Modulation of Interleukin 2 Activity by Δ^9-Tetrahydrocannabinol after Stimulation with Concanavalin A, Phytohemagglutinin, or Anti-CD3 Antibody (43494C)

YASUNOBU NAKANO, SUSAN H. PROSS,[1] AND HERMAN FRIEDMAN
Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa, Florida 33612

Abstract. **The effects of Δ^9-tetrahydrocannabinol (THC) on lymphocyte proliferation and interleukin (IL) 2 activity was investigated using adult murine spleen cells stimulated with either the mitogens concanavalin A, phytohemagglutinin, or anti-CD3 antibody. THC was found to suppress mitogen-induced proliferation, but to enhance anti-CD3-antibody-induced proliferation. These results reflected THC-induced suppression of Ly2 cells following concanavalin A or phytohemagglutinin stimulation and THC-induced enhancement of Ly2 cells following CD3 stimulation. The combination of THC and concanavalin A or phytohemagglutinin resulted in suppressed IL-2 activity, whereas the combination of THC and anti-CD3 antibody resulted in enhanced IL-2 activity. This drug-related modulation of IL-2 activity corresponded to the changes in blastogenic activity as well as to variations in numbers of Tac positive cells. These results suggest that the dysregulation in immune responses following THC treatment, either suppression or enhancement, may relate to the effects of THC on IL-2 production.**

[P.S.E.B.M. 1992, Vol 201]

It is now widely recognized that drugs of abuse such as marijuana, cocaine, and opiates suppress the immune response system. In this regard, previous studies in this and other laboratories have shown that Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, has strong immunomodulator effects, both *in vivo* and *in vitro* (1–9). For example, previous studies showed that THC suppresses the proliferative capacity *in vitro* of spleen cells stimulated with plant mitogens, such as phytohemagglutinin (PHA) or concanavalin A (Con A), which are mitogens for T lymphocytes. When spleen cells are treated with graded doses of THC *in vitro*, the ability of the cells to undergo blastogenesis when stimulated with relatively low doses of these mitogens is compromised.

T lymphocytes can be characterized in the helper or suppressor/cytotoxic T cell lineage by specific antigenic markers readily determined by immunofluorescent staining techniques. Helper T cells (L3T4 cells) are recognized by their CD4 surface antigen marker, whereas suppressor/cytolytic T cells (Ly2 cells) have the CD8 surface marker (10). All T cell lymphocytes, however, have the CD3 surface antigen marker, since monoclonal anti-CD3 antibody reacts with T lymphocytes in general (11). The antibody to this pan T cell marker can also stimulate T lymphocytes to undergo blastogenesis *in vitro*. In the present study, it was found that THC treatment of spleen cells *in vitro* with anti-CD3 antibody results in enhancement of blastogenesis. This is in contrast to the suppression of mitogenesis evident when spleen cells are treated with the same doses of THC *in vitro* and stimulated with plant mitogens. This study shows that interleukin (IL) 2 activity was increased by stimulation with anti-CD3 antibody and THC in contrast to the decrease seen following stimulation with plant mitogens and THC treatment. Furthermore, the THC modulation of IL-2 activity directly related to drug-induced alterations in Tac positive cells.

[1] To whom requests for reprints should be addressed at Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, 12901 Bruce B. Downs Boulevard, Tampa, FL 33612.

Received January 21, 1992. [P.S.E.B.M. 1992, Vol 201]
Accepted May 6, 1992.

0037-9727/92/2012-0165$3.00/0

Methods and Materials

Animals. BALB/c mice, 8–12 weeks of age, were obtained from Jackson Laboratory, Bar Harbor, ME. The animals were kept in groups of 8–10 under pathogen-free conditions in plastic mouse cages and fed mouse pellets and water *ad libitum*.

Marijuana Component. Tetrahydrocannabinol was obtained from the National Institute on Drug Abuse, Rockville, MD, in absolute ethanol. The alcohol was evaporated with N_2 gas and THC reconstituted in dimethyl sulfoxide and further diluted to the desired concentration in warm medium, as described previously (2).

Reagents. The medium used for this study was RPMI 1640 obtained from Gibco Laboratories, Grand Island, NY, and contained 10% fetal calf serum obtained from Hyclone Laboratories, Logan, UT. The mitogens used were Con A, obtained from Sigma Chemical Co., St. Louis, MO, and PHA, obtained from Burroughs Wellcome Co., Greenville, NC. The monoclonal anti-CD3 antibody (1 mg/ml) was obtained from Pharmigen Co., San Diego, CA. All plant mitogens were used at a final concentration of 5 μg/ml of medium and the anti-CD3 antibody was used at a dilution containing 1 μg/ml of medium.

Lymphoid Cell Preparation. The spleen was removed from individual mice after cervical dislocation. Single cell suspensions were prepared with a Stomacher 80-Lab Blender (Tekmar Co., Cincinnati, OH) and washed by centrifugation in Hanks' balanced salt solution. The cells were resuspended at a concentration of 2×10^6 cells/ml in RPMI 1640 medium containing fetal calf serum, antibiotics, and 2-mercaptoethanol (5×10^{-5} *M*). Cell viability exceeded 95%, as determined by trypan blue dye exclusion.

Lymphocyte Proliferation Assay. To determine lymphocyte blastogenesis, the spleen cells were dispensed in 0.1-ml volumes into individual wells of 96-well flat-bottom microtiter plates (Costar, Cambridge, MA). The stimulators and the THC were each added in 0.05-ml volumes to the wells in the plates, which were then incubated at 37°C for 48 hr in 5% CO_2 and air. At this time, the plates were pulsed with 0.5 μCi of [^{3}H]thymidine for 18 hr. The cells were harvested on glass fiber filters and the incorporated radioactivity was determined by standard liquid scintillation counting. Counts per minute ± SE were calculated for triplicate cultures. All experiments were repeated at least four times. Results were expressed as Δcpm where Δcpm = cpm experimental cultures − cpm THC alone × 10^3.

Interleukin 2 Activity. IL-2 activity was assayed by using CTLL-2 cells, an IL-2-dependent T cell line. The CTLL-2 cells (1×10^4 cells/well) were cultured in the presence of 2-fold-diluted, 48-hr culture supernatants of splenocytes stimulated by Con A, PHA, or anti-CD3 antibody in 96-well microplates for 24 hr at 37°C. During the last 4 hr of culture, 1.0 μCi of [^{3}H]thymidine was added to each microplate well. The CTLL-2 cells in the cultures were harvested, and the incorporated radioactivity was counted in a liquid scintillation counter. IL-2 production was assessed as activity expressed in units, employing the probit analysis of Gillis *et al.* (12). We define 1 unit of IL-2 activity as the reciprocal of the dilution required to give 50% of the maximum response.

Flow Cytometry. Spleen cells (1×10^6/ml) were incubated in 25-cm^2 tissue culture flasks for 72 hr with appropriate mitogen. Immunofluorescence analysis was performed by either two- or three-color analysis. The cell surface markers used were Ly2, L3T4 (Becton Dickinson, Mountain Valley, CA), and Tac (Life Technologies, Long Island, NY). For two-color analysis, 50 μl of the cell culture were placed in 12 × 75-mm tubes with either a fluorescein isothiocyanate- or phycoerythrin-labeled monoclonal antibody in 50 μl of phosphate-buffered saline. After 30 min of incubation in the dark on ice, the cells were washed in phosphate-buffered saline and resuspended in phosphate-buffered saline containing 1% paraformaldehyde. Samples were kept in the dark on ice until analyzed. Fluorescence-activated cell sorter analysis was performed using a Becton Dickinson FACScan. The lymphocytes were identified by forward versus side (90°) light scatter. Viable lymphoid cells were then selected for immunofluorescence analysis and the appropriate gates were set. Thresholds for positives were set using isotype-matched antibodies.

Statistics. A Student's *t* test with two-tailed alternative was used.

Table I. Effect of THC on Spleen Cell Blastogenic Response to Con A, PHA, and Anti-CD3 Antibody

THC concentration[a] (μg/ml)	Blastogenic response Δcpm (×10^3)[b]		
	Con A	PHA	Anti-CD3
None (control)	111.1 ± 9.0	58.7 ± 5.3	75.6 ± 10.9
1.0	110.0 ± 9.6	53.6 ± 4.3	83.2 ± 7.1
3.0	101.3 ± 7.9	44.1 ± 3.2[c]	123.3 ± 9.4[c]
5.0	69.2 ± 8.1[c]	42.3 ± 5.4[c]	138.4 ± 13.2[c]
7.0	26.5 ± 4.7[d]	21.6 ± 4.3[d]	89.3 ± 6.2

[a] Indicated concentration of THC added to 10^6 mouse spleen cells stimulated with 5.0 μg/ml of mitogen or 1.0 μg/ml of anti-CD3.
[b] Average Δcpm ± SE determined for cell cultures stimulated with indicated mitogen or antibody; Δcpm calculated as cpm experimental − cpm THC alone.
[c] $P < 0.05$ versus control.
[d] $P < 0.01$ versus control.

Results

Stimulation of murine spleen cell suspensions with the plant mitogens Con A and PHA resulted in blastogenesis. THC markedly suppressed this response (Table I). Treatment of spleen cells with anti-CD3 antibody also resulted in blastogenesis. In contrast to the situation with Con A and PHA, THC in combination with

Table II. Numbers of Splenocytes with Ly2 and L3T4 Marker after Culture with THC and Con A, PHA, and Anti-CD3 Antibody[a]

THC concentration (μg/ml)	Ly2 cell number (×10⁴)[b]			L3T4 cell number (×10⁴)[c]		
	Con A	PHA	Anti-CD3	Con A	PHA	Anti-CD3
None (control)	85.4 ± 3.2	76.7 ± 4.8	42.6 ± 2.8	58.5 ± 3.4	45.1 ± 3.1	57.2 ± 4.3
1.0	80.3 ± 4.7	68.9 ± 4.3	56.1 ± 3.4	57.5 ± 3.4	43.7 ± 3.6	56.4 ± 3.8
3.0	76.5 ± 2.5	48.5 ± 3.7[d]	73.5 ± 4.3[d]	52.3 ± 4.8	40.2 ± 4.2	55.1 ± 3.3
5.0	42.7 ± 3.9[d]	15.3 ± 2.5[e]	77.6 ± 3.9[d]	47.9 ± 2.7	37.4 ± 2.6	54.5 ± 2.9
7.0	12.3 ± 5.9[e]	2.5 ± 2.5[e]	42.1 ± 4.3	42.8 ± 5.4	34.3 ± 3.8	43.4 ± 2.5

[a] Data expressed as mean ± SE. Numbers of splenocytes determined by

$$\frac{\text{No. cells after culture}}{\text{ml}} \times \%\text{subpopulation.}$$

[b] Before stimulation, cell number per culture was 10.5 ± 2.1 (×10⁴).
[c] Before stimulation, cell number per culture was 20.1 ± 3.5 (×10⁴).
[d] $P < 0.05$ versus control.
[e] $P < 0.01$ versus control.

Table III. Effect of THC on Spleen Cell IL-2 Production to Con A, PHA, and Anti-CD3 Antibody

THC concentration (μg/ml)	IL-2 production (units/ml)[a]		
	Con A	PHA	Anti-CD3
None (control)	27.9 ± 1.5	12.5 ± 1.2	15.8 ± 1.3
1.0	26.3 ± 1.9	12.1 ± 1.8	20.3 ± 2.1
3.0	23.2 ± 1.7	9.7 ± 1.7[b]	28.8 ± 1.9[b]
5.0	18.9 ± 1.5[b]	8.7 ± 1.3[b]	31.6 ± 1.5[c]
7.0	17.8 ± 1.4[c]	6.7 ± 1.4[c]	18.0 ± 1.5

[a] The concentration of IL-2 in experimental samples was determined by the formula:

$$[\text{IL-2}] = \frac{\text{experimental reciprocal titer}}{\text{standard reciprocal titer}}$$

where a titer is defined as the dilution that yields 50% of maximum CTLL-2 [^{3}H]thymidine incorporation.
[b] $P < 0.05$ versus control.
[c] $P < 0.01$ versus control.

Table IV. Numbers of Splenocytes with Tac Marker after Culture with THC and Con A, PHA, and Anti-CD3 Antibody[a]

THC concentration (μg/ml)	Tac positive cell number (×10⁴)[b]		
	Con A	PHA	Anti-CD3
None (control)	43.8 ± 3.2	45 ± 3.4	33.2 ± 2.8
1.0	40.4 ± 2.8	44 ± 1.8	35.7 ± 1.5
3.0	37.5 ± 2.3	37.9 ± 1.7[c]	41.8 ± 2.5[c]
5.0	27.4 ± 1.9[c]	33.3 ± 3.2[c]	46.3 ± 2.6[c]
7.0	21.9 ± 2.4[d]	24.5 ± 2.5[d]	31.2 ± 2.1

[a] See footnote to Table II for clarification.
[b] Before stimulation, cell number was 10.6 ± 2.4 (×10⁴).
[c] $P < 0.05$ versus control.
[d] $P < 0.01$ versus control.

CD3 stimulation served to enhance the cellular proliferation. This enhancing effect was seen only at the lower doses of THC. The suppressive effect of 7 μg/ml of THC, which was apparent after stimulation with plant mitogens, was not seen after anti-CD3 stimulation. Appropriate dimethyl sulfoxide controls (both high and low dose) eliminated the vehicle as the source of these effects.

The responding cells were analyzed by the fluorescence-activated cell sorter to determine which subpopulations of T cells were responding to the stimulators alone or with THC. Table II shows that in all experimental situations, the Ly2 and the L3T4 cells responded to either the plant mitogens or to the anti-CD3 antibody. THC suppressed the proliferation of the Ly2 cells to either Con A or PHA, and enhanced the proliferation of these cells to anti-CD3 antibody.

In order to better understand the mechanisms of the induced Ly2 proliferation, IL-2 activity was determined. Unstimulated spleen cultures showed less than 2 units/ml of IL-2 activity. Table III shows that Con A and PHA alone increased the IL-2 activity. This effect was suppressed by THC. Anti-CD3 antibody alone also increased IL-2 activity. In contrast to the results with plant mitogens, but in concordance with the blastogenic data, the combination of 3 or 5 μg/ml of THC with anti-CD3 antibody up-regulated the IL-2 activity. Interestingly, whereas the higher doses of THC suppressed mitogen-induced IL-2 activity, the same dose of drug in combination with anti-CD3 antibody served only to reduce the THC-related enhancement to control levels.

The numbers of Tac positive cells were determined after THC treatment in Con A-, PHA-, and anti-CD3 antibody-stimulated cultures (Table IV). Similar to the IL-2 data, THC suppressed the numbers of Tac positive cells in Con A- and PHA-stimulated spleen cultures. There was enhancement, but no suppression, seen in THC-treated cultures stimulated with anti-CD3 antibody.

Discussion

It is apparent from the results of this study that marijuana, specifically its major psychoactive component THC, has the ability to not only suppress the

blastogenic response of murine spleen cells *in vitro*, but also to enhance such a response, dependent upon the stimulator used to induce blastogenesis. The results of previous studies in this laboratory, as well as in others, have shown that THC, in graded doses, can suppress the blastogenic responsiveness of murine spleen cells stimulated *in vitro* with a wide variety of plant mitogens, including PHA and Con A as well as a nonspecific immunostimulator from gram-negative bacteria, i.e., lipopolysaccharide (2, 4). These findings suggest that T lymphocytes, as well as B lymphocytes, are susceptible to the immunosuppressive activity of THC, at least *in vitro*. Although the doses of THC used in these *in vitro* studies range from 10 to 50 times those found in marijuana smokers (13), the physiological effects may, nevertheless, be quite relevant. Measurements *in vivo* are generally limited to body fluids, including plasma and blood. It would be expected that cellular and tissue levels may excede these.

In the present study, however, it was found that THC can enhance as well as suppress a selected response. The blastogenic response of murine spleen cells stimulated with anti-CD3 antibody, which reacts with a surface antigenic marker present on all T lymphocytes, was increased by simultaneous treatment of the cells with relatively low doses of THC. Further analysis showed that although both the Ly2 and L3T4 subpopulations of T cells were stimulated by Con A and PHA, suppression induced by THC was directed primarily to the Ly2 subpopulation. Similarly, anti-CD3 antibody stimulated both T cell subpopulations and, once again, the Ly2 cells were the most affected by the addition of THC.

Mitogen induced T cell proliferation relates directly to IL-2 activation (14, 15). In this regard, it was found that treatment of spleen cells with either the plant mitogens or the anti-CD3 antibody resulted in increased IL-2 activity as well as Tac positive cells. The THC-related suppression of Con A- and PHA-induced blastogenesis corresponded with the THC-induced decrease in IL-2 activity and Tac positive cells. Similarly, the THC-related enhancement of anti-CD3 antibody-induced blastogenesis corresponded with the THC-induced increase in IL-2 activity and Tac positive cells.

In conclusion, THC has been shown to either up-regulate or down-regulate T lymphocyte proliferation (particularly Ly2 cells) depending upon the method of stimulating the cells. This drug-induced immunomodulation is clearly evidenced in terms of its effect on IL-2 activity, both in terms of enhancement and suppression. The specific immunomodulatory effect of THC on both Tac positive cells and Ly2 cell numbers has been found to relate directly to its effect on IL-2 activity and is influenced by the method of cell stimulation. It is not clear why the immunomodulation by THC is dependent upon whether the cells are stimulated with Con A, PHA, or anti-CD3 antibody. However, since stimulation of the CD3 receptor may relate more directly to stimulation of T cells by antigens, this particular dysregulation by THC may prove to be biologically relevant.

This work was supported by a grant from the U.S. Public Health Service National Institute of Drug Abuse No. DA06385. The typing skills of Sally Baker and Ilona Friedman are gratefully acknowledged.

1. Nahas G, Morishima A, Desoize B. Effects of cannabinoids on macromolecular synthesis and replication of cultured lymphocytes. Fed Proc **36**:1748–1752, 1977.
2. Klein TW, Newton CA, Widen R, 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.
3. Cabral GA, Lockmuller JC, Mishkin EM. Delta-9-tetrahydrocannabinol decreases alpha/beta interferon response to herpes simplex virus type 2 in the B6C3F1 mouse. Proc Soc Exp Biol Med **181**:305–311, 1986.
4. Pross SH, Klein TW, Newton CA, Smith J, Widen R, Friedman H. Differential suppression of T-cell subpopulations by THC (delta-9-tetrahydrocannabinol). Int J Immunopharmacol **12**: 539–544, 1990.
5. Bradley SG, Munson AE, Dewey WL, Harris LS. Enhanced susceptibility of mice to combinations of delta-9-tetrahydrocannabinol and live or killed gram negative bacteria. Infect Immun **17**:325–329, 1977.
6. Specter S, Rivenbark M, Newton C, Kawakami Y, Lancz G. Prevention and reversal of delta-9-tetrahydrocannabinol induced depression of natural killer cell activity by interleukin 2. Int J Immunopharmacol **11**:63–69, 1989.
7. Djeu JY, Wang M, Friedman H. Adverse effect of delta-9-tetrahydrocannabinol on human neutrophil function. Adv Exp Med Biol **28**:57–62, 1991.
8. Watzl B, Scuder P, Watson RR. Marijuana components stimulate human peripheral blood mononuclear cell secretion of interferon-gamma and suppress interleukin-1 alpha *in vitro*. Int J Immunopharmacol **13**:1091–1097, 1991.
9. Luo YD, Patel MK, Wiederhold MD, Ou DW. Effects of cannabinoids and cocaine on the mitogen-induced transformations of lymphocytes of human and mouse origins. Int J Immunopharmacol **14**:49–56, 1992.
10. Swain S. T cell subsets and the recognition of MHC class. Immunol Rev **74**:129–142, 1983.
11. Clevers H, Alarcon B, Wileman T, Terhorst C. The T cell receptor/CD3 complex: A dynamic protein ensemble. Annu Rev Immunol **6**:629–662, 1988.
12. Gillis S, Ferm MM, Ou W, Smith KA. T cell growth factor: Parameters of production and a quantitative microassay for activity. J Immunol **120**:2027–2032, 1978.
13. Hawks RL. The constituents of cannabis and the disposition and metabolism of cannabinoids. In: Hawks RL, Ed. The Analysis of Cannabinoids in Biological Fluids, Research Monograph, 42. Bethesda, MD: National Institute on Drug Abuse, pp125–137, 1982.
14. Cantrell DA, Smith KA. The interleukin-2 T cell system: A new cell growth model. Science **224**:1312–1316, 1984.
15. Smith KA. Interleukin-2: Inception, impact and implication. Science **240**:1169–1176, 1988.