

INHIBITION BY DELTA-9-TETRAHYDROCANNABINOL OF TUMOR NECROSIS FACTOR ALPHA PRODUCTION BY MOUSE AND HUMAN MACROPHAGES

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Abstract — Suppression by delta-9-tetrahydrocannabinol (THC) of tumor necrosis factor (TNF) production by macrophages has not been reported previously. The present study evaluated the effect *in vitro* of THC on soluble TNF- α production by cultured murine peritoneal macrophages. THC at 5 or 10 $\mu\text{g}/\text{ml}$ added to medium [RPMI 1640 containing 10 ng LPS/ml, mouse IFN- γ (100 u/ml), and 0.5% bovine serum albumin (BSA)] used to induce TNF significantly decreased TNF- α production by BALB/c mouse macrophages. Macrophages pretreated with THC at 0.1, 0.5, or 1.0 $\mu\text{g}/\text{ml}$ in protein-free medium for 3 h at 37°C, prior to TNF induction, also showed a decreased ability to produce TNF- α in a dose-dependent manner. Increasing the protein concentration from 0.5 to 5% BSA in the medium which was used to induce TNF prevented the inhibitory activity of THC. Human peripheral blood adherent cells treated with THC-containing medium produced less TNF- α than controls that were not exposed to THC. Thus, our data provide evidence that THC can inhibit TNF production by mouse and human macrophages. The drug's activity is concentration dependent and is related to the amount of serum protein in the medium used to induce this cytokine.

Individuals who smoke marijuana have been reported to have decreased cellular immunity (Nahas, Suci-Foca, Armand & Morishima, 1974). *In vitro* studies of the effects of marijuana's major psychoactive component, delta-9-tetrahydrocannabinol (THC), on immune functions show that THC is able to inhibit both human and mouse natural killer cell activity (Specter, Klein, Newton, Mondragon, Widen & Friedman, 1986; Kawakami, Klein, Newton, Djeu, Specter & Friedman, 1988), lymphocyte proliferation in response to mitogens (Klein, Newton, Widen & Friedman, 1985; Specter, Lancz & Hazelden, 1990b), and phagocytosis of yeast by macrophages (Lopez-Cepero, Friedman, Klein & Friedman, 1986; Specter, Lancz & Goodfellow, 1991). Moreover, THC was found to suppress mitogen-induced increases in mouse thymocyte cytosolic calcium (Yebara, Klein & Friedman, 1992), mouse splenocyte antibody forming capacity (Klein & Friedman, 1990), mouse delayed hypersensitivity responses (Smith, Harris,

Uwaydah & Munson, 1978), and interferon (IFN) induction in mouse spleen cell cultures (Blanchard, Newton, Klein, Stewart & Friedman, 1986). However, the effect of THC on tumor necrosis factor (TNF) production by macrophages has not been reported.

TNF- α , which is secreted principally by activated macrophages (Old, 1988), has been shown recently to be produced in greater amounts if stimulation of murine peritoneal macrophages is performed in medium containing *Salmonella enteritidis* lipopolysaccharide (LPS) and 0.5% bovine serum albumin (BSA) in place of 10% fetal bovine serum (FBS) (Z.M. Zheng & S. Specter, unpublished observations). The use of BSA resulted in a reduction in the serum protein in the medium used to examine the effect of THC on TNF- α production. This is important since medium containing 10% FBS inhibits THC activity. The present report provides evidence that THC decreases TNF- α production (stated in the broad sense to encompass production

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and release) by **both mouse peritoneal macrophages** and adherent cells (**macrophages**) isolated from human peripheral blood.

EXPERIMENTAL PROCEDURES

Reagent preparations

RPMI 1640 (Gibcoj containing 2 mM glutamine, 20 mM HEPES (pH 7.3). and penicillin/streptomycin (100 u/ml and 100 µg/ml, respectively) is referred to as "standard" medium. This medium when supplemented with 10% FBS (Hyclone Laboratories, Inc. Logan, UT) and 5×10^{-5} M 2-mercaptoethanol is referred to as "complete" medium.

Phenol-extracted LPS from *S. enteritidis* (Sigma Chemical Co., St. Louis, MO) was diluted in sterile pyrogen-free PBS to yield a stock concentration of 1 mg/ml, which was aliquored and stored at -20°C .

THC was obtained from the National Institute on Drug Abuse (NIDA), Bethesda, MD. The stock THC was dried to remove ethanol and then dissolved in dimethyl sulfoxide (DMSO) to yield a stock concentration of 20 mg/ml as described previously (Specter *et al.*, 1990b).

Mouse macrophage culture and TNF- α induction

Macrophages were obtained by washing the peritoneal cavity of 8-18-week-old female BALB/c mice with 5 ml of cold Hanks' balanced salt solution (HBSS) containing 50 mM HEPES. The cells were washed twice by centrifugation at 300 g for 10 min at room temperature and resuspended in standard medium. The cells were counted, adjusted to 1×10^6 cells/ml, and then plated in flat-bottom 96-well plates (Costar, Cambridge, MA) at 100 µl/well. After incubation for 2 h at 37°C in 5% CO_2 and 95% air, nonadherent cells were removed by washing with standard medium, and the adherent cells were used for TNF- α induction.

For THC pretreatment, adherent cells were treated with standard medium containing 0.5% DMSO (the diluent for THC), or 0.1, 0.5, or 1 µg (3.2×10^{-6} M) THC/ml for 3 h at 37°C in 5% CO_2 . Unbound THC was removed by washing cells twice, as indicated above, using standard medium and cell viability was determined using trypan blue dye exclusion. Next, "induction" medium [standard medium containing LPS (10 ng/ml), mouse IFN- γ (100 u/ml, Genzyme, Cambridge, MA), and 0.5% BSA (Sigma)] was added to yield a final volume of 100 µl/well for TNF- α induction.

The effect of simultaneous addition of THC and induction medium to adherent cells was tested using 100 µl/well induction medium containing either DMSO or THC at 1, 5, or 10 µg/ml for 6 h at 37°C in 5% CO_2 . The higher concentrations of THC in this protocol are necessitated by the presence of 0.5% BSA in this medium as compared with no serum protein in the pretreatment protocol. Supernatant fluids from adherent cell cultures were then collected and stored at -20°C for use in the TNF- α assay.

Human monocyte macrophage preparation and TNF- α induction

Buffy coats were obtained from volunteers seen at the Southwest Florida Blood Bank (Tampa, FL). Human peripheral blood mononuclear cells (PBMC) were separated from the buffy coat by centrifugation through Ficoll - Hypaque (Pharmacia LKB Biotech. Inc., Piscataway, NJ). The PBMC were then incubated in 225 cm² plastic flasks (Costar) at 37°C for 2 h to allow cells to adhere to the surface. Nonadherent cells were removed by washing with PBS. Adherent cells (PBAC) were rinsed with cold HBSS (without Mg^{2+} and Ca^{2+}) and removed using a cell scraper (Costar). Cells were adjusted to 1×10^6 /ml using complete medium, plated in flat-bottom 96-well plates at 100 µl/well, and incubated overnight at 37°C in 5% CO_2 . The adherent cells were then treated to induce TNF as described above for murine macrophages, except that LPS was increased to 100 ng/ml and 100 u/ml human IFN- γ (Sigma) was used in place of mouse IFN- γ . The supernatant fluids from these cultures were collected after 6 h incubation at 37°C in 5% CO_2 and stored at -20°C for TNF- α assay.

Titration of TNF- α by bioassay

Lysis of a TNF-sensitive murine cell line, WEHI-164, using a ^{51}Cr release cytotoxicity assay (Laskov *et al.*, 1990; Eskandari, Nguyen, Kunkel & Remick, 1990) was used to measure TNF- α activity. WEHI-164 cells (1×10^6) were labeled with 100 µCi of $\text{Na}^{51}\text{Cr}(\text{O}_4)_2$ (Amersham, Arlington Heights, IL) for 1 h at 37°C . The labeled cells were washed three times with 10 ml of complete medium to remove excess label and adjusted to 5×10^5 cells/ml. One hundred microliters of macrophage supernatant fluids (TNF) were added to 100 µl of labeled WEHI-164 cells in 96-well flat-bottom microtiter plates. Three cultures were prepared for each supernatant. After 6 h at 37°C , the ^{51}Cr released into 100 µl of the supernatant fluid from lysed target cells

was collected and counted in a gamma counter as a measure of cell death. Results in triplicate wells of each macrophage supernatant are averaged and expressed as percent specific cytotoxicity as calculated by the following formula:

$$\% \text{ Specific cytotoxicity} = \frac{\text{experimental counts/min} - \text{spontaneous counts/min}}{\text{maximum counts/min} - \text{spontaneous counts/min}} \times 100.$$

Spontaneous release was determined from wells containing ^51Cr -labeled WEHI-164 cells incubated in standard medium. Maximum release was obtained from incubation of ^51Cr -labeled WEHI-164 cells with 100 μl of 10% sodium dodecyl sulfate. Spontaneous release in the 6 h assay was in the range of 15–20% of maximum release. The number of TNF- α units in each experiment was calculated by interpolation of samples along a curve of percent cytotoxicity created using dilutions of a murine TNF- α standard (Genzyme) and then using regression analysis (Duncan, Knapp & Miller, 1983) in each experiment.

Confirmation that the cytotoxic activity of macrophage supernatant fluids for WEHI-164 cells was due to TNF- α was made by mixing 0.99 ml of macrophage supernatant fluids with 10 μl of rabbit anti-mouse TNF-Q polyclonal antibody (Genzyme, 1 μl of the antibody can neutralize about 1000 u of mouse TNF- α in a bioassay) at room temperature for 1 h. Subsequently, 100 μl of antibody treated supernatant fluids, in triplicate, were added to 100 μl of the ^51Cr -labeled WEHI cells and TNF activity was measured.

Statistical analysis

Statistical analysis was done using the one-tailed analysis of a pooled two sample *t*-test (Duncan *et al.*, 1983). Experimental values were compared with the vehicle control value only. *P* values < 0.05 were considered significant.

RESULTS

Inhibition by THC of TNF- α production by murine peritoneal macrophages

Addition of JHC to macrophage cultures during TNF- α induction in five experiments showed that THC significantly decreased TNF- α production by macrophages after a 6 h induction period (Table 1). THC at 5 $\mu\text{g}/\text{ml}$ diminished TNF- α production by

Table 1. Inhibition by THC of TNF- α production by murine peritoneal macrophages

THC (μg)	TNF- α — unit/ml [†] (mean) $\pm$ S.D.)	% of controls
0 [‡]	115.0 $\pm$ 85.3	100.0
1.0	67.6 $\pm$ 49.5	46.6
5.0	31.3 $\pm$ 30.7*	21.6
10.0	7.3 $\pm$ 6.9**	5.0

P* < 0.025; *P* < 0.005. Statistical analysis by a one-tailed pooled two sample *t*-test.

[†]Mean number of TNF- α units from triplicates of five experiments (each experiment contained the pooled macrophages from at least five animals) when supernatant fluids were collected from cultures of adherent cells incubated in induction medium for 6 h.

[‡]0.5% DMSO vehicle only.

78.4% and at 10 $\mu\text{g}/\text{ml}$ by 95% when compared with the TNF- α level of controls. Similar results were observed in two separate experiments when macrophage supernatants were serially collected after 2, 4, or 6 h incubation in induction medium and assayed for TNF- α production (Fig. 1). Again, THC significantly reduced TNF- α secretion from macrophages after a 6 h induction period (*P* < 0.025 at both 5 and 10 μg THC/ml, respectively), as well as after a 4 h induction period (*P* < 0.05 at both 5 and 10 μg THC/ml). There was little or no TNF- α production after a 2 h induction period even in the absence of THC. Inhibition by THC of TNF- α production by macrophages was not due to neutralization of TNF- α activity in our bioassay, since THC (5–10 $\mu\text{g}/\text{ml}$) addition to TNF- α -containing supernatants or standard TNF- α -containing solutions did not decrease TNF activity (data not shown). By contrast, activity of TNF- α could be abrogated by the addition of anti-mouse TNF- α polyclonal antibody to macrophage supernatant fluids (data not shown).

Effect of pretreatment or simultaneous treatment with THC on macrophage viability and TNF- α production

In the pretreatment experiments, THC was added to standard medium (no BSA and FBS) and murine macrophages were then incubated in this THC-containing medium for 3 h (see Experimental Procedures). When JHC was used at higher concentrations (5–10 $\mu\text{g}/\text{ml}$), toxicity to the macrophages in medium free of serum proteins was observed (data not shown). However, THC could be used effectively at 10 times less concentration, i.e. 0.1, 0.5, and 1 $\mu\text{g}/\text{ml}$ in the pretreatment studies. As shown in Table 2, murine macrophages pretreated

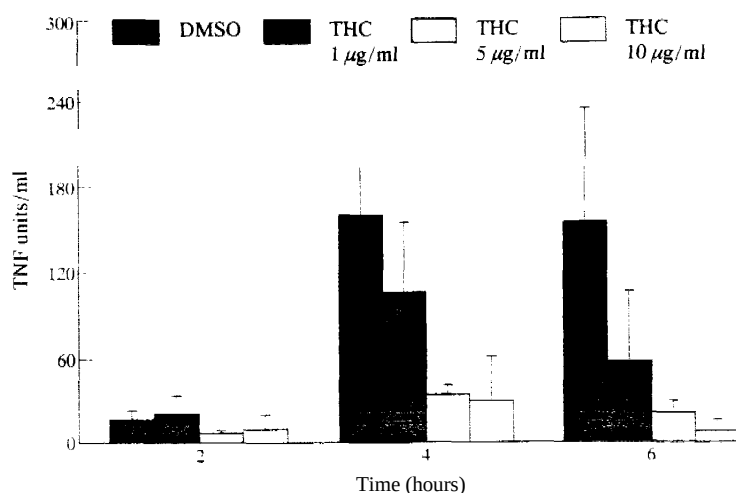


Fig. 1. Effect of THC on TNF- α production by murine macrophages when the length of the induction period was varied. Macrophage supernatants were serially collected after 2, 4, or 6 h incubation in induction medium with or without THC and then assayed for TNF- α production using a TNF-sensitive murine cell line, WEHI-164, labeled with "Cr. Data in this figure were calculated from triplicate cultures of two experiments. Each experiment contained the pooled macrophages from at least five animals. $P < 0.05$ at both 5 and 10 μg THC/ml compared with 0.5% DMSO vehicle after 4 h induction, $P < 0.025$ at both 5 and 10 μg THC/ml compared with 0.5% DMSO vehicle after 6 h induction.

Table 2. Comparative effect of THC pretreatment and simultaneous treatment on soluble TNF- α production by murine peritoneal macrophages

THC ($\mu\text{g/ml}$)	Cell viability [†] (%)		TNF-a (unit/ml)	% of controls
	After 3 h pre.	After 6 h ind.		
Pretreatment [‡] (three animals)				
0 [§]	91.7 $\pm$ 5.0 ⁿ	70.3 $\pm$ 13.8	89.7 $\pm$ 61.5	100.0
0.1	90.0 $\pm$ 3.5	71.7 $\pm$ 12.6	99.0 $\pm$ 40.8	99.2
0.5	85.3 $\pm$ 4.7	69.3 $\pm$ 19.0	42.5 $\pm$ 41.3	47.4
1.0	54.7 $\pm$ 13.8	33.3 $\pm$ 20.2	4.7 $\pm$ 6.8*	5.2
Simultaneous treatment [†] (three animals)				
0	ND	85.0 $\pm$ 9.9	160.0 $\pm$ 54.0	100.0
1.0	ND	89.5 $\pm$ 2.1	88.0 $\pm$ 34.0	55.0
5.0	ND	84.5 $\pm$ 11.7	64.0 $\pm$ 5.8**	40.0
10.0	ND	81.5 $\pm$ 9.2	6.8 $\pm$ 1.0***	4.3

* $P < 0.05$; ** $P < 0.025$; *** $P < 0.005$.

[†]Bytrypan blue exclusion.

*Cells were treated with THC-containing RPMI1640 without FBS and BSA for 3 h before TNF induction was initiated.

[‡]0.5% DMSO vehicle only.

[§]Mean $\pm$ S.D. was obtained from the triplicate wells of three individual animals.

[†]THC was added to TNF induction medium and TNF- α production was induced for 6 h before macrophage supernatants were collected.

ND — no pretreatment of macrophages was done.

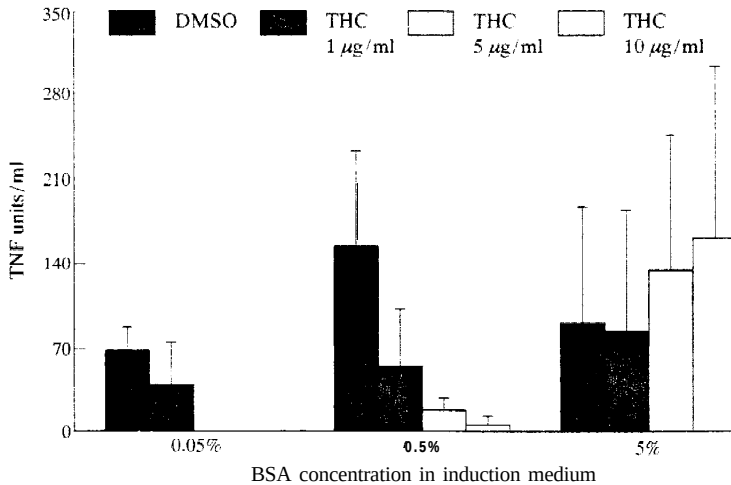


Fig. 2. Effect of BSA concentration on THC inhibitory activity to TNF- α production by murine macrophages. 411 macrophage supernatant fluids tested in triplicates for TNF- α were collected after a 6 h incubation period in induction medium containing THC or DMSO. Data in this figure were calculated from 3 experiments. Each experiment contained the pooled macrophages from at least 10 animals. $P < 0.025$ at both 5 and 10 μ g THC/ml compared with 0.5% DMSO vehicle after a 6 h incubation period in induction medium containing 0.5% BSA.

for 3 h with standard medium containing THC were sensitive to inhibition of TNF- α production by the drug at low concentrations. Although macrophage viability in the pretreated group at 1 μ g THC/ml was decreased to about 50% of the controls, the decrease in TNF- α production at this concentration of THC was almost 95%. The "simultaneous" treatment studies clearly showed that inhibition of TNF- α production by macrophages exposed to THC at 5 and 10 μ g/ml was not related to drug toxicity for the cells (Table 2).

Inhibition by THC of TNF- α production by murine macrophages is serum protein-dependent

As shown in Fig. 2, inhibition by THC of TNF- α production by macrophages is clearly affected by the concentration of BSA in the induction medium when both drug and BSA were added simultaneously. The induction medium containing 0.05% BSA only had a weak TNF- α induction capacity and THC at 5–10 μ g/ml in this low BSA-containing medium was toxic to the macrophages. However, induction medium containing 0.5% BSA strongly induced TNF- α production. Also, THC at 5–10 μ g/ml in the medium significantly inhibited TNF- α production ($P < 0.025$), but was not toxic to the cells. In contrast, the induction medium containing 5% BSA had a highly variable ability to induce TNF- α as compared with medium containing 0.5% BSA. Furthermore, THC at 5–10 μ g/ml in the 5% BSA-containing

Table 3. Effect of THC on TNF- α production by human peripheral blood adherent mononuclear cells[†]

THC @g/ml [†]	% of DMSO controls (mean $\pm$ S.D.)
1	50.5 $\pm$ 27.3
5	26.6 $\pm$ 21.6*

* $P < 0.05$.

[†]There were 12 responders to TNF- α induction. Data in this table were calculated from triplicate cultures of 9 responders. Results from other three responders are shown in Fig. 3.

*Simultaneous addition of THC and induction medium to adherent cells was tested for 6 h at 37°C in 5% CO₂.

medium did not inhibit TNF- α production and in some cases increased TNF- α production by macrophages. Similar results to these obtained using 5% BSA were observed using induction medium containing 10% FBS (data not shown).

Inhibition by THC of TNF- α production by human blood peripheral adherent cells

Buffy coats from 19 different normal healthy individuals were used to isolate PBAC for this study. TNF- α activity in supernatant fluids could be detected from only 12 subjects, who were referred to as LPS-responders. As seen in Table 3, THC at 5 μ g/ml significantly inhibited TNF- α production by PBAC (1 $\times$ 10⁶ cells/ml). Graded numbers of PBAC (1–4 $\times$ 10⁶ cells/ml) from three buffy coats

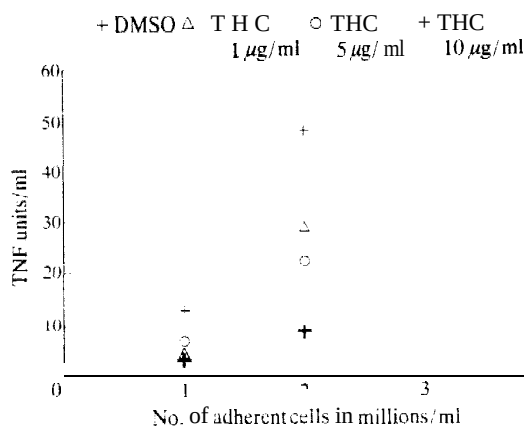


Fig. 3. Effect of THC on TNF- α production by different numbers of human peripheral blood adherent cells. The amount of TNF- α was titrated using the cultured adherent cell supernatants collected at a 6 h incubation period in induction medium. Representative data are shown from a total of three buffy coats.

were used to evaluate whether altering the number of cells exposed to THC affected its ability to inhibit TNF- α production. The results from this study showed that TNF- α production by PBAC was suppressed by THC at all cell concentrations tested despite increased TNF production in control cultures containing increased cell numbers (Fig. 3).

DISCUSSION

These studies have demonstrated that the addition of THC to medium used to induce TNF- α resulted in the suppression of production of this monokine from either mouse or human macrophages. This inhibition of TNF- α production was related to the incubation conditions including both THC concentration and the concentration of serum proteins present in the medium. This latter observation is consistent with previous studies which indicate that serum can inhibit the ability of THC to suppress lymphocyte blastogenic transformation (Klein *et al.*, 1985). Since serum protein was necessary for strong induction of TNF, addition of THC simultaneously with the induction medium (containing LPS, IFN- γ and BSA or FBS) required higher concentrations of the drug (5–10 $\mu\text{g/ml}$) in order to inhibit induction. Inhibition of TNF induction when THC and LPS are added to macrophages simultaneously was noted both at suboptimal (4 h) and optimal (6 h) times of incubation for TNF induction. However, when

macrophages were pretreated with THC before TNF induction the presence of serum was not necessary during drug treatment. Under such conditions much lower concentrations of THC (0.5–1.0 $\mu\text{g/ml}$) were able to diminish the ability of macrophages to respond to the TNF induction protocol. Thus, murine macrophages exposed to low concentrations of THC for 3 h were diminished in their ability to produce TNF by greater than 50% using a standard induction medium. This effect was far greater than the reduction that could be accounted for by a loss in viability of the macrophages. Furthermore, the simultaneous treatment studies indicate that loss of TNF- α production was achieved without loss of viability. Thus, inhibition of TNF- α production due to incubation in the presence of THC is a pathophysiologic effect not a result of drug cytotoxicity. This is consistent with our earlier findings that inhibition by THC of natural killer cell cytotoxicity is a reversible phenomenon (Specter, Rivenbark, Newton, Kawakami & Lancz, 1989).

In contrast to these studies is the report of Klein & Friedman (1990) that THC did not diminish interleukin 1 (IL-1) production by mouse macrophages. In those studies macrophages were treated by up to 10 μg THC/ml in the presence of 10% FBS and IL-1 levels were increased in macrophage culture supernatant fluids. These contrasting results regarding two different monokines could be the result of differing culture conditions. It is possible that at the high protein concentrations, THC may be bound to serum/culture proteins and thus a very low concentration of THC may be available to affect (enhance) TNF- α production. Alternatively, these data could indicate that the mechanisms by which THC affects cytokine production may vary. This is an important observation since these two sets of data suggest that the THC effect is not merely a broad inhibition of protein synthesis but that the production of different proteins may be selectively affected. Incubation of macrophages to produce IL-1 under conditions using less serum, or perhaps BSA, are needed to clarify this discrepancy.

In an effort to improve TNF production by human PBAC the cell number was increased from 1 to 4 $\times 10^6$ cells/ml. Results indicated that 2 $\times 10^6$ cells/ml yielded optimal amounts of TNF. Regardless of the cell concentration THC was still able to inhibit TNF production. It is interesting to note that not all macrophage cultures from human subjects were able to produce TNF; only 12/19 responded to induction medium even when cells were incubated with a concentration of LPS 10 times greater than that used for mouse macrophages.

The mechanism by which THC inhibits TNF production is not yet clear, nor is the significance of this observation. Because THC is lipophilic and can be incorporated into cell membranes (Specter, Lancz & Friedman, 1990a) it is possible that cell membrane alterations may contribute to the inhibitory effects of the drug for macrophages. Recent studies have indicated that THC can cause a decrease in the expression of interleukin 2 receptors on lymphocyte membranes (K. Trisler & S. Specter, unpublished observations). This may have relevance concerning TNF, since TNF- α occurs in two forms, soluble TNF- α which is secreted by activated macrophages and membrane-bound TNF- α on the macrophage surface (Chensue, Remick, Shmyr-Forsch, Beals & Kunkel, 1988). Presently, we have only measured a decrease in soluble TNF- α but it is likely that expression of both forms of TNF- α are affected by THC. This could contribute to a loss in macrophage cytotoxicity for tumor cells and will be examined.

In addition, other studies in progress (Diaz *et al.*, submitted for publication) suggest that THC can affect second messenger functions, including cyclic AMP production. There is currently no information

regarding whether THC affects transcription or translation related to TNF production. Thus, while inhibition of TNF production is demonstrated by these studies it is presently unclear as to the level of cellular activity that is altered. This problem is currently the focus of research efforts.

The significance of the ability of THC to diminish TNF production by macrophages remains to be determined. Clearly, in light of the demonstration that THC decreases a variety of immune functions, the loss of TNF activity reflects the likelihood that inhibition of production of soluble mediators may provide a selective advantage for ubiquitous, opportunistic pathogens when an individual has used marijuana. Thus, in the immunocompromised host especially, the decrease in production of soluble mediators such as TNF could have devastating consequences regarding the progression of infections or tumors.

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REFERENCES

- BLANCHARD, D. K., NEWTON, C., KLEIN, T. W., STEWART, W. E. & FRIEDMAN, H. (1986). In vitro and in vivo suppressive effects of delta-9-tetrahydrocannabinol on interferon production by murine spleen cells. *Int. J. Immunopharmac.*, **8**, 819–824.
- CHENSUE, S. W., REMICK, D. G., SHMYR-FORSCH, C., BEALS, T. F. & KUNKEL, S. L. (1988). Immunohistochemical demonstration of cytoplasmic and membrane-associated tumor necrosis factor in murine macrophages. *Am. J. Pathol.*, **133**, 564–572.
- DUNCAN, R. C., KNAPP, R. G. & MELLER, M. C. (eds) (1983). *Introductory Biostatistics for the Health Sciences*. Wiley, New York.
- ESKANDARI, M. K., NGUYEN, D. T., KUNKEL, S. L. & REMICK, D. G. (1990). WEHI 164 subclone 13 assay for TNF: sensitivity, specificity, and reliability. *Immun. Invest.*, **19**, 69–79.
- KAWAKAMI, Y., KLEIN, T. W., NEWTON, C., DJEU, J. Y., SPECTER, S. & FRIEDMAN, H. (1988). Suppression by delta-9-tetrahydrocannabinol of interleukin Z-induced lymphocyte proliferation and lymphokine-activated killer cell activity. *Int. J. Immunopharmac.*, **10**, 485–488.
- KLEIN, T. W. & FRIEDMAN, H. (1990). Modulation of murine immune cell function by marijuana components. In *Drugs of Abuse and Immune Function* (ed. Watson, R. R.), pp. 87–111. CRC Press, Boca Raton.
- KLEIN, T. W., NEWTON, C. A., WIDEN, R. & FRIEDMAN, H. (1985). The effects of delta-9-tetrahydrocannabinol and 11-hydroxy-delta-9-tetrahydrocannabinol on T-lymphocyte and B-lymphocyte mitogen responses. *Int. J. Immunopharmac.*, **7**, 451–466.
- LASKOV, R., LANCZ, G., RUDDLE, N. H., MCGRATH, K. M., SPECTER, S., KLEIN, T. W., DJEU, J. & FRIEDMAN, H. (1990). Production of tumor necrosis factor (TNF- α) and lymphotoxin (TNF- β) by murine pre-B and B cell lymphomas. *J. Immunol.*, **144**, 3424–3430.
- LOPEZ-CEPERO, M., FRIEDMAN, M., KLEIN, T. W. & FRIEDMAN, H. (1986). Tetrahydrocannabinol-induced suppression of macrophage spreading and phagocytic activity in vitro. *J. Leuk. Biol.*, **39**, 679–686.
- NAHAS, G. G., SUCIU-FOCA, N., ARMAND, J.-P. & MORISHIMA, A. (1974). Inhibition of cellular immunity in marijuana smokers. *Science*, **183**, 419–420.
- OLD, L. J. (1988). Tumor necrosis factor. *Scient. Am.*, **258** (5), 59–75.

- SMITH, S. H., HARRIS, L. S., UWAYDAH, I. M. & MUNSON, A. E. (1978). Structure-activity relationships of natural and synthetic cannabinoids in suppression of humoral and cell-mediated immunity. *J. Pharmac. exp. Ther.*, **207**, 165 – 170.
- SPECTER, S. C., KLEIN, T. W., NEWTON, C., MONDRAGON, M., WIDEN, R. & FRIEDMAN, H. (1986). Marijuana effects on immunity: suppression of human natural killer cell activity by delta-9-tetrahydrocannabinol. *Int. J. Immunopharmac.*, **8**, 741 – 745.
- SPECTER, S., LANCZ, G. & FRIEDMAN, H. (1990a). Marijuana and immunosuppression in man. In *Drugs of Abuse and Immune Function* (ed. Watson, R. R.), pp. 73 – 85. CRC Press, Boca Raton.
- SPECTER, S., LANCZ, G. & GOODFELLOW, D. (1991). Suppression of human macrophage function *in vitro* by delta-9-tetrahydrocannabinol. *J. Leuk. Biol.*, **50**, 423 – 426.
- SPECTER, S., LANCZ, G. & HAZELDEN, J. (1990b). Marijuana and immunity: tetrahydrocannabinol mediated inhibition of lymphocyte blastogenesis. *Int. J. Immunopharmac.*, **12**, 261 – 267.
- SPECTER, S., RIVENBARK, M., NEWTON, C., KAWAKAMI, Y. & LANCZ, G. (1989). Prevention and reversal of delta-9-tetrahydrocannabinol induced depression of natural killer cell activity by interleukin-2. *Int. J. Immunopharmac.*, **11**, 63 – 69.
- YEBRA, M., KLEIN, T. W. & FRIEDMAN, H. (1992). Delta-9-tetrahydrocannabinol suppresses concanavalin A induced increase in cytoplasmic free calcium in mouse thymocytes. *Life Sci.*, **51**, 151 – 160.