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Δ^9-Tetrahydrocannabinol Decreases Alpha/Beta Interferon Response to Herpes Simplex Virus Type 2 in the B6C3F1 Mouse[1] (42258)

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Abstract. This study was undertaken to determine the effect of Δ^9-tetrahydrocannabinol (Δ^9-THC) on polyinosinic:polycytidylic acid [poly(I):poly(C)]-induced, and on herpes simplex virus type 2 (HSV-2)-induced, alpha/beta interferon in the B6C3F1 mouse. Animals were administered Δ^9-THC, or the diluent, intraperitoneally for 4 consecutive days or at various time intervals prior to administration of the interferon inducer. Poly(I):poly(C) or HSV-2 was injected intravenously on Day 4. Animals receiving poly(I):poly(C) and treated with Δ^9-THC at doses ranging from 5 to 100 mg/kg exhibited significantly lower titers of interferon than mice given poly(I):poly(C) and the diluent. Diminished interferon titers occurred in HSV-2-infected animals treated with Δ^9-THC in doses exceeding 15 mg/kg when compared to virus-infected animals given the diluent. This suppression of early interferon persisted through 24 hr. © 1986 Society for Experimental Biology and Medicine.

The incidence of herpes genitalis has reached near epidemic proportions within the sexually active segment of the human population in the United States (1). This increased incidence has occurred at a time when marijuana is in extensive use. Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the major psychoactive component of marijuana, and has been shown to be immunosuppressive (2). It diminishes both cellular and humoral responses in a variety of animal models including man (3, 4). The drug has also been shown to decrease host resistance to a number of infectious agents. Morahan *et al.* (3) have shown that mice treated with Δ^9-THC are more susceptible to *Listeria monocytogenes* and to herpes simplex virus type 2 (HSV-2). Studies conducted in this laboratory have demonstrated that Δ^9-THC decreases host resistance to HSV-2 vaginal infection in guinea pigs (5). This decreased resistance was elicited early in the infectious process in that drug-treated animals experienced rapid onset of primary herpes genitalis when compared to virus-infected control animals. These results indicated that an early factor of host resistance, such as interferon to HSV-2, was impaired by Δ^9-THC. The objective of this study was to determine whether Δ^9-THC suppressed virus-induced alpha/beta interferon.

Materials and Methods. *Cells.* Vero cells were obtained from M. A. Bioproducts (Whittaker, Md.). Vero cells were cultured in Eagle's minimal essential medium (EMEM), supplemented with 10% fetal calf serum (FCS), 2% glutamine, 2% nonessential amino acids, 2% vitamins, and penicillin/streptomycin plus fungizone. Medium was buffered with Hepes and sodium bicarbonate. Mouse L929 cells were obtained from Dr. Lawrence Schook of the Department of Microbiology and Immunology at the Virginia Commonwealth University. Mouse L929 cells were cultured in Dulbecco's medium (DMEM) supplemented as described for Vero cell cultures. Cultures were maintained in closed flasks at 37°C.

Virus. Herpes simplex virus type 2, strain HSV-2-FMC-P180, was isolated from cervical tissue of a gynecological patient. The virus was propagated in Vero cells and was shown to have a stock titer of 1×10^8 plaque-forming units (PFU)/ml by plaque assay (6). Vesicular stomatitis virus (VSV), Indiana strain, was

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obtained from the American Tissue Type Collection (Rockville, Md.). VSV stocks were prepared in mouse L929 cells and were titered by plaque assay as 1×10^8 PFU/ml. Virus stocks were stored at −80°C until used.

Drugs and chemicals. Δ^9-Tetrahydrocannabinol (Δ^9-THC) and cannabidiol were provided by Dr. L. Harris of the Department of Pharmacology and Toxicology at the Virginia Commonwealth University. Cannabinoids were prepared as stock solutions of 100 mg/ml in diluent consisting of emulphor:ethanol:saline (1:1:8). Polyinosinic:polycytidylic acid [poly(I):poly(C)] (mol wt = 100,000) was purchased from Sigma Chemical Company (St. Louis, Mo.). Actinomycin D was donated by Dr. J. Formica of the Department of Microbiology and Immunology, Virginia Commonwealth University. Cyclophosphamide was purchased from Sigma Chemical Company.

Standard interferon and interferon antiserum. Interferon standards and standard interferon antisera were purchased from Lee Biomolecular Research Company (San Diego, Calif.). Alpha + beta mouse interferon, 8.8 $\times 10^5$ IU/ml (1.7×10^6 IU/mg), lot 82010, was reconstituted with sterile deionized water and was stored at 4°C until used. Rabbit anti-mouse interferon (alpha + beta), 1×10^4 units/ml, lot 84005, was reconstituted in sterile deionized water and stored at 4°C until used.

Animals. Female B6C3F1 virus-free mice were purchased from Frederick Cancer Research Center (Frederick, Md.). These mice were selected for this study since we have previously employed this model to demonstrate that Δ^9-THC decreases host resistance to HSV-2 primary infection following either intravaginal (25) or intravenous administration (personal observations). Animals were quarantined for 1 week prior to initiation of the study. Sentinel animals were tested serologically for evidence of infection with mouse hepatitis virus or with Sendai virus and were shown to be free of virus infection. The mice, 8–9 weeks of age (20 g), were given free access to food and water.

Drug administration. Mice were administered Δ^9-THC intraperitoneally (ip) in doses of 5, 25, 50, or 100 mg/kg in a 0.2-ml vol. The Δ^9-THC was administered for 4 consecutive days (i.e., Days 1 to 4). Control animals received the diluent or cannabidiol (100 mg/kg) for 4 consecutive days. Mice were then inoculated on Day 4 in the tail vein with 0.2 ml of poly(I):poly(C), 1×10^7 PFU of HSV-2, or saline. In one set of experiments, to determine how long after Δ^9-THC administration suppression of poly(I):poly(C)-induced interferon could be effected, animals received a single injection of vehicle or Δ^9-THC (50 mg/kg) at 24, 12, 4, or 1 hr prior to poly(I):poly(C) treatment (200 μg). Mice also received Δ^9-THC or vehicle at 1 hr following poly(I):poly(C) injection.

Interferon induction. Mice were injected intravenously (iv) via the tail vein with 5, 10, 15, 20, 50, and 200 μg poly(I):poly(C) coupled to equal amounts of DEAE–Dextran (7) in a 0.2-ml vol and were bled via the brachial artery at 2, 4, 6, and 8 hr postinoculation. Blood was maintained for 1 hr at room temperature and serum was separated by centrifugation (800*g*) at 4°C. Mice were injected in the tail vein with HSV-2 (1×10^7 PFU/0.2 ml) and were bled by cardiac puncture at 4, 6, 8, 10, 15, and 24 hr post-virus-inoculation. Sera were harvested and stored, as described above, at −80°C until employed in interferon assays.

Interferon assay. Sera from all mice were acid-treated with 0.5 *M* HCl until pH 2.0 was obtained, and were maintained at this pH for 4 hr at room temperature and overnight at 4°C (8). Sera were neutralized with 0.5 *M* NaOH and were diluted 1:10 in DMEM. All assays were performed in 96-well flat-bottomed microtiter plates (Costar, Cambridge, Mass.). Diluted serum and L929 cell suspension (4×10^4 cells/well) were added to each well and plates were incubated at 37°C for 24 hr in a humidified 5% CO_2 atmosphere. Next, VSV (200 PFU) was added and the plates were incubated at 37°C (5% CO_2) until plaques were observed in VSV-infected wells preincubated with normal mouse serum.

Wells inoculated only with cells, or with cells plus virus, were included in each microtiter plate as controls. Normal serum controls were also included in each plate. In addition, a plate with a standard interferon control was processed along with each assay.

Determination of interferon titer. The titer was defined as the reciprocal of the highest serum dilution resulting in a 50% reduction of VSV plaques in L929 cell monolayers when compared to nonserum treated, VSV-inocu-

lated controls and to VSV-inoculated normal serum controls. In addition, plates were analyzed for interferon titer spectrophotometrically. Using this assay, percentage protection was determined following quantitation of crystal violet eluted from stained L929 monolayers. The crystal violet stain was eluted with 100 μl/well of 50% ethanol containing 0.2 *M* HCl and the absorbance of the solution was read at 500 nm. Percentage protection was defined using

$$\% \text{ P} = 100\% \times [(A_i - A_v)/(A_c - A_v)]$$

where A_i is the absorbance for the interferon dilution, A_v is the absorbance for the virus controls, and A_c is the absorbance of the cell controls. Interferon serum titers were converted to international units (IU) by comparing percentage protection rendered by each mouse serum sample to the percentage protection rendered by the stadard interferon. One interferon unit (IU) was defined as that interferon dilution which elicited 50% protection in cultures inoculated with VSV.

Characterization of alpha/beta interferon. Mouse sera were serially diluted twofold horizontally along each row of a 96-well microtiter plate. The sera, then, were "block-titrated" against an equal volume of serial twofold dilutions of rabbit anti-mouse interferon (alpha + beta) along each column. Thus, the anti-alpha + anti-beta antibody ranged in dilution from 1×10^4 units/ml to approximately 80 units/ml along the vertical axis. Following incubation, the mixtures containing the interferon–anti-interferon complexes were transferred to L929 cell monolayers in 96-well flat-bottom microculture plates (Costar, Cambridge, Mass.). A 50-μl vol of VSV (200 PFU) was added to each well and the plates were incubated. In each plate a set of diluted mouse sera not incubated with anti-interferon antisera was added to L929 monolayers.

Statistical analysis. Data were examined statistically using Student's *t* test, one-way parametric ANOVA, and by linear regression analysis (9).

Results. *Optimal time of induction of interferon by poly(I):poly(C) and by HSV-2.* Peak production of poly(I):poly(C)-induced interferon in the female B6C3F1 mouse, following intravenous injection of 200 μg of the inducer, was shown to be 4 hr postinoculation. These observations are in agreement with those obtained by other investigators employing other mouse strains (10, 11). The optimal time of HSV-2-elicited interferon in mice administered 1×10^7 PFU of the virus intravenously was 6–8 hr postinoculation and is in general agreement with that recorded by Engler *et al.* (12) using C3H, C57BL, and BALB/c mice.

Protein inhibitors. Actinomycin D and cyclophosphamide (CTX) were used as positive controls to evaluate the effect of protein inhibitors on HSV-2-elicited interferon. Mice ($n = 8$ per group) were inoculated intraperitoneally for 4 consecutive days with 25 μg/kg actinomycin D or 50 mg/kg cyclophosphamide. On Day 4, animals were inoculated (iv) with 1×10^7 PFU of HSV-2 and were bled 6 hr postinfection. Mean interferon titers in control animals, receiving only HSV-2, were 5.4×10^4 IU. In contrast, the mean interferon titers elicited in virus-infected mice administered actinomycin D or cyclophosphamide were significantly suppressed ($P < 0.05$) to 1.1×10^4 or 8.1×10^3 IU, respectively.

Effect of Δ^9-THC on poly(I):poly(C)-induced interferon. In order to evaluate the effect of Δ^9-THC on poly(I):poly(C)-induced interferon, mice were assigned to groups ($n = 8$ per group) and were subjected to a 4-day regimen (i.e., Days 1–4) of 5, 25, 50, or 100 mg/kg of Δ^9-THC or drug vehicle (ip). Sera for measurement of interferon titers were obtained 4 hr following introduction of the poly(I):poly(C) on Day 4. Poly(I):poly(C)-injected control animals had a mean interferon titer of 4×10^4 IU. Diluent-treated, poly(I):poly(C)-injected control animals exhibited a mean interferon titer of 4.8×10^4 IU. In contrast, animals receiving Δ^9-THC at doses ranging from 5 to 100 mg/kg yielded serum interferon titers of less than 9×10^3 IU (Fig. 1).

To determine how long after Δ^9-THC administration the suppression of poly(I):poly(C)-induced interferon could be effected, mice ($n = 4$) were exposed to a single inoculum (50 mg/kg) of the cannabinoid at 24, 12, 4, and 1 hr prior to, and 1 hr after, administration of the synthetic polynucleotide. Mice were bled 4 hr following poly(I):poly(C) injection. As indicated in Table I, significant suppression ($P < 0.05$ to $P < 0.08$) was observed at all time periods when canabinoid administration proceded that of poly(I):poly(C). Significant

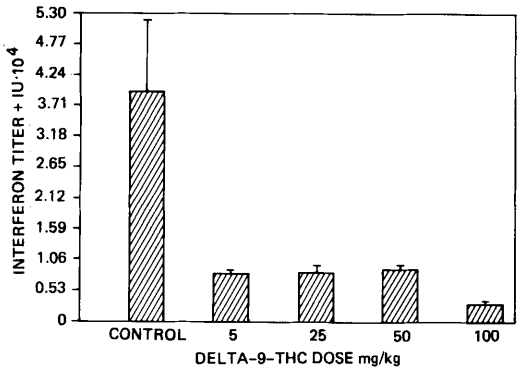

FIG. 1. Titers of poly(I):poly(C)-induced interferon 4 hr postinoculation. Animals (n = 8 per group) were administered vehicle, 5, 25, 50, or 100 mg/kg Δ^9-THC and were inoculated intravenously with 200 μg poly(I):poly(C). Δ^9-THC in doses ranging from 5 to 100 mg/kg significantly suppressed alpha/beta interferon induction by poly(I):poly(C) (Student's t test; $P < 0.01$).

suppression was not observed for animals receiving Δ^9-THC 1 hr following the poly(I):poly(C) administration.

Effect of Δ^9-THC on HSV-2-induced interferon. The effect of Δ^9-THC on HSV-2-induced interferon was assessed. Additionally, a group of eight animals was treated with 100 mg/kg cannabidiol, a nonpsychoactive component of cannabis. Sera were obtained 6 hr after challenge with HSV-2 on Day 4. A significant reduction in interferon titer ($P < 0.01$) occurred in HSV-2-infected animals treated with 50 mg/kg (4×10^3 IU) or with 100 mg/kg (3.5×10^3 IU) of Δ^9-THC when compared to non-drug treated, virus-infected controls (6.5×10^4 IU). No drug-related suppression of interferon was observed in virus-infected animals administered 25 mg/kg of Δ^9-THC (Fig. 2). Sera of animals treated with cannabidiol demonstrated a mean interferon titer of 4.3×10^4 IU.

Time kinetics of Δ^9-THC-mediated suppression of HSV-2-induced interferon. The effect of Δ^9-THC on HSV-2-induced alpha/beta interferon over a 24-hr period was examined (Fig. 3). Virus-infected, diluent-treated animals produced interferon as early as 4 hr postinfection (i.e., 5.1×10^4 IU). Titers increased in a linear manner through 8 hr postinfection (r = 0.97) to a mean titer of 8.7×10^4 IU. Titers of 5.6×10^4 and 5.2×10^4 IU were recorded for 10 and 15 hr postinfection, respectively. By 24 hr postinfection, a mean interferon titer of 1×10^5 IU occurred in diluent controls. Thus two maxima, at 8 and 24 hr postinfection, were observed in virus-infected, vehicle-treated animals. In con-

TABLE I. INTERVAL OF POLY(I):POLY(C)-INDUCED INTERFERON SUSCEPTIBILITY TO PRIOR ADMINISTRATION OF Δ^9-TETRAHYDROCANNABINOL

Time of sample[a] (hr)	Interferon titer ± SE (IU)	
	Vehicle control[b]	Δ^9-THC treated[b]
−24	6.08 ± 2.11	0.23 ± 0.04[c]
−12	10.34 ± 3.49	1.60 ± 0.52[c]
−4	2.13 ± 0.91	0.08 ± 0.03[d]
−1	3.34 ± 0.91	0.30 ± 0.11[c]
+1	4.86 ± 1.72	2.23 ± 1.33

[a] Mice (n = 4 per group) received either a single dose of Δ^9-THC (50 mg/kg) or vehicle at −24, −12, −4, −1, or +1 hr relative to intravenous administration of 200 μg of poly(I):poly(C). All animals were bled by cardiac puncture at 4 hr post-poly(I):poly(C) administration.

[b] Titers are expressed as interferon international units (IU) × 10^4.

[c] Student's t test; $P < 0.05$.

[d] Student's t test; $P < 0.10$.

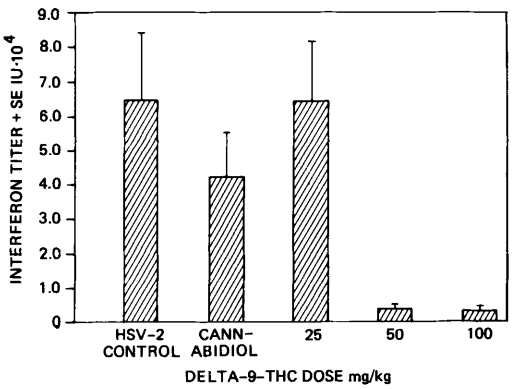

FIG. 2. Titers of HSV-2-induced alpha/beta interferon 6 hr post-viral-inoculation. Animals (n = 8 per group) were administered 25, 50, or 100 mg/kg Δ^9-THC and were infected intravenously with 1×10^7 PFU HSV-2. One group of mice was dosed with 100 mg/kg cannabidiol and was injected with virus. Significant suppression of alpha/beta interferon titer was observed at 50 and 100 mg/kg Δ^9-THC (Student's t test; $P < 0.01$). Interferon suppression, comparable to that observed in animals receiving 50 or 100 mg/kg of Δ^9-THC, was not noted in animals treated with 100 mg/kg cannibidiol.

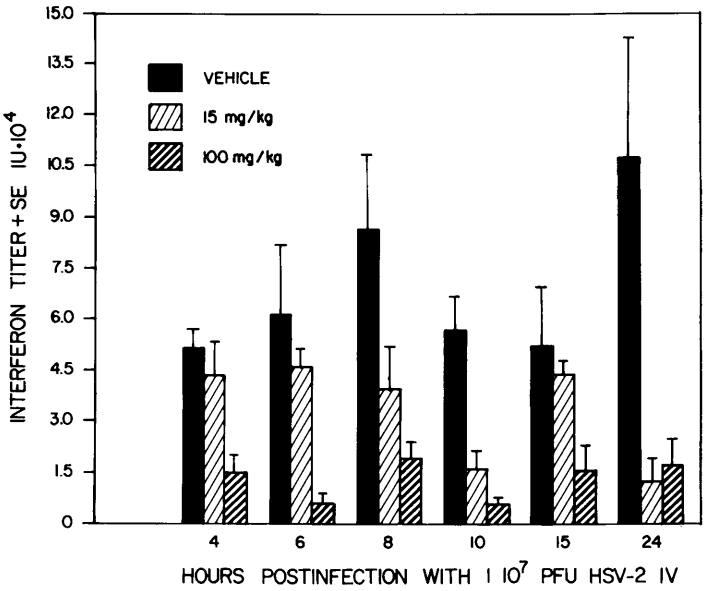

FIG. 3. Alpha/beta interferon titers recorded at 4, 6, 8, 10, 15, and 24 hr post-HSV-2-inoculation. Animals (n = 8 per group) were administered vehicle, 15, or 100 mg/kg Δ^9-THC and were injected intravenously with 1×10^7 PFU HSV-2. Significant suppression (Student's t test; $P < 0.05$) of HSV-2-induced interferon over a 24-hr period was noted in serum of animals dosed with 100 mg/kg Δ^9-THC at each recorded time period. Significant suppression ($P < 0.05$) of alpha/beta interferon was noted also for animals dosed with 15 mg/kg Δ^9-THC at 10, 15, and 24 hr post-HSV-2-injection.

trast, virus-infected animals treated with 100 mg/kg Δ^9-THC demonstrated significantly lower titers ($P < 0.05$) than vehicle controls at all time periods tested. Virus-infected animals treated with 15 mg/kg of Δ^9-THC demonstrated a significant suppression ($P < 0.05$) of mean interferon titer at 10, 15, and 24 hr postinfection.

Characterization of alpha/beta interferon. In order to confirm the presence of alpha/beta interferon in mouse sera, and to demonstrate specifically that Δ^9-THC affected alpha/beta interferon activity, sera of mice injected with HSV-2 were block-titrated against rabbit anti-mouse alpha/beta interferon. Treatment of mouse sera with anti-alpha/beta interferon antiserum neutralized the antiviral activity in mice inoculated with poly(I):poly(C) or with HSV-2 as evidenced by demonstrable linearity of decreased protection to VSV in block-titrated microtiter plates.

Discussion. It has been observed by several laboratories (2–5) and cited in clinical reports (13) that Δ^9-THC decreases host resistance to a variety of infectious agents. Studies conducted in this laboratory have demonstrated that Δ^9-THC diminishes host resistance to primary HSV-2 vaginal infection in both guinea pigs (5) and mice (14) producing more rapid onset of disease, more severe disease sequelae, and a higher number of mortalities when compared to virus-infected, vehicle controls. These observations, coupled with recognition that Δ^9-THC induces immunosuppressive effects, suggest that early factors of host resistance to HSV-2 are targeted by this cannabinoid. Alpha/beta interferon plays an important role in host resistance to virus infections (15). In a study in which susceptibility of eight different inbred mouse strains to HSV-2 infection was compared, HSV-resistant mice were found to produce high titers of interferon 2–4 hr post-viral inoculation (16). Less resistant mice, however, lacked this early response.

In the present investigation, Δ^9-THC was shown to significantly decrease poly(I):poly(C)-induced interferon in animals treated for 4 consecutive days with the cannabinoid in doses ranging from 5 to 100 mg/kg. This decrease in poly(I):poly(C)-induced interferon was also effected with a single dose (50 mg/

kg) of Δ^9-THC administered at times ranging from 24 to 1 hr prior to administration of the synthetic polynucleotide. These data suggest that Δ^9-THC need not be administered in close temporal relationship to the interferon inducer in order to bring about suppression, so long as the administration of the cannabinoid precedes that of the inducer by 1 to 24 hr. Studies are in progress to determine whether the interval of Δ^9-THC susceptibility extends beyond 24 hr prior to administration of poly(I):poly(C). Poly(I):poly(C) is a potent inducer of both alpha and beta interferon in mouse fibroblasts (17) and the plasma of mice (11). Both alpha and beta interferon have been shown to exhibit a spectrum of antiviral activities to a number of viruses (15) including HSV (16, 18). Thus, these results suggest that Δ^9-THC contributed to a decreased host resistance by targeting production of alpha/beta interferon. The fact that alpha/beta interferon was targeted by Δ^9-THC is supported by the observation that the anti-viral activity in mouse sera was neutralized by prior incubation with anti-alpha/beta interferon antiserum. Furthermore, the antiviral activity in mouse sera was stable at pH 2.0, reached peak activity at 4–6 hr following intravenous injection with poly(I):poly(C) or with HSV-2, and was inhibited by actinomycin D indicating that cell transcription was necessary for eliciting the antiviral effect. Experiments utilizing specific anti-alpha interferon and anti-beta interferon antibodies, coupled with experiments entailing exogenous interferon administration to mice to reverse the effects of Δ^9-THC on HSV2 resistance, will allow for determination of the extent to which each of the interferon components is affected by Δ^9-THC. The suppressive effect of Δ^9-THC on early interferon, however, does not preclude that additional parameters of early host resistance to primary infection may be affected by the cannabinoid. Studies are in progress to determine the effect of Δ^9-THC on natural killer (NK) cell activity (19), and on macrophage extrinsic activity (20) to HSV primary infection.

Significant suppression of HSV-2-induced interferon was obtained at 6 hr post-viral inoculation in animals treated with Δ^9-THC. This suppression was not a consequence of generalized toxicity since previous studies have demonstrated that Δ^9-THC at these doses induces immunosuppressive effects in mice in the absence of toxicity (4). Animals treated with 100 mg/kg Δ^9-THC exhibited significant suppression of interferon for the entire 24-hr period when compared to vehicle controls. These results suggest that Δ^9-THC compromises host resistance to HSV-2 during an early, and critical phase, of virus replication. Indeed, both alpha and beta interferon have been shown to block replication of, and cell fusion induced by, herpes simplex viruses (18). Thus, Δ^9-THC treatment of mice may lead to enhanced virus replication. Indeed, we have shown that Δ^9-THC administration to guinea pigs and mice infected intravaginally with HSV-2 results in significantly higher yields of virus shed from the vagina when compared to virus-infected, vehicle control animals (5).

Because Δ^9-THC has similarities in structure and pharmacological action to steroids (4), and steroids have been shown to potentiate viral replication *in vitro* (21) and *in vivo* (22), the effect of cannabidiol administration on induction of early interferon was examined. Cannabidiol administration to HSV-2-infected mice did not significantly decrease levels of virus-induced alpha/beta interferon. These results suggest that the decreased resistance to HSV-2 in experimental animals is not a consequence of steroid-induced hyperproduction of virus, but rather a result of suppression induced by the major psychoactive component of marijuana, Δ^9-THC.

In the present investigation relatively high doses of Δ^9-THC (i.e., 100 mg/kg) were employed since the mouse is less susceptible to Δ^9-THC than man. However, the doses of cannabinoid which were administered are comparable to those attainable in man after appropriate extrapolation of drug dose (mg/kg) versus animal mass/surface ratio (mg/m^2). A dose of 100 mg/kg in the mouse corresponds approximately to a dose of 10 mg/kg in man (23). Human Δ^9-THC intake in excess of 15 mg/kg/day has been reported for some cannabis users (24).

1. Sumaya CV, Marx J, Ullis F. Genital infection with herpes simplex virus in a university student population. Sex Transm Dis 7:16–20, 1980.
2. Levy JA, Heppner GH. Alterations of immune reactivity by haloperidol and delta-9-tetrahydrocannabinol. J Immunopharmacol 3:93–109, 1981.

3. Morahan PS, Klykken PC, Smith SH, Harris LS, Munson AE. Effects of cannabinoids on host resistance to *Listeria monocytogenes* and herpes simplex virus. Infect Immun **23**:670–674, 1979.
4. Munson AE, Fehr KD. Immunological effects of cannabinoids. In: Fehr KO, Kalant H, eds. Adverse Health and Behavioral Consequences of Cannabis Use. Working Papers for the ARS-WHO Scientific Meeting, Toronto, 1981. Addiction Research in Toronto, pp258–353, 1983.
5. Cabral GA, Mishkin EM, Holsapple MP. Delta-9-tetrahydrocannabinol decreases host resistance to herpes simplex virus type 2 vaginal infection in the guinea pig. Marihuana '84. Proceedings of the Oxford Symposium on Cannabis. Oxford, IRL Press, p431–438, 1985.
6. Rapp F. Variants of herpes simplex virus: Isolation, characterization, and factors influencing plaque formation. J Bacteriol **86**:985–991, 1963.
7. Smetana O, Eylan E, Kariv N. The effect of poly I:C and IUDR on the inhibition of HSV in rabbit eyes. Med Microbiol Immunol **171**:99–112, 1982.
8. Zawatzky R, Kirchner H, Demayer-Guignard J, Demayer E. An X-Linked locus influences the amount of circulating interferon induced in the mouse by herpes simplex virus type 1. J Gen Virol **63**:325–332, 1982.
9. Zar JH. Biostatistical Analysis. Englewood Cliffs, N.J., Prentice-Hall, 1974.
10. Baron S, duBuy H, Buckler CE, Johnson ML, Park J, Billian A, Sarma P, Huebner RJ. Induction of interferon and viral resistance in animals by polynucleotides. Ann NY Acad Sci **173**:568–580, 1970.
11. Machida H, Kawade Y, Watanabe Y. Antigenicity of mouse interferons induced *in vitro* and *in vivo* by poly(I):poly (C). J Interferon Res **4**:347–353, 1984.
12. Engler H, Zawatzky R, Kirchner H, Armerding D. Experimental infection of inbred mice with herpes simplex virus. IV. Comparison of interferon production and natural killer cell activity in susceptible and resistant adult mice. Arch Virol **74**:239–247, 1982.
13. Juel-Jenson BE. Cannabis and recurrent herpes simplex. Brit Med J **iv**:296, 1972.
14. Holsapple MP, McNerney PJ, Mishkin EM, Cabral GA. *In vivo* and *in vitro* effects of delta-9-tetrahydrocannabinol on immune responsiveness and vaginal resistance to herpes simplex virus type II (HSV2) infection by B6C3F1 mice. Proceedings of the Oxford Symposium on Cannabis. Oxford, IRL Press, p439–450, 1985.
15. Friedman RM. Antiviral activity of interferons. Bacteriol Rev **41**:543–567, 1977.
16. Pedersen EB, Haahr S, Mogensen SC. X-Linked resistance of mice to high doses of herpes simplex virus type 2 correlates with early interferon production. Infect Immun **42**:740–746, 1983.
17. Yamamoto Y, Kawade Y. Antigenicity of mouse interferons: Distinct antigenicity of the two L cell interferon species. Virology **103**:80–88, 1980.
18. Chatterjee S, Lakeman AD, Whitley RJ, Hunter E. Effect of cloned human interferons on the replication of and cell fusion induced by herpes simplex virus. Virus Res **1**:81–87, 1984.
19. Engler H, Zawatzky R, Goldbach A, Schroder CH, Weyand C, Hammerling GJ, Kirchner H. Experimental infection of inbred mice with herpes simplex virus. II. Interferon production and activation of natural killer cells in the peritoneal exudate. J Gen Virol **55**:25–30, 1981.
20. Morahan PS, Connor JR, Leary KR. Viruses and the versatile macrophage. Brit Med Bull **41**:15–21, 1985.
21. Costa J, Yee C, Troost T, Rabson AS. Effect of dexamethasone on herpes simplex virus type 2 infection *in vitro*. Nature **252**:745–746, 1974.
22. Baker DA, Plotkin SA. Enhancement of vaginal infection in mice by herpes simplex virus type II with progesterone. Proc Soc Exp Biol Med **158**:131–134, 1978.
23. Klaasen CD, Doull J. Evaluation of safety. Toxicologic evaluation. In: Doull, J, Klaasen CD, Amdur MO, eds. Toxicology: The Basic Science of Poisons. New York, Macmillan p11, 1980.
24. Tennant FS, Groesbeck CJ. Psychiatric effects of hashish. Arch Gen Psychiatry **27**:133–136, 1982.
25. Mishkin EM, Cabral GA. Delta-9-tetrahydrocannabinol decreases host resistance to herpes simplex virus type 2 vaginal infection in the B6C3F1 mouse. J Gen Virol, In press.

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