Δ^9-Tetrahydrocannabinol Decreases Cytotoxic T Lymphocyte Activity to Herpes Simplex Virus Type 1-Infected Cells (43452)

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Abstract. **The purpose of this study was to examine the effect of Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive component of marijuana, on T lymphocyte functional competence against herpes simplex virus Type 1 (HSV1) infection. Spleen cells from C3H/HeJ (H-2^k) mice primed with HSV1 and exposed to Δ^9-THC were examined for anti-HSV1 cytolytic T lymphocyte (CTL) activity. Flow cytometry was used to determine whether Δ^9-THC altered T cytotoxic (Lyt-2^+) and T helper (L3T4^+) lymphocyte numbers or cell ratios. Nomarski optics microscopy was used to determine whether effector lymphocytes from drug-treated mice were able to bind to virally infected L929 (H-2^k) target cells. Cytotoxicity assays demonstrated that CTL from mice exposed to Δ^9-THC were deficient in anti-HSV1 cytolytic activity. Δ^9-THC *in vivo* treatment had little effect on the number of T lymphocytes expressing the Lyt-2 or L3T4 antigens. Nomarski optics microscopy revealed that the CTL from the drug-treated mice were able to bind specifically to the HSV1-infected targets. However, Δ^9-THC *in vivo* exposure affected CTL cytoplasmic polarization toward the virus-infected target cell. CTL granule reorientation toward the effector cell-target cell interface following cell conjugation occurred at a lower frequency in co-cultures containing CTL from drug-treated mice. These results suggest that Δ^9-THC elicits dysfunction in CTL by altering effector cell-target cell postconjugation events.** [P.S.E.B.M. 1992, Vol 200]

Delta-9-tetrahydrocannabinol (Δ^9-THC) is the major psychoactive component of marijuana. It is widely acknowledged that this compound induces a variety of suppressive and inhibitory effects on the host immune response (1–13). The immunosuppressive effects of Δ^9-THC include inhibition of mitogen-induced T lymphocyte proliferation (2, 6, 11). Splenocytes exposed to Δ^9-THC *in vitro* or *in vivo* also produce less interferon after mitogen stimulation (1). However, little information is available on the effects of Δ^9-THC on individual populations of T lymphocytes. An *in vitro* study has demonstrated that Δ^9-THC preferentially inhibits proliferation of the cytotoxic/suppressor (Lyt-2^+) lymphocyte population, but the cytolytic capabilities of these lymphocytes have not been investigated (12).

Cytolytic T lymphocytes (CTL) play an important role in the immune response in their capacity to lyse virally infected cells (14–17), tumor cells (18, 19), and allogeneic or xenogeneic transplants (20, 21). In executing these functions, CTL undergo discrete sequential phases (22). First, recognition and adhesion occurs between the lymphocyte and the target cell. Following the formation of CTL-target cell conjugates, a cytoplasmic polarization occurs within the effector cell. The microtubule-organizing center and Golgi apparatus of the CTL are reoriented toward the target cell contact area (23–26). This reorientation directs the movement of granules containing cytolytic molecules to the CTL-target cell interface. Then the CTL delivers the "lethal hit" to the target cell (27). In murine systems, polyclonal populations of antiviral CTL are usually restricted by major histocompatibility complex (MHC) Class I antigens and are Lyt-2^+ cells.

The purpose of the present study was to examine

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the effect of Δ^9-THC on anti-herpes simplex virus Type 1 (HSV1) CTL function following primary *in vivo* induction. Δ^9-THC administration was shown to suppress CTL-mediated lysis of HSV1-infected syngeneic cells. Fluorescence-activated cell sorter scan (FACScan) analysis, in concert with Nomarski optics microscopy, suggests that Δ^9-THC elicits CTL dysfunction by affecting CTL-target cell postconjugation events.

Materials and Methods

Mouse Virus Infection and Drug-Dosing Regimen. Eight-week-old, virus-free, female C3H/HeJ mice (H-2^k) (Charles River Breeding Laboratories, Wilmington, MA) were quarantined for 1 week. Mice ($n = 4$ per group) were administered Δ^9-tetrahydrocannabinol (15 mg/kg or 100 mg/kg) or vehicle (ethanol: emulphor:saline, 1:1:18) in a volume of 0.01 ml/g body wt ip on Days 1–4, 8–11, and 15–18. Mice received an intraperitoneal injection of 1×10^7 plaque-forming units (PFU) of infectious HSV1 on Day 21. Spleens were removed 7 days later (15).

Mice were sacrificed by cervical dislocation and their spleens were aseptically removed. Single-cell suspensions were prepared by teasing cells through 80-mesh sieves into Hanks' balanced salt solution (Gibco, Grand Island, NY). After one wash, spleen cells were depleted of erythrocytes with distilled H_2O. Then, cells were washed twice more and cell aggregates were removed. Total viable cell counts were performed with a hemacytometer using the trypan blue exclusion method (28). Cells were resuspended in RPMI 1640 (Gibco) containing 5% heat-inactivated fetal calf serum (FCS; Flow Laboratories, McLean, VA) to serve as effector cells in the cytotoxicity assays.

Target Cells. L929 mouse fibroblasts (H-2^k) (CCL-1; American Type Culture Collection, Rockville, MD) were grown in RPMI 1640 supplemented with 10% FCS, 1.5% sodium bicarbonate, 25 m*M* Hepes buffer, 1% glutamine, 1% minimal essential medium vitamins, 1% nonessential amino acids, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. The cells were maintained at 37°C in 5% CO_2 in 75-cm^2 tissue culture flasks and were subcultured twice weekly.

Virus. Herpes simplex virus type 1, strain KOS was propagated in green monkey kidney (Vero) cells and were shown by plaque assay (29) to contain 4×10^8 PFU/ml. HSV1 stocks were stored at −80°C until used in experiments. Virus suspensions to be used for *in vivo* infection were sonicated (70 kcycles/sec, 1 min), diluted in Hanks' balanced salt solution at a concentration of 5×10^7 PFU/ml, and injected intraperitoneally in 0.2 ml into mice. A replicate inoculum was assessed for infectivity by plaque assay in order to confirm the number of PFU introduced into the mice.

Cytotoxicity Assay. L929 target cells were suspended in 1 ml of RPMI 1640 containing 5% FCS and were infected with HSV1 at a multiplicity of infection of 10 and simultaneously labeled with 100 μCi of $Na_2{}^{51}CrO_4$/(1×10^6) cells for a total of 4 hr at 37°C. Then, cells were washed three times with warm medium and suspended at a concentration of 1×10^5 cells/ml in RPMI 1640 with 5% FCS. Uninfected, radiolabeled L929 cells were used as control target cells in the cytotoxicity assays. Infected or uninfected target cells were added to effector cells in 96-well round-bottom microtiter plates at a concentration of 1×10^4 cells/well for an effector cell to target cell ratio of 100:1 or 50:1. Cytotoxicity assays were begun 6 hr after the initiation of infection with HSV1 and were carried out for 4 hr in a 5% CO_2 humidified incubator. All assays were performed in quadruplicate. Results were reported as percentage of specific release according to the following formula:

$$\% \text{ specific release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100\%$$

Total releasable ^{51}Cr was obtained by exposing radiolabeled target cells in 1 *N* NaOH.

FACScan Analysis. Spleen cells from each treatment group were collected, depleted of erythrocytes, washed, and suspended (1×10^7 cells/ml) in cold phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 0.1% sodium azide. Cells (100 μl) were incubated with 2 μl each of phycoerythrin-labeled anti-L3T4 (clone GK1.5) and fluorescein isothiocyanate (FITC)-labeled anti-Lyt-2 (clone 53-6.7) antibodies (Becton-Dickinson, Mountain View, CA) for 30 min at 4°C in the dark. Cells were washed twice with cold PBS and data on 10^5 viable cells were obtained using a FACScan flow cytometer (Becton-Dickinson) equipped with an Argon laser emitting 15 mW at 488 nm. Data were analyzed by a Hewlett-Packard 9000 computer (Portland, OR) using Consort 30 and LYSYS Programs (Becton-Dickinson).

Cell Depletion. Spleen cells from each treatment group to be used for Nomarski optics and fluorescence microscopy were depleted of macrophages by adherence to plastic for 2 hr at 37°C in a humidified atmosphere (30). The remaining lymphocytes were used as effector cells in the co-cultures and were examined by Nomarski optics microscopy and fluorescence microscopy.

Nomarski Optics Microscopy. L929 target cells, grown to approximately 70% confluence on coverslips, were infected with HSV1 at a multiplicity of infection of 10 for 4 hr. Spleen cells from each of the treatment groups were depleted of erythrocytes and macrophages and were added to the target cells to yield an effector to target cell ratio of 5:1. Co-cultures were incubated

for 1 hr, 4 hr, or 6 hr and fixed with 2% glutaraldehyde in 0.2 *M* sodium cacodylate buffer (pH 7.0) for 30 min at room temperature. Coverslips were mounted on glass slides in the fixative solution and the monolayers were examined and photographed on an Olympus BH-2 light microscope equipped with Nomarski optics (Opelco, Washington, DC). For each treatment group, at least four random microscopic fields (original magnification ×20) were examined. A total of at least 100 conjugates for each treatment group was examined.

Fluorescence Microscopy. L929 target cells infected with HSV1 were grown on coverslips as described above. Spleen cells from mice receiving HSV1 and vehicle injections were depleted of erythrocytes and macrophages and were added to the target cells to yield an effector to target cell ratio of 5:1. Co-cultures were incubated for 1 hr or 4 hr and fixed with acetone for 5 min at room temperature and allowed to air dry. The coverslips were equilibrated in PBS for 20 min and then blocked with PBS containing 1% bovine serum albumin and 0.01% sodium azide for 15 min. The cells were rinsed in PBS and incubated for 1 hr with monoclonal antibody 145-2C11, which reacts with the epsilon chain of the CD3 complex associated with the T cell receptor (originally obtained from J. A. Bluestone of the Ben May Institute, Chicago, IL) (31). After the incubation with primary antibody, the cells were rinsed three times in PBS and incubated with a 1/32 dilution of FITC-labeled anti-hamster IgG antibody and 0.01% Evans blue for 1 hr in the dark. Once again, the cells were rinsed three times in PBS and allowed to air dry. The coverslips were mounted on glass slides with Aqua-mount and allowed to harden overnight at 4°C. The co-cultures were examined and photographed on an Olympus BH-2 microscope.

Results

Effect of Δ^9-THC on Spleen Mass and Splenocyte Cell Number. Δ^9-THC administration had little affect on either the spleen mass or the total number of viable splenocytes of C3H/HeJ mice (Table I). Spleen mass averaged 142 mg for mice treated with drug, vehicle, or placebo (0.85% saline) and not exposed to virus. An approximate 25% increase in spleen mass was noted for mice receiving HSV1, even when they were treated with low doses of Δ^9-THC. For these mice, spleen mass averaged 186 mg. Spleen mass from mice receiving HSV1 and high drug dose (i.e., 100 mg/kg) was slightly lower. Similarly, Δ^9-THC did not significantly affect the total number of cells from spleens of either HSV1-recipient or nonrecipient mice. Individual mice yielded 5×10^7 cells/spleen on the average.

Effect of Δ^9-THC on CTL Activity. The effect of Δ^9-THC on anti-HSV1 CTL function following *in vivo* priming with 10^7 PFU of HSV1 was assessed using a chromium release assay. Typical results from one of three separate experiments are shown in Table II. C3H/HeJ mice (H-2^k) upon infection with HSV1 generated CTL activity (29%) toward H-2-restricted, virus-infected L929 (H-2^k) target cells, but not toward uninfected target cells. The anti-herpes CTL activity was decreased in a dose-related manner when mice were exposed to 15 mg/kg or 100 mg/kg Δ^9-THC. The CTL response generated from mice exposed to 100 mg/kg of Δ^9-THC was decreased more than 2-fold when compared with that generated from virus-infected mice receiving vehicle.

Effect of Δ^9-THC on Murine Splenocytes. Spleen cells from each treatment group were analyzed by two-color FACScan analysis using phycoerythrin-labeled anti-L3T4 and FITC-labeled anti-Lyt-2 antibodies to discriminate between T helper and T cytotoxic/suppressor cells, respectively (Fig. 1). Vehicle controls were composed of approximately 16% L3T4$^+$ lymphocytes and 6% Lyt-2$^+$ lymphocytes yielding a L3T4$^+$:Lyt-2$^+$ ratio of approximately 2.5:1 (Fig. 1A). As expected, the percentage of Lyt-2$^+$ lymphocytes increased in the splenocyte populations obtained from mice infected with HSV1 (Fig. 1B). For the virus-infected mice, the increase in Lyt-2$^+$ cells, consistent with the induction

Table I. Effect of Δ^9-THC on Spleen Mass and Cell Number

Treatment[a]	Virus inoculum	Spleen mass (mg) ±SE	Cells/spleen
Vehicle	−	142 ± 9.7	3.0×10^7
Vehicle	+	188 ± 8.0	5.4×10^7
Δ-9-THC 15 mg/kg	+	184 ± 6.8	6.2×10^7
Δ-9-THC 100 mg/kg	+	152 ± 7.9	4.7×10^7

[a] C3H/HeJ mice (H-2^k) were subjected to three rounds of 4 days each of intraperitoneal injections of vehicle (ethanol:emulphor:saline; 1:1:18) or Δ-9-THC interspersed with 3-day rest periods. Mice receiving HSV1 were inoculated intraperitoneally 3 days later with 10^7 PFU. Splenocytes were harvested 7 days later for use in the cytotoxicity assays.

Table II. Effect of Δ^9-THC on Anti-Herpes CTL Activity

Treatment[a]	Virus inoculum	Percentage of cytolysis ± SE	
		L929-HSV1	L929
Vehicle	−	4.6 ± 2.6	2.1 ± 0.5
Vehicle	+	28.9 ± 2.4	6.7 ± 1.6
Δ-9-THC 15 mg/kg	+	19.2 ± 5.6	0.9 ± 0.9
Δ-9-THC 100 mg/kg	+	12.7 ± 2.0	2.4 ± 1.3

[a] C3H/HeJ mice (H-2^k) were subjected to three rounds of 4 days each of intraperitoneal injections of vehicle (ethanol:emulphor:saline; 1:1:18) or Δ-9-THC interspersed with 3-day rest periods. Mice receiving HSV1 were inoculated intraperitoneally 3 days later with 10^7 PFU. Splenocytes were harvested 7 days later and were assessed for cytolytic activity against ^{51}Cr-labeled HSV1-infected or uninfected murine L929 cells (H-2^k).

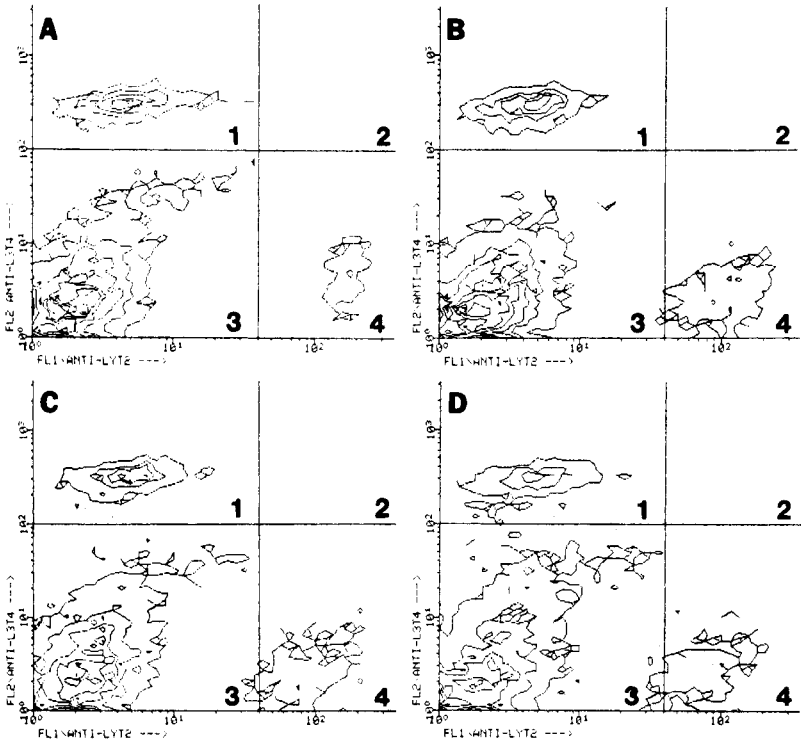

Figure 1. FACScan contour plot analysis of splenocytes simultaneously stained for murine T helper (L3T4$^+$) and T cytotoxic/suppressor (Lyt-2$^+$) cells. Mice (H-2^k) were subjected to the drug-dosing regimen described in Materials and Methods. FACScan analysis was performed using a Becton-Dickinson Flow Cytometer equipped with an Argon laser emitting 15 mW at 488 nm. Data on 10^5 viable cells, as determined by vital dye staining using propidium iodide and gating out orange-positive cells using the machine's compensation network, were collected and analyzed by a Hewlett-Packard 9000 computer using Consort 30 and LYSYS programs (Becton-Dickinson). Phycoerythrin-labeled anti-L3T4 was employed to identify T helper cells on the ordinate (Quadrant 1), while fluoresceinated anti-Lyt-2 was used to identify T cytotoxic/suppressor cells on the abscissa (Quadrant 4). The contour plot at the origin of each graph (Quadrant 3) designates cells that are L3T4$^-$, Lyt-2$^-$. (A) Profile of splenocytes of vehicle-treated mice: Quadrant 1, 16.3%; Quadrant 2, 0.6%; Quadrant 3, 76.3%; and Quadrant 4, 6.8%. (B) Profile of splenocytes of vehicle-treated mice inoculated with virus: Quadrant 1, 15.8%; Quadrant 2, 0.4%; Quadrant 3, 71.9%; and Quadrant 4, 11.9%. Note the increase in Lyt-2$^+$ cells in Quadrant 4. (C) Profile of splenocytes of mice receiving 15 mg/kg of Δ^9-THC and inoculated with virus: Quadrant 1, 13.5%; Quadrant 2, 0.5%; Quadrant 3, 75.3%; and Quadrant 4, 10.7%. (D) Profile of splenocytes of mice receiving 100 mg/kg of Δ^9-THC and inoculated with virus: Quadrant 1, 14.6%; Quadrant 2, 0.6%; Quadrant 3, 73.0%; and Quadrant 4, 11.8%. Δ^9-THC had no effect on the number of T helper cells or on the intensity of L3T4 cell surface expression from either HSV1-infected or uninfected mice. Δ^9-THC did not affect the approximate 2-fold increase in Lyt-2$^+$ cells for HSV1-infected mice. However, the drug diminished the intensity in staining of Lyt-2$^+$ cells.

of anti-HSV1 CTL, resulted in a L3T4$^+$:Lyt-2$^+$ ratio of approximately 1.3:1. Δ^9-THC administration did not affect the approximate 2-fold increase in the number of Lyt-2$^+$ cells that was recorded for vehicle-treated mice inoculated with HSV1 when compared with uninfected, vehicle-treated mice (Fig. 1, C and D). However, differences in the intensity of Lyt-2$^+$ staining were noted. Vehicle-treated mice exhibited a contour plot for Lyt-2$^+$ cells, for which Lyt-2$^+$ staining exceeded $\log_{10}2$ intensity. Splenocytes of HSV1-infected mice not receiving drug exhibited Lyt-2$^+$ staining, for which the intensity ranged from $\log_{10}1.4$ to $\log_{10}2.1$. In contrast, a decrease in the intensity of Lyt-2$^+$ staining was noted for splenocytes obtained from mice treated with 15 mg/kg or 100 mg/kg of Δ^9-THC.

Effect of Δ^9-THC on Effector Cell to Target Cell Attachment. The effect of Δ^9-THC *in vivo* treatment on the capacity of CTL to form conjugates with syngeneic HSV1-infected target cells was examined. Splenocytes were depleted of erythrocytes and macrophages and examined by fluorescence microscopy and Nomarski optics microscopy. Fluorescence microscopy demonstrated CD3$^+$ CTL, obtained from mice inoculated with HSV1, attached to HSV1-infected target cells (Fig. 2). Virtually 100% of the CD3$^+$ cells observed by fluorescence microscopy were found bound to virally infected target cells. Furthermore, the majority of lymphocytes attached to target cells were CD3$^+$; approximately 10% of the effector cells bound to the target cells were CD3$^-$. Δ^9-THC had no effect on the time kinetics of attachment of CTL to their targets based on monitoring of conjugate formation at 1 hr, 4 hr, and 6 hr after co-cultivation. Δ^9-THC *in vivo* exposure did not affect the capacity of CTL to bind to HSV1-infected targets, nor did it affect the number of CTL effector cells that bound to the targets. However, Δ^9-THC altered the apparent capacity of CTL to effect lysis of target cells (Figs. 3 and 4). CTL obtained from HSV1-

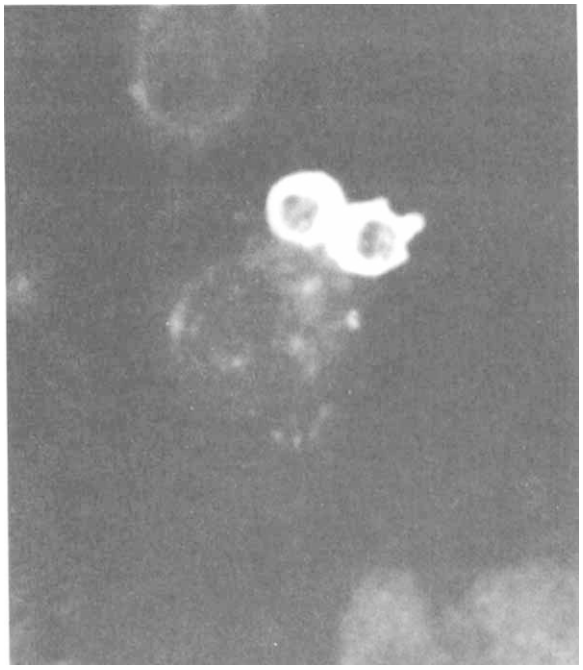

Figure 2. Fluorescence microscopy of $CD3^+$ CTL, obtained from HSV1-infected mice, attached to HSV1-infected target cells. Mice ($H\text{-}2^k$) were subjected to the drug-dosing regimen described in Materials and Methods. Splenocytes were depleted of erythrocytes and macrophages and co-cultured with HSV1-infected or uninfected L929 target cells ($H\text{-}2^k$). Fluorescence microscopy indicated that effector cells bound to HSV1-infected L929 targets demonstrated a $CD3^+$ phenotype indicative of a T lymphocyte rather than a B lymphocyte or an NK cell.

infected, vehicle-treated mice did not form conjugates with uninfected L929 target cells (Fig. 3A). However, CTL were observed conjugated to HSV1-infected target cells as early as 1 hr after co-cultivation. Greater than 50% of target cells conjugated with CTL exhibited cell surface blebs, cytoplasmic macrovacuolization, and/or cell surface plasma membrane disruption. By 4 hr after conjugation, numerous lysed target cells were observed in these co-cultures (Fig. 3B). In contrast, co-cultures containing CTL from Δ^9-THC-treated mice (15–100 mg/kg) exhibited relatively few target cells (approximately 10%) that expressed surface blebs, cell-surface alterations, or that were lysed. These conjugates contained target cells demonstrating normal morphology (Fig. 3C). In addition, CTL conjugated to HSV1-infected target cells exhibited a cytoplasmic polarization in which granules were oriented toward the effector cell to target cell attachment area (Fig. 4A). However, CTL from the drug-treated mice that were conjugated to virus-infected targets did not exhibit cytoplasmic polarization in that cytoplasmic granules were not preferentially oriented toward the effector cell to target cell attachment junction (Fig. 4B). For these CTL, cytoplasmic granules were distributed throughout the cytoplasm.

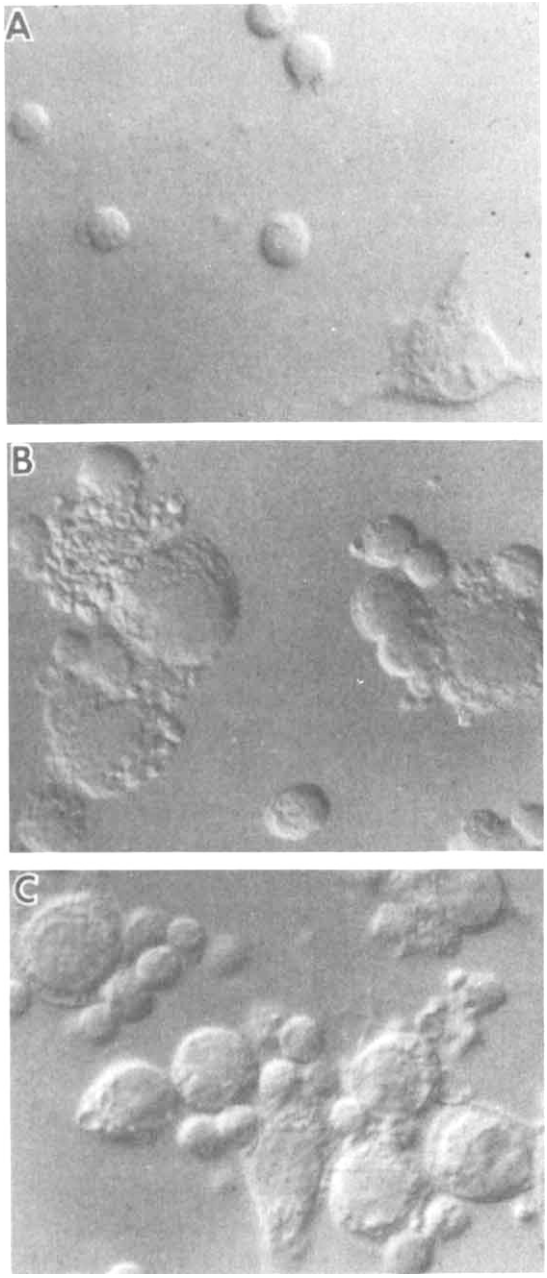

Figure 3. Nomarski optics microscopy of CTL-HSV1-infected L929 conjugates. Mice ($H\text{-}2^k$) were subjected to the drug-dosing regimen described in Materials and Methods. Splenocytes were depleted of erythrocytes and macrophages and co-cultured with HSV1-infected or uninfected L929 target cells ($H\text{-}2^k$) for 1 hr, 4 hr, or 6 hr. (A) CTL and uninfected L929 cell co-cultures at 4 hr after incubation. CTL from vehicle-treated, HSV1-infected mice do not form conjugates with uninfected L929 target cells. (B) CTL and HSV1-infected L929 cell co-cultures at 4 hr after cultivation. CTL from vehicle-treated, HSV1-infected mice conjugate to, and readily lyse, HSV1-infected L929 cells. (C) CTL from Δ^9-THC-treated mice (100 mg/kg) and HSV1-infected L929 cell co-cultures at 6 hr after incubation. Note that CTL bind to the target cell, but do not effect lysis.

Discussion

Δ^9-Tetrahydrocannabinol, the major psychoactive component of marijuana, has been shown to be immunosuppressive *in vitro* (6, 10) and to suppress the

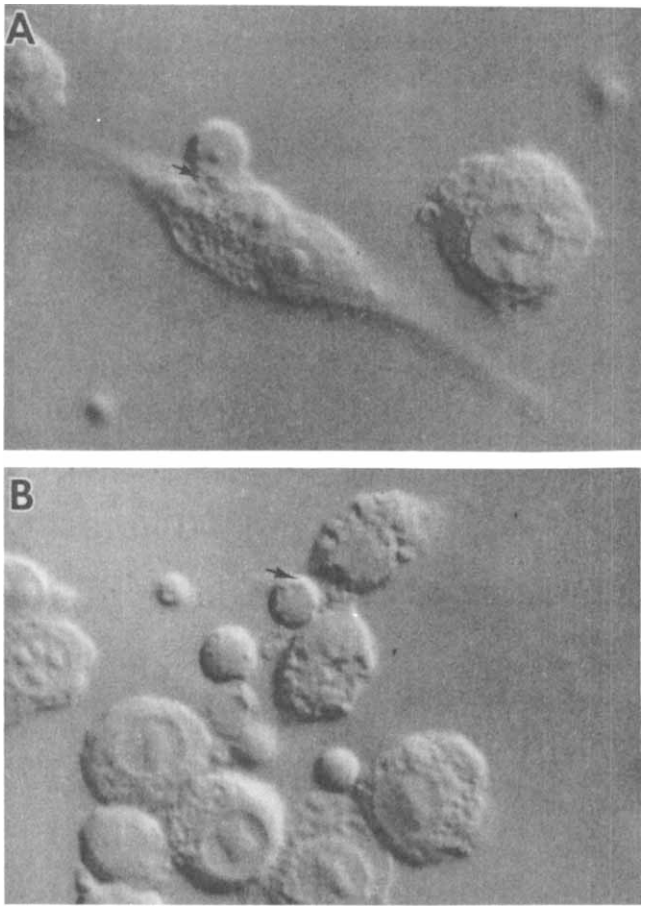

Figure 4. Nomarski optics microscopy of cytoplasmic polarization of CTL bound to HSV1-infected target cells. (A) CTL from vehicle-treated, HSV1-infected mice conjugated to target HSV1-infected L929 cell showing cytoplasmic polarization as evidenced by granule reorientation toward the effector cell to target cell attachment junction. (B) CTL from drug-treated (100 mg/kg) mice showing that the cytoplasmic granules are not oriented toward the effector cell to target cell junction. Arrows identify CTL granules.

immune system in a number of animal models (4, 8). These latter studies demonstrated that the cannabinoid decreases host resistance to herpes simplex virus Type 2 vaginal infection in guinea pigs and mice. Morahan *et al.* (9) demonstrated that Δ^9-THC diminished host resistance to *Listeria monocytogenes* in addition to HSV. These observations indicate that this drug has the capacity to alter the immune system in a number of animal models in a manner that affects both antiviral and antibacterial activities.

It has been well established that H-2-restricted cytolytic T lymphocytes play an important role in the recovery from virus infections, including those elicited by HSV (15, 32–40). Since HSV elicited higher morbidity and mortality in animals exposed to Δ^9-THC, the present study was conducted to determine whether the drug altered anti-HSV1 CTL function. Δ^9-THC was shown to inhibit *in vitro* the anti-HSV1 cytotoxic activity of splenocytes of mice primed *in vivo* with HSV1. Splenocytes of HSV1-infected, vehicle-treated mice generated CTL activity (e.g., 29% specific release) at a level comparable to that previously reported as typical following *in vivo* primary induction with HSV1 (15). In contrast, splenocytes of mice treated with Δ^9-THC (15 mg/kg or 100 mg/kg) exhibited a drug dose-related decrease in CTL activity. However, *in vivo* drug exposure with doses as high as 100 mg/kg failed to totally eliminate CTL activity. *In vivo* drug doses exceeding 100 mg/kg were not employed in attempts to further reduce anti-HSV1 CTL activity, since these higher doses have been shown to elicit hypothermia and toxicity in mice (personal observation), and the resultant elimination of CTL function could represent a generalized toxicological effect rather than a direct effect on CTL. These results suggest that Δ^9-THC at the doses employed fails to completely inhibit anti-HSV1 CTL activity or that other cell types within splenocytes, such as natural killer (NK) cells, also exert antiviral activity and are relatively resistant to Δ^9-THC. Although it has been shown that exposure to Δ^9-THC *in vitro* decreases NK cell cytolytic activity (13), it is unlikely that NK cells are responsible for the cytolytic activity in our system, since the effector cells bound to the virally infected targets were small, $CD3^+$ lymphocytes. NK cells, on the other hand, are typically large granular lymphocytes that do not express CD3 on their surface. Nevertheless, NK cells may contribute to the anti-HSV1 cytolytic activity or indirectly effect lysis of the virally infected targets.

The most obvious alterations in T cell function from drug exposure could be due to an alteration in the total number of reactive T cells. An alternative possibility could be that the ratio of regulatory T cells might be altered from the normal 2:1 T helper to T cytotoxic cell ratio. In order to determine whether Δ^9-THC affected total T lymphocyte cell numbers of mice in different experimental groups, spleen masses and total T cell numbers of virus-infected, drug-treated, or untreated mice were calculated. Δ^9-THC administration did not exert a major effect on spleen mass. Spleens of mice receiving virus, as expected, exhibited an approximate 25% increase in mass when compared with spleens of mice not exposed to virus. No major differences in spleen mass were noted for mice receiving drug versus vehicle. In addition, total splenocyte numbers did not differ among the experimental groups in relation to spleen mass regardless of whether or not they received virus and/or Δ^9-THC. These observations indicate that the drug at the doses employed did not exert a toxicological effect on virus-exposed animals, which would have resulted in a decrease in total cell number.

Since Δ^9-THC had little affect on either spleen mass or total splenocyte numbers, experiments were conducted using FACScan analysis in order to determine whether Δ^9-THC affected individual populations of T

lymphocytes. Results of double staining with FITC-labeled anti-Lyt-2 and phycoerythrin-labeled anti-L3T4 antibodies to identify T cytotoxic/suppressor and T helper cells, respectively, indicated that Δ^9-THC had no major effect on the number of T helper cells from uninfected or HSV1-infected mice. Splenocytes of drug-treated mice exhibited an approximate 2-fold increase in Lyt-2^+ cells following exposure to virus. Δ^9-THC treatment did not affect the increase in Lyt-2^+ cell number in mice exposed to HSV1. This increase was comparable to that recorded for HSV1-infected, vehicle-treated mice. The FACScan analysis indicated, in addition, that Δ^9-THC at doses of 100 mg/kg or less did not affect the total number of Lyt-2^+ cells. However, differences in the intensity of Lyt-2^+ staining were noted after Δ^9-THC exposure. The drug-diminished staining intensity of Lyt-2^+ cells suggests a depletion either in the number of Lyt-2 surface molecules or in a cell population for which Lyt-2 staining is normally high.

CTL, NK cell, and macrophage cytolytic antiviral activities are similar in that they are multistep processes in which the first step consists of conjugation of the effector cell to its target cell. The attachment step is followed by post-conjugation events which either effect cytolysis of the target cell or inhibit virus replication within the target cell (41, 42). CTL versus NK cell and macrophage cell contact-dependent events are distinctive, however, in that CTL activities are MHC-restricted. In order to determine the step in CTL contact-dependent effector function affected by Δ^9-THC, experiments were conducted to determine whether the drug altered the capacity of anti-HSV1 CTL to attach to their syngeneic HSV1-infected targets. Δ^9-THC did not alter the capacity of CTL to attach to the virus-infected murine L929 cells. However, drug exposure altered the capacity of these effector cells to lyse their targets. CTL from virus-infected, vehicle-treated mice elicited cell surface blebs on, and macrovacuolization within, HSV1-infected target cells by as early as 1 hr after conjugation. By 6 hr after conjugation, numerous lysed target cells with CTL attached to them were observed. In contrast, conjugates containing CTL from drug-treated mice rarely exhibited blebs or lysed target cells even by 6 hr after co-cultivation. These results are in agreement with our cytotoxicity data indicating a decrease in cytolytic activity of CTL from HSV1-infected mice treated with Δ^9-THC. The ability of CTL from drug-treated mice to attach to target cells is in agreement with previous studies that indicated that peritoneal macrophages of mice receiving *Propionibacterium acnes* in concert with Δ^9-THC exhibited decreased antiviral activity, but were not precluded from attaching to tumor cells or virus-infected target cells (43). These observations suggest that Δ^9-THC may exert a common mode of action on effector cell mediators of both MHC-restricted and non-MHC-restricted cell contact-dependent cytolytic activities. Furthermore, these studies indicate that Δ^9-THC does not inhibit effector cell to target cell conjugation, but, rather, alters effector cell postconjugation events.

CTL effector cells become rapidly polarized following target cell attachment and position their microtubule-organizing center and Golgi complex toward the target cell contact area (23–26). This reorientation of cytoskeletal elements is requisite for cell contact-dependent cytotoxicity, since blocking of this step with tubulin blockers (e.g., Nocodazole) prevents target cell lysis (24). The effector cell, then, undergoes vectorially oriented granule exocytosis which results in the release of pore-forming protein/perforin monomer into the intercellular space (23, 40, 44–46). Ca^{2+}-Dependent assembly of membrane lesions occurs on the target cell with consequent polymerization of the pore-forming protein monomer into a functional channel in the membrane. Target cell death, then, occurs as a result of the formation of structural and functional membrane lesions which allow for the irreversible and lethal equilibration of water, electrolytes, and macromolecules. Thus, an intact cytoskeletal system is requisite for the rapid cytoskeletally directed vectorial traffic of Golgi apparatus-derived vesicles to the leading edge of the CTL and the subsequent injection of new membrane mass into the target cell (47, 48).

In the present study, Nomarski optics microscopy revealed that cytoplasmic granules of CTL conjugated to target HSV1-infected cells were found directed toward the proximal edge of the effector cell to target cell attachment junction. In contrast, conjugates containing CTL from drug-treated mice exhibited granules that were randomly distributed in the cytoplasm and not necessarily oriented toward the effector cell to target cell junction. These observations suggest that Δ^9-THC may alter the capacity of CTL to undergo cytoplasmic polarization and cytoskeletal reorientation after conjugation with the virus-infected target cell. Δ^9-THC has been shown to bring about a morphological disruption of cellular membranes (49, 50) and of cytoskeletal elements (51). Perturbations of cellular membranes could cause a disruption of cytoskeletal elements with the effector cell, resulting in the failure to transport CTL granules containing effector molecules to the virus-infected cell. Indeed, we have shown that Δ^9-THC administered *in vitro* elicits membrane perturbation of cell surface and cytoplasmic membranes and disrupts the assembly and orientation of cytoskeletal elements in rat neuroblastoma cells (51).

The perturbations in cellular membranes may be attributed to the binding of the highly lipophilic Δ^9-THC molecule. Such binding has been shown to affect membrane fluidity and, subsequently, alter selective permeability (52). Alterations in cell-surface-membrane-selective permeability with the attendant increase

in intracellular sodium have been proposed as a mode that results in disruption of cytoskeletal elements (44).

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