal anti-STAT1α antibody. The immunoprecipitate was probed first with a monoclonal anti-STAT1α antibody immunoblotting assay and then reprobed with an anti-phosphotyrosine immunoblotting assay. The results demonstrated clearly that the IFN-γ-induced p91 with tyrosine phosphorylation was, in fact, tyrosine-phosphorylated STAT1α (top band in Fig. 2, A and B). The lower kDa band in Fig. 2A and B was STAT1β (p84), since monoclonal anti-STAT1α antibody has a cross-reaction to STAT1β and PY20 antibody could also stain tyrosine-phosphorylated STAT1β

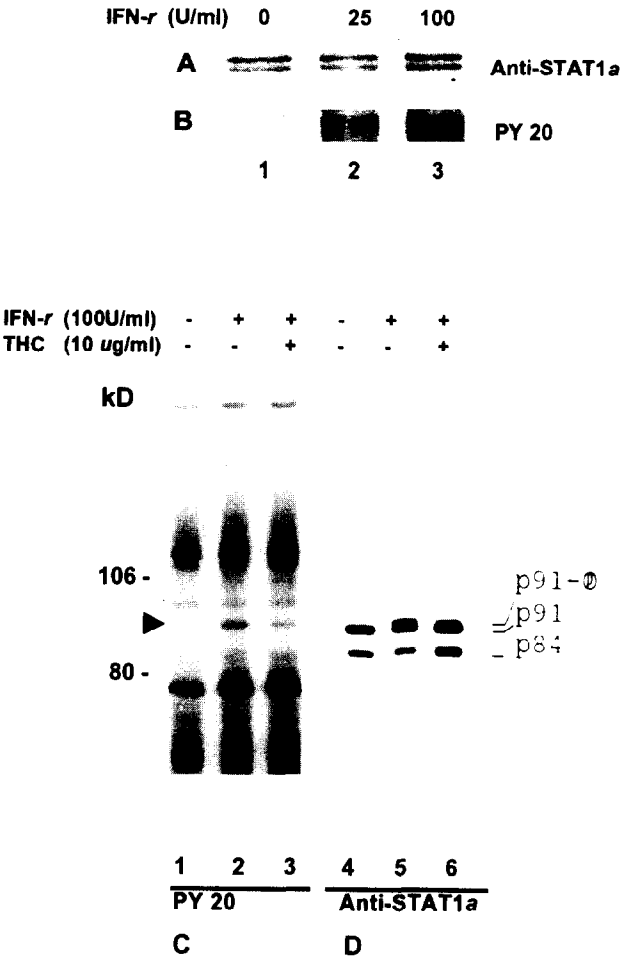



**FIG. 2. Identification by immunoprecipitation and immunoblotting of IFN-γ-induced tyrosine-phosphorylated protein as a p91-kDa STAT1α protein. Murine resident peritoneal macrophages ($6 \times 10^6$ cells/well) in 6-well plates were cultured for 3 days in standard RPMI 1640 medium (no serum) and then stimulated for 30 min at 37° with different stimulators in RPMI 1640 as indicated. Macrophages were washed and lysed in 0.25 mL of 1% Triton X-100 lysis buffer [21]. Lysates were spun at approximately 10,000 *g* in an Eppendorf centrifuge for 15 min at 4°. The supernatant fluids were collected and immunoprecipitated with 2 μg of mouse monoclonal anti-STAT1α antibody. The immunoprecipitates were subjected to 12% SDS–PAGE and electrotransferred to a pre-wet nitrocellulose (NC) membrane overnight at 20 V for anti-STAT1α (panel A) and then anti-phosphotyrosine (panel B) immunoblot assay. Alternatively, the cytoplasmic extracts prepared were subjected directly to 7.5% SDS–PAGE and electrotransferred to NC membrane as above for anti-phosphotyrosine (panel C) and then anti-STAT1α immunoblot assay (panel D). The arrow indicates IFN-γ-induced tyrosine-phosphorylated protein (panel C) and the isoform of the tyrosine-phosphorylated STAT1α (panel D).**

(Fig. 2B). When cytoplasmic extracts were prepared from macrophages incubated with IFN-γ plus THC, protein tyrosine phosphorylation of p91 was inhibited so dramatically that very little tyrosine-phosphorylated p91 was detectable in these extracts (lane 3 in Fig. 2C). Image analysis on the p91 band shows that THC reduced about 70% of the IFN-γ-induced protein tyrosine phosphorylation as compared with controls treated with IFN-γ only [lane 3 (IOD value 0.45) vs lane 2 (IOD value 1.46) in Fig. 2C). Further immunoblot assay of the same membrane using monoclonal anti-STAT1α antibody demonstrated that suppression by THC of IFN-γ-induced tyrosine phosphorylation of p91 actually diminished its isoform formation, a protein with a slightly higher molecular weight after phosphorylation (lane 6 in Fig. 2D).

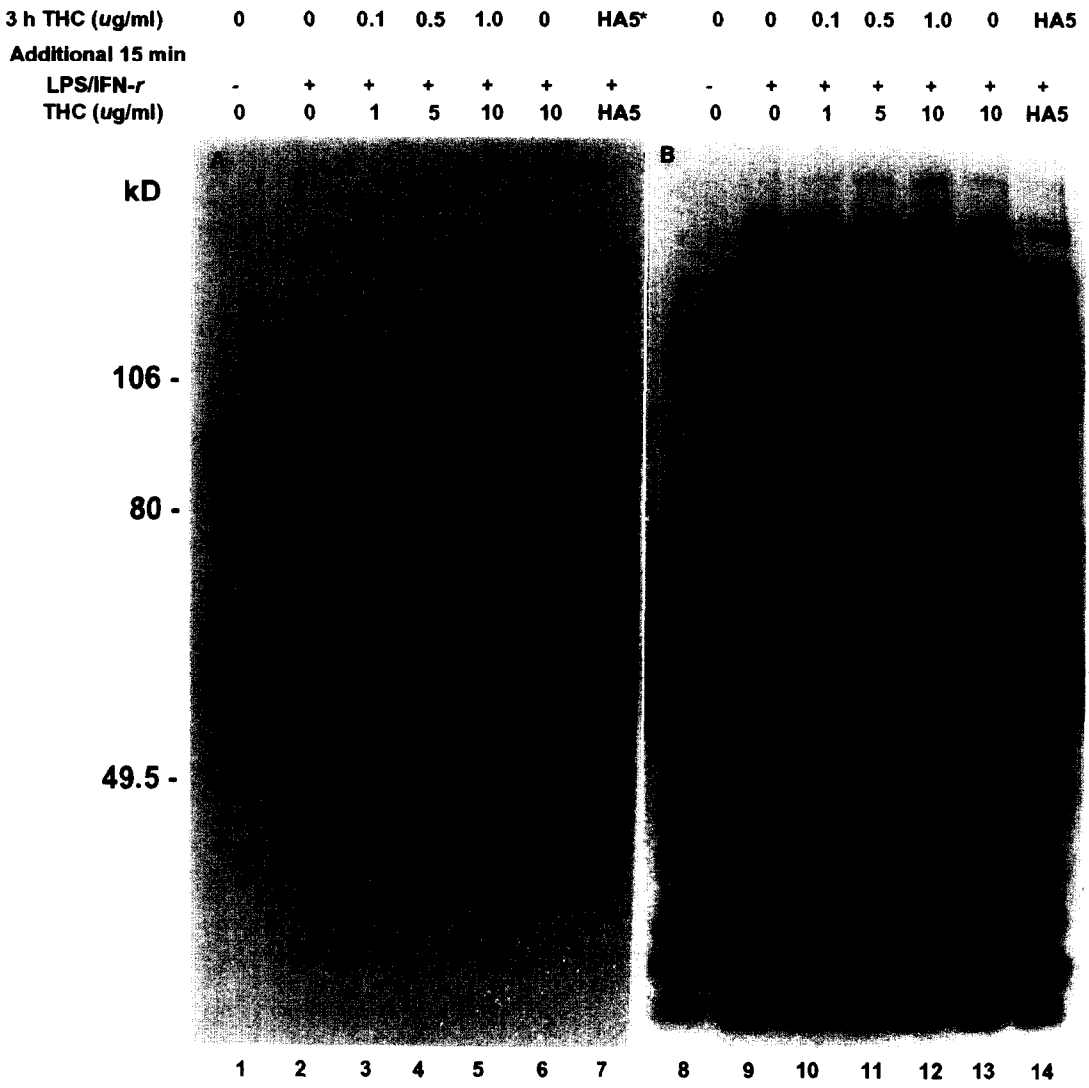
### *Concentration Curve of THC Suppression on STAT1α Protein Tyrosine Phosphorylation Induced by IFN-γ*

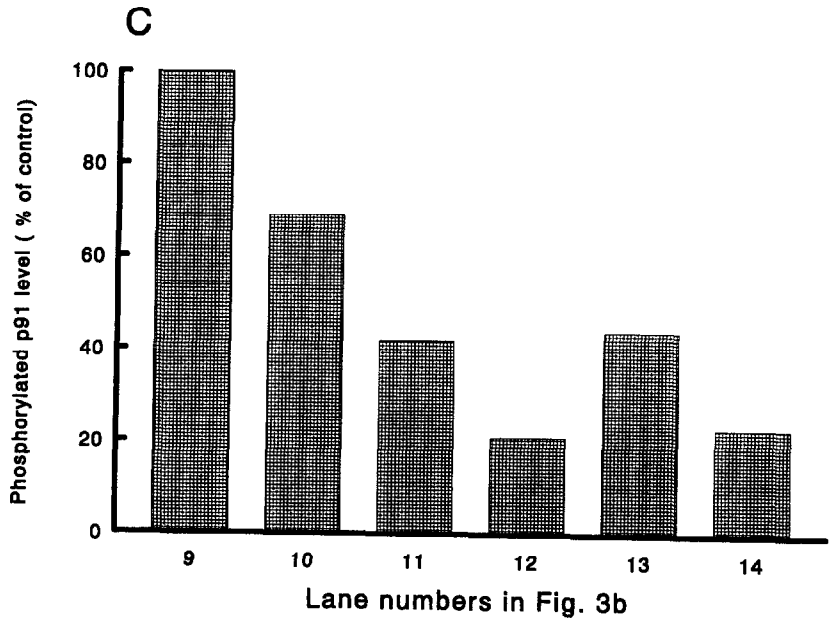
Finally, evidence that protein tyrosine phosphorylation of STAT1α is sensitive to THC treatment was provided by a concentration curve study. Since bacterial LPS is also able to induce tyrosine phosphorylation of many proteins [24–28] but not STAT1α in macrophages in our studies, experiments were designed to include LPS as an IFN-γ co-inducer not only to mimic our previous macrophage activation protocol [17, 18, 21, 22], but also to determine whether tyrosine phosphorylation of other proteins is sensitive to THC. This approach would provide information for us to explain THC-induced suppression of TNF-α release by LPS/IFN-γ-activated macrophages [17, 18]. A combination of THC pre and simultaneous treatment was chosen for the concentration curve study since the protocol is most effective for altering macrophage function [21]. THC concentrations were reduced in the pretreatment since the 5–10 μg THC/mL used for simultaneous treatment in medium supplemented with 0.5% BSA was very toxic to the cells using the 3 hr incubation for pretreatment in medium free of BSA or other added proteins [21]. As shown in Fig. 3, LPS/IFN-γ stimulation of macrophages for 15 min induced tyrosine phosphorylation of several proteins including STAT1α (lanes 2 and 9, arrows). When macrophages were pretreated for 3 hr with a low concentration of THC prior to LPS/IFN-γ stimulation (lanes 3–5 and 10–12) and/or treated simultaneously with a high concentration of THC during LPS/IFN-γ stimulation (lanes 6 and 13), only the formation of the STAT1α isoform, due to tyrosine phosphorylation, was obviously blocked in a concentration-dependent manner (Fig. 3A). The reduction of STAT1α isoform formation was correlated to the inhibition by THC of p91 protein tyrosine phosphorylation (Fig. 3, B and C). As we predicted, the combination treatment [pre (0.1 μg THC/mL) plus simultaneous (10 μg THC/mL) treatment] had a greater (2-fold) suppression of STAT1α tyrosine phosphorylation than the simultaneous (10 μg/mL) treatment alone (lane 12 vs lane 13 in Fig. 3, B and C). Herbimycin A, a tyrosine kinase inhibitor [29, 30], was included as a positive control in these experiments. Similar to THC, suppression by herbimycin A of protein tyrosine phosphorylation resulted in only a small amount of tyrosine-phosphorylated STAT1α formation (lanes 7 and 14 in Fig. 3).

## DISCUSSION

IFN-γ is a cytokine produced by T cells and natural killer cells that is important in host defense, cellular growth and differentiation, and immunopathogenesis [31]. The receptor for IFN-γ (IFN-γR) is part of a family of cytokine receptors, and binding of IFN-γ to various epithelial and fibroblast cells initiates a rapid tyrosine phosphorylation of IFN-γR, Jak1, Jak2, and STAT1α [7, 32]. The present study has focused on the IFN-γ-induced tyrosine phosphorylation of STAT1α in macrophages and has demonstrated that induction by IFN-γ of STAT1α tyrosine phosphorylation is required as an immediate early signal event for macrophage activation. Furthermore, our results have shown that IFN-γ-induced STAT1α tyrosine phosphorylation is highly sensitive to THC treatment when compared with other protein tyrosine phosphorylation induced by LPS/IFN-γ activation. Although it has been reported that tyrosine phosphorylation of IFN-γR and Jak proteins precedes that of STAT1α [32], it remains unclear at present how THC inhibits this signal transduction pathway during macrophage activation. Since tyrosine phosphorylation of other proteins induced by LPS/IFN-γ seems insensitive to THC treatment (Fig. 3B), the results may imply that the tyrosine phosphorylation of STAT1α induced by IFN-γ may be targeted specifically by THC treatment. Nevertheless, a more extensive study on whether IFN-γ-induced tyrosine phosphorylation of IFN-γR and Jak proteins is sensitive to THC

**FIG. 3. Concentration curve of THC suppression on STAT1α protein tyrosine phosphorylation induced by IFN-γ. Murine macrophages at 6 × $10^6$ cells/well in a 6-well plate were cultured for 3 days in standard RPMI 1640 medium and then incubated with or without THC in RPMI 1640 for 3 hr. The THC-treated and -untreated macrophages were subsequently changed to 0.5% BSA-supplemented RPMI 1640 medium containing either no IFN-γ/LPS, IFN-γ (100 U/mL)/LPS (100 ng/mL), IFN-γ/LPS plus THC, or IFN-γ/LPS plus HA (herbimycin A) as indicated in the figure and incubated for 15 min at 37°. Then the macrophages were washed and lysed into the sample buffer as described in Fig. 1. The cell lysates were run on 7.5% SDS–PAGE, immunoblotted first with monoclonal murine anti-STAT1α antibody (panel A) and then reprobed with PY-20 (panel B) as indicated in Figs. 1 and 2. Panel C shows the results from image analysis on tyrosine-phosphorylated p91 (STAT1α) protein in panel B. The IOD of each band in each lane has been converted into the percent of control (see lane 9 of panel B). The arrows indicate the isoform of tyrosine-phosphorylated STAT1α (A) and IFN-γ-induced p91 protein tyrosine phosphorylation (B).**


3 h THC (ug/ml) 0 0 0.1 0.5 1.0 0 HA5* 0 0 0.1 0.5 1.0 0 HA5
Additional 15 min
LPS/IFN-r - + + + + + + - + + + + + +
THC (ug/ml) 0 0 1 5 10 10 HA5 0 0 1 5 10 10 HA5
kD
106 -
80 -
49.5 -
A
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14
HA5* = herbimycin A (5 ug/ml)


C
Phosphorylated p91 level ( % of control)
100
80
60
40
20
0
9 10 11 12 13 14
Lane numbers in Fig. 3b

treatment is needed to confirm whether STAT1α is affected directly or if another step in this activation pathway is affected.

Macrophage activation in response to external stimuli is a complex process composed of two stages: a priming stage and a triggering stage [33]. IFN-γ released by activated T-lymphocytes is mainly responsible for the priming activity [34, 35], whereas bacterial LPS classically provides the triggering signal [36]. After activation, macrophages release TNF-α as an important mediator involved in cytokine networks and in host defense mechanisms [37]. Albeit the molecular mechanism of macrophage priming by IFN-γ is poorly understood, IFN-γ priming of macrophages is known to be important for TNF-α production during LPS stimulation in our unpublished observations as well as from other reports [33, 38]. Since IFN-γ-induced STAT1α tyrosine phosphorylation usually appears earlier than LPS-induced protein tyrosine phosphorylation [21], we believe that STAT1α may be an important protein involved in the IFN-γ priming process. Thus, suppression by THC of IFN-γ-induced protein tyrosine phosphorylation of STAT1α and THC's reduction of TNF-α release by LPS/IFN-γ-activated macrophages in our previous studies [17, 18] suggests that a relationship may exist between tyrosine-phosphorylated STAT1α and TNF-α release by macrophages.

Although marijuana and its active ingredient, THC, have been used in medicine for many years [39], the exploitation of the possible medical benefits has been limited by its powerful psychoactive properties and long-term toxic effects on brain [40], lung, and reproductive function [41] as well as immune defenses [13, 42]. Our results support the concept that THC is immunosuppressive. This suppressive effect may be mediated through receptor-mediated or non-receptor-mediated processes, since THC receptors have not been definitely found on macrophages [43–45].

*This study was supported by a grant (DA04141) from the U.S. Public Health Service via the National Institute on Drug Abuse.*

## References


1. Darnell JE Jr, Kerr IM and Stark GR, Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signal proteins. *Science* **264:** 1415–1421, 1994.
2. Taniguchi T, Cytokine signaling through nonreceptor protein tyrosine kinases. *Science* **268:** 251–255, 1995.
3. Hill CS and Treisman R, Transcriptional regulation by extracellular signals: Mechanisms and specificity. *Cell* **80:** 199–211, 1995.
4. Schindler C, Shuai K, Prezioso VR and Darnell JE Jr, Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science* **257:** 809–813, 1992.
5. Stahl N, Farruggella TJ, Boulton TG, Zhong Z, Darnell JE Jr and Yancopoulos GD, Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* **267:** 1349–1353, 1995.
6. Shuai K, Schindler C, Prezioso VR and Darnell JE Jr, Activation of transcription by IFN-γ: Tyrosine phosphorylation of a 91-kD DNA binding protein. *Science* **258:** 1808–1812, 1992.
7. Greenlund AC, Farrar MA, Viviano BL and Schreiber RD, Ligand-induced IFNγ receptor tyrosine phosphorylation couples the receptor to its signal transduction system (p91). *EMBO J* **13:** 1591–1600, 1994.
8. David M and Larner AC, Activation of transcription factors by interferon-alpha in a cell-free system. *Science* **257:** 813–815, 1992.
9. Larner AC, David M, Feldman GM, Igarashi K-i, Hackett RH, Webb DSA, Sweitzer SM, Petricoin EF III and Finbloom DS, Tyrosine phosphorylation of DNA binding proteins by multiple cytokines. *Science* **261:** 1730–1733, 1993.
10. Perez C, Wietzerbin J and Benech PD, Two *cis*-DNA elements involved in myeloid-cell-specific expression and gamma interferon (IFN-γ) activation of the human high-affinity Fcγ receptor gene: A novel IFN regulatory mechanism. *Mol Cell Biol* **13:** 2182–2192, 1993.
11. Fu X-Y, Schindler C, Improta T, Aebersold R and Darnell JE Jr, The proteins of ISGF-3, the interferon α-induced transcriptional activator, define a gene family involved in signal transduction. *Proc Natl Acad Sci USA* **89:** 7840–7843, 1992.
12. Shuai K, Stark GR, Kerr IM and Darnell JE Jr, A single phosphotyrosine residue of Stat91 required for gene activation by interferon-γ. *Science* **261:** 1744–1746, 1993.
13. Nahas GG, Suciu-Foca N, Armand J-P and Morishima A, Inhibition of cellular immunity in marijuana smokers. *Science* **183:** 419–420, 1974.
14. Klein TW, Newton CA, Widen R and Friedman H, The effect of delta-9-tetrahydrocannabinol and 11-hydroxy-delta-9-tetrahydrocannabinol on T-lymphocyte and B-lymphocyte mitogen responses. *J Immunopharmacol* **7:** 451–466, 1985.
15. Specter SC, Klein TW, Newton C, Mondragon M, Widen R and Friedman H, Marijuana effects on immunity: Suppression of human natural killer cell activity by delta-9-tetrahydrocannabinol. *Int J Immunopharmacol* **8:** 741–745, 1986.
16. Specter S, Lancz G and Goodfellow D, Suppression of human macrophage function *in vitro* by $\Delta^9$-tetrahydrocannabinol. *J Leukoc Biol* **50:** 423–426, 1990.
17. Zheng Z-M, Specter S and Friedman H, Inhibition by delta-9-tetrahydrocannabinol of tumor necrosis factor alpha production by mouse and human macrophages. *Int J Immunopharmacol* **14:** 1445–1452, 1992.
18. Zheng ZM, Specter S and Friedman H, Serum proteins affect the inhibition by delta-9-tetrahydrocannabinol of tumor necrosis factor alpha production by mouse macrophages. *Adv Exp Med Biol* **335:** 89–93, 1993.
19. Fischer-Stenger K, Dove Pettit DA and Cabral GA, $\Delta^9$-Tetrahydrocannabinol inhibition of tumor necrosis factor-α: Suppression of post-translation events. *J Pharmacol Exp Ther* **267:** 1558–1565, 1993.
20. Blanchard DK, Newton C, Klein TW, Stewart WE II and Friedman H, *In vitro* and *in vivo* suppressive effects of delta-9-tetrahydrocannabinol on interferon production by murine spleen cells. *Int J Immunopharmacol* **8:** 819–824, 1986.
21. Zheng ZM and Specter S, Suppression by delta-9-tetrahydrocannabinol of lipopolysaccharide-induced and intrinsic tyrosine phosphorylation and protein expression in mouse peritoneal macrophages. *Biochem Pharmacol* **47:** 2243–2252, 1994.
22. Zheng ZM, Specter SC and Lancz G, Bovine serum albumin preparations enhance *in vitro* production of tumor necrosis factor α by murine macrophages. *Immunol Invest* **24:** 737–756, 1995.
23. Ruff-Jamison S, Chen K and Cohen S, Induction by EGF and interferon-γ of tyrosine phosphorylated DNA binding proteins in mouse live nuclei. *Science* **261:** 1733–1736, 1993.
24. Weinstein SL, Gold MR and DeFranco AL, Bacterial lipopolysaccharide stimulates protein tyrosine phosphorylation in macrophages. *Proc Natl Acad Sci USA* **88:** 4148–4152, 1991.
25. Weinstein SL, Sanghera JS, Lemke K, DeFranco AL and

Pelech SL, Bacterial lipopolysaccharide induces tyrosine phosphorylation and activation of mitogen-activated protein kinases in macrophages. *J Biol Chem* **267:** 14955–14962, 1992.
26. Weinstein SL, June CH and DeFranco AL, Lipopolysaccharide-induced protein tyrosine phosphorylation in human macrophages is mediated by CD14. *J Immunol* **151:** 3829–3838, 1993.
27. Dong Z, O'Brian CA and Fidler IJ, Activation of tumoricidal properties in macrophages by lipopolysaccharide requires protein-tyrosine kinase activity. *J Leukoc Biol* **53:** 53–60, 1993.
28. Dong Z, Qi X and Fidler IJ, Tyrosine phosphorylation of mitogen-activated protein kinases is necessary for activation of murine macrophages by natural and synthetic bacterial products. *J Exp Med* **177:** 1071–1077, 1993.
29. Uehara Y and Fukazawa H, Use and selectivity of herbimycin A as inhibitor of protein tyrosine kinases. *Methods Enzymol* **201:** 370–379, 1991.
30. Levitzki A and Gazit A, Tyrosine kinase inhibition: An approach to drug development. *Science* **267:** 1782–1788, 1995.
31. Farrar MA and Schreiber RD, The molecular cell biology of interferon-γ and its receptor. *Annu Rev Immunol* **11:** 571–611, 1993.
32. Igarash K-i, Garotta G, Ozmen L, Ziemiecki A, Wilks AF, Harpur AG, Larner AC and Finbloom DS, Interferon-γ induces tyrosine phosphorylation of interferon-γ receptor and regulated association of protein tyrosine kinases, Jak1 and Jak2, with its receptor. *J Biol Chem* **269:** 14333–14336, 1994.
33. Manthey CL, Perera P-Y, Salkowski CA and Vogel SN, Taxol provides a second signal for murine macrophage tumoricidal activity. *J Immunol* **152:** 825–831, 1994.
34. Meltzer MS, Macrophage activation for tumor cytotoxicity: Characterization of priming and trigger signals during lymphokine activation. *J Immunol* **127:** 179–183, 1981.
35. Nicolet CM and Paulnock DM, Promoter analysis of an interferon-inducible gene associated with macrophage activation. *J Immunol* **152:** 153–162, 1994.
36. Hayes MP and Zoon KC, Priming of human monocytes for enhanced lipopolysaccharide responses: Expression of alpha interferon, interferon regulatory factors, and tumor necrosis factor. *Infect Immun* **61:** 3222–3227, 1993.
37. Vassalli P, The pathophysiology of tumor necrosis factor. *Annu Rev Immunol* **10:** 411–452, 1992.
38. Yu S-F, Koerner TJ and Adams DO, Gene regulation in macrophage activation: Differential regulation of genes encoding for tumor necrosis factor, interleukin-1, JE, and KC by interferon-γ and lipopolysaccharide. *J Leukoc Biol* **48:** 412–419, 1990.
39. Caswell A, Marijuana as medicine. *Med J Aust* **156:** 497–498, 1992.
40. Block RI and Ghoneim MM, Effects of chronic marijuana use on human cognition. *Psychopharmacology* **110:** 219–228, 1993.
41. Nahas GG and Latour C, The human toxicity of marijuana. *Med J Aust* **156:** 495–497, 1992.
42. Gupta S, Grieco MH and Cushman P Jr, Impairment of rosette-forming T lymphocytes in chronic marijuana smokers. *N Engl J Med* **291:**874–877, 1974.
43. Munro S, Thomas KL and Abu-Shaar M, Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365:** 61–65, 1993.
44. Lynn AB and Herkenham M, Localization of cannabinoid receptors and nonsaturable high density cannabinoid binding sites in peritoneal tissues of the rat: Implications for receptor-mediated immune modulation by cannabinoids. *J Pharmacol Exp Ther* **268:** 1612–1623, 1994.
45. Klein TW, Newton C, Zhu W, Daaka Y and Friedman H, $\Delta^9$-Tetrahydrocannabinol, cytokines, and immunity to *Legionella pneumophila*. *Proc Soc Exp Biol Med* **209:** 205–212, 1995.