Δ^9-Tetrahydrocannabinol Suppresses Macrophage Extrinsic Antiherpesvirus Activity (43356)

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Abstract. **The effect of Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, on macrophage intrinsic and extrinsic antiherpesvirus activities was examined. THC had no effect on the capacity of the macrophage-like cells RAW264.7, J774A.1, and P388D$_1$ to take up virus. In addition, replication of virus within macrophages did not occur regardless of drug treatment, indicating that THC had no effect on macrophage intrinsic antiviral activity. In contrast, the cannabinoid exerted a dose-dependent inhibition of macrophage extrinsic antiviral activity. This activity describes that macrophage function by which these cells suppress virus replication within xenogeneic cells in an interferon-independent manner. The inhibitory effect of THC on extrinsic antiviral activity was greatest on RAW264.7 and J774A.1 cells, followed by P388D$_1$ cells. These macrophage-like cells regained their extrinsic antiviral activity in a time-related fashion following removal of the drug. These results indicate that THC inhibits macrophage extrinsic antiherpesvirus activity, but has no effect on intrinsic antiviral activity. However, the suppressive effect of THC on extrinsic antiviral activity is reversible upon removal of the drug.** [P.S.E.B.M. 1992, Vol 199]

Delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, has been shown to elicit a variety of immunosuppressive effects *in vivo* and *in vitro*. These include dysfunction in lymphocyte response to mitogens and particulate antigens (1, 2), a decrease in T cell rosette formation (3, 4), inhibition of natural killer cell activity (5, 6), suppression of leukocyte migration (7), and perturbation of macrophage morphology, function, and motility (8-10). THC, in addition, has been shown to decrease host resistance to bacterial and virus infections. Morahan *et al.* (11) demonstrated that mice treated with various cannabinoids experienced a dose-related decrease in resistance to both *Listeria monocytogenes* and herpes simplex virus (HSV) type 2. We have demonstrated that exposure of mice and guinea pigs to THC exacerbates primary HSV2 genital infection (12).

Macrophages play a critical role in host immunity, especially at the primary site of infection. These cells are actively involved in resistance to intracellular infection (13), act as accessory cells in the immune response in their capacity as antigen-presenting cells and as producers of immunomodulatory signals (14, 15), and function as cytotoxic effectors against tumor cells (16) and virus-infected cells (17). Macrophages also exert intrinsic and extrinsic antiviral activities. Intrinsic activity is the process by which macrophages ingest and degrade virus, and thereby are nonpermissive for productive virus infection (18–21). This degradation of virus by macrophages facilitates clearance of virus from the local site of infection. Extrinsic antiviral activity is the process by which macrophages elicit an interferon-independent, cell contact-dependent reduction of virus growth in infected, virus-susceptible cells (22). Both intrinsic and extrinsic antiviral activities play an important role in the control of localized virus infection and in limiting the spread of virus within the infected host.

Drug-induced alteration of macrophage antiviral activities could have a major effect on host resistance

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Received March 1, 1991. [P.S.E.B.M. 1992, Vol 199]
Accepted September 23, 1991.

0037-9727/92/1992-0255$3.00/0

to venereally transmitted pathogens, particularly at the primary site of exposure. Thus, the objective of this study was to define the effect of THC on macrophage intrinsic and extrinsic antiviral activities. Results of this investigation demonstrate that THC has no effect on intrinsic anti-HSV2 activity, but suppresses macrophage extrinsic antiviral activity to HSV2.

Materials and Methods

Cell Cultures. The macrophage-like cell lines RAW264.7 (TIB71), J774A.1 (TIB67), and P388D_1 (TIB63) were obtained from the American Type Culture Collection (Rockville, MD). Macrophages were grown in complete RPMI 1640 medium (RPMI 1640 supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% nonessential amino acids, 1% antibiotic/antimycotic [10,000 units/ml of penicillin, 25 μg/ml of amphotericin K, and 10,000 μg/ml of streptomycin], 1.5% Hepes, 1.5% sodium bicarbonate, and 1% minimal essential medium vitamins). Green monkey kidney (Vero) cells were grown in RPMI 1640 supplemented as described above. Cells were grown and maintained at 37°C in a humidified 5% CO_2 atmosphere.

Virus. Herpes simplex virus type 2 (strain HSV-FMC-P180) was isolated from the cervical tissue of a gynecological patient with carcinoma *in situ* at the Medical College of Virginia, Virginia Commonwealth University (Richmond, VA). The virus was plaque-purified and was typed as HSV2 by partial restriction endonuclease digestion, as described previously (23).

Virus stocks were prepared by infecting confluent monolayers of Vero cells at a multiplicity of infection of 0.01 and harvesting the virus when 90% of the cells manifested typical HSV-induced cytopathic effect. Virus titer was determined by plaque assay (24) using Vero cells grown in 24-well tissue culture plates (Corning, Inc., Corning, NY). A virus stock with a titer of 1×10^8 plaque-forming units (PFU) per milliliter was employed in these studies.

THC Preparation. THC (FW = 316) was obtained from the National Institute on Drug Abuse, Rockville, MD. THC was prepared from a stock solution of 100 mg/ml in 95% ethanol. The stock solution was appropriately diluted in complete RPMI 1640 medium such that 10 μl was added per milliliter of medium to yield final concentrations of 10^{-5} *M*, 10^{-6} *M*, or 10^{-7} *M* THC and 0.1% ethanol. The vehicle consisted of 10 μl of 9.5% ethanol per milliliter of RPMI 1640 medium to yield a final concentration in medium of 0.1% ethanol. Placebo cultures consisted of RPMI 1640 medium inoculated with 10 μl/ml of the same medium.

***In Vitro* Exposure of Macrophages to THC.** For assessment of the effect of THC on intrinsic antiviral activity, confluent monolayers of macrophages in 24-well cluster plates (5×10^5 cells/well) were pretreated with THC (10^{-5} *M*, 10^{-6} *M*, or 10^{-7} *M*), vehicle (0.1% ethanol), or placebo (medium only) for 48 hr. Following washing with sterile phosphate-buffered saline (PBS), the macrophage-like cells were exposed to HSV2 and were evaluated for intrinsic antiviral activity. For assessment of the effect of THC on macrophage extrinsic antiviral activity, macrophages in 75-cm^2 culture flasks were exposed to THC (10^{-5} *M*, 10^{-6} *M*, or 10^{-7} *M*), vehicle, or placebo for 48 hr. Alternatively, macrophages were exposed to the drug or vehicle for 48 hr and then were maintained in THC-free medium for 48 or 120 hr. Then, cells were scraped into the medium with a rubber policeman, assessed for viability by trypan blue exclusion, enumerated, and added to virus-infected Vero cell monolayers for assessment of extrinsic antiviral activity.

Assay of Intrinsic Antiviral Activity. Following exposure to THC, vehicle, or placebo, macrophage-like cells were washed with sterile PBS, and a 0.1-ml inoculum calculated to contain 50 PFU of HSV2 was added to each confluent macrophage monolayer in 24-well tissue culture plates. Replicate cultures containing Vero cell monolayers were inoculated with an equal volume of HSV2 in order to quantitate the number of input infectious virus on the basis of plaques elicited. Macrophages were adsorbed with HSV2 for 1, 2, 3, 4, 24, and 48 hr. At the end of each of the designated time periods, the supernatant from each culture was removed and the corresponding cell monolayer was lysed by three rapid freeze-thaw cycles. The corresponding supernatant and cell lysate of each macrophage culture were quantitated for infectious virus by plaque assay.

Assay of Extrinsic Antiviral Activity. Confluent Vero cell monolayers (5×10^5 cells) in 24-well cluster plates were inoculated with a 0.1-ml vol of HSV2 such that each of the first two columns of wells received 500 PFU. The remaining four columns of wells received 250, 125, 60, or 30 PFU, respectively. Following incubation for 1.5 hr at 37°C in a 5% CO_2 environment to allow for virus adsorption, the Vero cell monolayers were washed three times with sterile PBS (37°C) to remove unadsorbed virus. Then, suspensions (1 ml) of placebo-, vehicle-, or THC-treated macrophages were added to the Vero cell monolayers such that the four rows of wells contained macrophage to Vero cell cocultures at effector cell to target cell (E:T) ratios of 5:1, 3:1, and 1:1. Duplicate plates contained wells with virus-infected (500 PFU to 30 PFU) Vero cells or with uninfected Vero cells in the absence of macrophages. Additionally, uninfected Vero cells were cocultured with macrophages at E:T ratios of 5:1 to 1:1 to monitor for potential macrophage-mediated cytotoxicity against the Vero cells. Trypan blue exclusion staining demonstrated that the macrophage lines did not effect cytolysis of uninfected Vero cells. Following the addition of macrophages, the culture plates were centrifuged (800 rpm, 15 min) to allow for effector cell to target cell

contact and 1 ml of 2% methylcellulose in complete RPMI 1640 medium was added to each well. After 3 days of incubation at 37°C in a 5% CO_2 environment, the plates were fixed with 4% formaldehyde in PBS, stained with 0.5% crystal violet, and enumerated for virus plaques. Each series of experiments was performed six times. The number of plaques elicited in HSV2-infected Vero cell monolayers in the absence of macrophages was designated as 100%. Extrinsic antiviral activity was measured by calculating the percentage of plaques elicited in macrophage to HSV2-inoculated Vero cell cocultures when compared with similarly HSV2-inoculated Vero cell monolayers maintained in the absence of macrophages. The lower the percentage of plaques noted in cocultures, the greater the extrinsic macrophage antiviral activity.

Statistics. Virus titration data were expressed as $\log_{10}$. Means ± SE were obtained from data from the individual cultures. Student's two-tailed t test was employed to determine statistical significance of the data (25).

Table I. *In Vitro* Effect of THC on J774A.1 Macrophage Intrinsic Antiviral Activity

	Treatment[a]				
Time (hr)	Placebo[b]	VH	10^{-5} MTHC	10^{-6} MTHC	10^{-7} MTHC
1	28 (14)[c]	28 (12)	27 (18)	27 (16)	26 (16)
2	28 (13)	24 (12)	17 (15)	20 (14)	24 (15)
3	27 (13)	21 (11)	15 (13)	15 (12)	21 (14)
4	20 (9)	20 (11)	15 (10)	13 (9)	20 (10)
24	14 (4)	14 (9)	12 (10)	12 (9)	13 (10)
48	12 (2)	10 (4)	12 (5)	8 (5)	11 (4)

[a] J774A.1 macrophages were treated with THC, vehicle, or placebo for 48 hr, washed, and inoculated with approximately 50 PFU of HSV2. The number of input PFU was corroborated by counting plaques elicited on Vero cell control monolayers following their exposure to the same inoculum. At each of the indicated times after virus inoculation, the supernatant and monolayer lysate from each culture were recovered and were assessed for infectious virus by plaque assay.
[b] Placebo indicates medium only.
[c] Values denote PFU elicited by the culture supernatant. Values in parentheses denote PFU produced by the cell lysate from the corresponding culture.

Results

Effect of THC on Intrinsic Antiviral Activity. Virus uptake experiments were performed in order to directly evaluate the effect of THC on intrinsic antiviral activity. Infectious virus in each macrophage culture supernatant and corresponding cell lysate were quantitated by plaque assay. Virus plaques produced from the cell lysates served as a measure of infectious virus taken up by macrophages at the designated time periods. In addition, these plaques served as a measure of virus "survival" within the recipient macrophages. In contrast, virus plaques elicited by the culture supernatants (1–4 hr postinoculation) served as a measure of input infectious virus not taken up by the macrophages. Plaques elicited by the 24-hr or 48-hr postinoculation culture supernatants served as a measure of productive virus infection within macrophages, since by that time, $\log_{10}$-fold amounts of HSV2 are released into the culture medium by virus-permissive cells. Results from a representative experiment of the effect of THC on intrinsic antiviral activity of J774A.1 macrophages are listed in Table I. Plaque assay of control Vero cell monolayers yielded 45–55 PFU following inoculation with a 0.1-ml vol of virus suspension calculated to contain 50 PFU. At 1 hr after inoculation, the sum of infectious virus in the culture supernatants and corresponding cell monolayers, regardless of treatment regimen, ranged from 40 to 45 PFU. Thus, each culture was inoculated with a comparable amount of infectious virus. Lysates of vehicle-treated J774A.1 cells yielded 12 plaques following a 1-hr absorption period. By 48 hr after the virus inoculation, lysates of the J774A.1 cells yielded only four plaques. A parallel decrease in infectious virus from culture supernatants accompanied the decrease in infectious intracellular virus. By 24 hr, culture supernatants plus cell lysates yielded a total of 23 PFU. These results indicate that within the first 4 hr, approximately 50% of the input virus was taken up and degraded by the macrophages. By 48 hr after inoculation, approximately 30% of the total original input infectious virus remained in the macrophage cultures. Thus, the input virus was readily taken up and degraded by the macrophages. A similar temporal reduction in infectious virus was observed for J774A.1 cells treated with THC (10^{-5}–10^{-7}). Even after exposure to 10^{-5} M THC, macrophage intrinsic anti-HSV2 activity remained unaffected. At 1 hr after inoculation, cell lysates yielded 18 PFU of HSV2. By 48 hr after inoculation, cell lysates produced only 5 PFU. A temporal decrease in extracellular virus from the culture supernatants also was recorded (e.g., 27 PFU at 1 hr after inoculation versus 12 PFU at 48 hr after inoculation). Similar results were obtained when P388D_1 macrophages were employed in the intrinsic antiviral studies (data not shown). Thus, these results indicate that THC in the doses employed did not affect virus uptake by macrophages. Furthermore, the macrophages remained nonpermissive for HSV2 replication.

Effect of THC on Extrinsic Antiviral Activity. The effect of THC on extrinsic antiviral activity of P388D_1, RAW264.7, and J774A.1 macrophage-like cells was assessed. Macrophages were incubated with THC (10^{-5} M, 10^{-6} M, or 10^{-7} M), vehicle, or placebo for 48 hr and then were cocultured with virus-inoculated Vero cell monolayers at effector cell to target cell ratios of 5:1, 3:1, and 1:1. Phase contrast microscopy and Nomarski optics microscopy demonstrated that THC

treatment of macrophages had no effect on the attachment of macrophages to the virus-infected cells. Macrophage extrinsic antiviral activity was measured on the basis of suppression of plaque formation in macrophage to HSV2-infected Vero cell cocultures when compared with plaque formation in similarly HSV2-infected Vero cell monolayers maintained in the absence of macrophages.

Figure 1 illustrates the results of a typical experiment for assessing the effect of THC on macrophage extrinsic antiviral activity. Cocultures of vehicle-treated $P388D_1$ macrophages and HSV2-inoculated Vero cells at E:T ratios of 5:1 yielded a minimal number of virus plaques (Fig. 1A). Even at an E:T ratio of 1:1, extrinsic antiviral activity was exerted, since less than 50% of the input virus elicited plaques. In contrast, decreased extrinsic antiviral activity was evident for cocultures

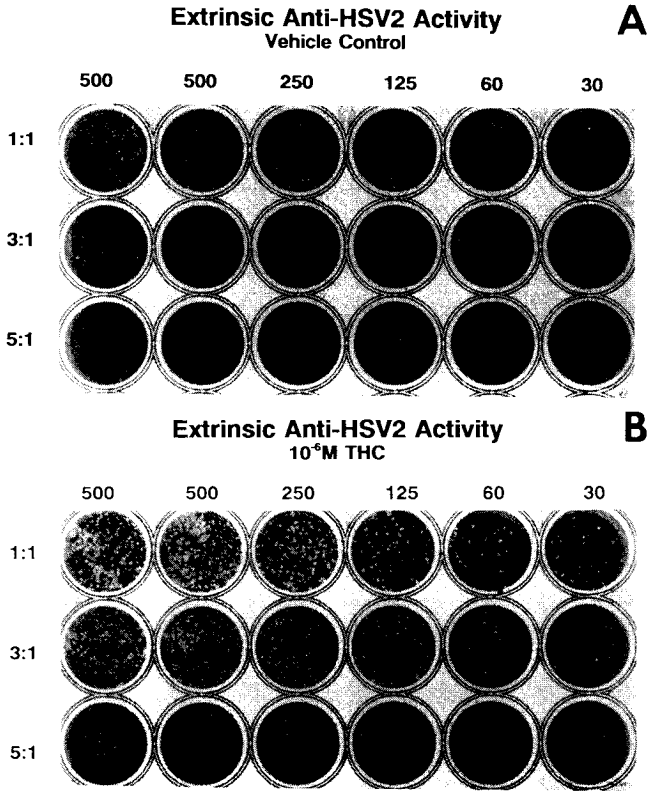

Figure 1. Effect of THC on macrophage extrinsic antiviral activity. $P388D_1$ macrophage-like cells were treated with (A) vehicle or with (B) 10^{-6} *M* THC for 48 hr. Then, macrophages were added to HSV2-infected Vero cell monolayers to yield effector cell to target cell (E:T) ratios of 5:1, 3:1, and 1:1. The number over each column designates the calculated number of PFU of HSV2 added to each Vero cell monolayer in that column. The number assigned to each row designates the macrophage to Vero cell E:T ratio for each row. (A) Cocultures containing $P388D_1$ macrophages treated with vehicle. There is a decrease in the number of virus plaques in direct correlation with increasing E:T ratios. At an E:T ratio of 5:1, a minimal number of plaques was elicited in all of the cocultures, regardless of the input number of infectious HSV2. (B) Cocultures containing $P388D_1$ macrophages pretreated (48 hr) with 10^{-6} *M* THC. Note the increase in the number of plaques in all cocultures at all E:T ratios, indicating a decrease in extrinsic antiviral activity.

containing macrophages exposed to THC (Fig. 1B). Cocultures at the highest E:T ratio (e.g., 5:1) expressed a much greater number of virus plaques than the comparable cocultures containing vehicle-treated macrophages. Cocultures at E:T ratios of 5:1, 3:1, and 1:1 expressed a greater than 2-fold increase in plaques when compared with cocultures containing vehicle-treated macrophages.

Figure 2 illustrates quantitatively the effect of THC on macrophage extrinsic antiviral activity. Vero cell control monolayers were inoculated with aliquots of virus suspension calculated to contain 120, 60, or 30 PFU of HSV2. The respectively infected monolayers, maintained in the absence of macrophages, were designated as producing the maximal number of virus plaques (i.e., 100%). The percentage of virus plaques elicited in cocultures of macrophages and virus-inoculated Vero cells, then, was determined by comparison to the 100% yield of the corresponding virus-inoculated Vero cell monolayer. Infected Vero cells cocultured with J774A.1 macrophages that were treated with placebo or vehicle yielded a minimal number of plaques when compared with HSV2-inoculated Vero cell control monolayers, indicating that the J774A.1 cells exerted extrinsic antiviral activity. Cocultures containing vehicle-treated or placebo-treated macrophages, and maintained at E:T ratios of 5:1 and 3:1, yielded less than 30% the number of plaques recorded for HSV2-infected Vero cell controls. Even at the lowest E:T ratio (1:1), macrophage extrinsic antiviral activity was exerted by placebo-treated and vehicle-treated cells, since cocultures yielded less than 58% of the plaques noted in control HSV2-infected Vero cell cultures. In contrast, cocultures containing THC-treated J774A.1 macrophages and HSV2-infected Vero cells yielded a dose-related increase in viral plaques indicative of a dose-related decrease in macrophage extrinsic anti-HSV2 activity. At E:T ratios of 1:1, THC eliminated nearly all of the macrophage extrinsic antiviral activity. Indeed, cocultures containing macrophages treated with 10^{-5} *M* THC yielded greater than 90% the number of plaques recorded for monolayers of control HSV2-infected Vero cells.

Similar results were obtained when RAW264.7 macrophages were employed as effector cells. At E:T ratios of 5:1 and 3:1, a minimal number of plaques was elicited in cocultures containing macrophages treated with vehicle or placebo (i.e., less than 40%). In contrast, a drug dose-related increase in plaque formation was noted at all E:T ratios for cocultures containing macrophages treated with 10^{-5} *M* through 10^{-7} *M* THC. At E:T ratios of 3:1 and 1:1, cocultures containing macrophages treated with 10^{-5} *M* or 10^{-6} *M* THC expressed greater than 70% the number of plaques observed in control HSV2-infected Vero cell cultures, indicating a loss of most extrinsic macrophage antiviral activity.

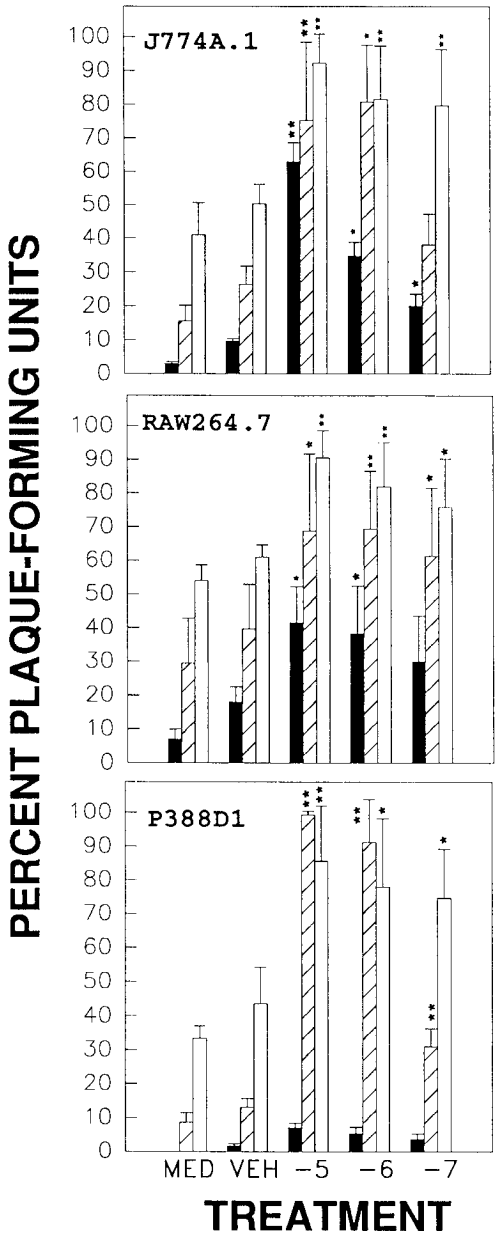

Figure 2. Effect of THC on macrophage extrinsic antiviral activity. J774A.1, RAW264.7, and P388D$_1$ macrophage-like cells were treated with THC (10^{-5}–10^{-7} *M*), vehicle, or placebo for 48 hr. Vero cell monolayers were inoculated with 60 PFU of HSV2. Macrophages, then were added to the Vero cell monolayers to yield E:T ratios of 5:1, 3:1, and 1:1. The ordinate represents the percentage of plaques elicited in macrophage to Vero cell cocultures when compared with the number of plaques elicited in HSV2-inoculated Vero cell monolayers not containing macrophages. Each bar designates the average of six experiments. The black, hatched, and white bars represent E:T ratios of 5:1, 3:1, and 1:1, respectively. The asterisks designate significant (* $P < 0.1$ or ** $P < 0.05$, respectively; Student's *t* test) increases in the percentage of plaques formed for each E:T ratio when compared with the matching E:T ratio of cocultures containing vehicle-treated macrophages. A drug dose-related increase in the relative percentage of plaques, indicative of a decrease in extrinsic anti-HSV2 activity, was noted for macrophages treated with THC (10^{-5}–10^{-7} *M*). MED, medium; VEH, vehicle; -5, -6, or -7 represent 10^{-5} *M*, 10^{-6} *M*, or 10^{-7} *M* THC, respectively.

This decrease was also noted for macrophages that were treated with 10^{-7} *M* drug and then added to HSV2-infected Vero cells at an E:T ratio of 1:1.

Also, THC was shown to diminish the extrinsic antiviral activity of the P388D$_1$ macrophage-like cells. Cocultures of placebo-treated or vehicle-treated P388D$_1$ macrophages and HSV2-infected Vero cells at E:T ratios of 5:1 and 3:1 produced less than 20% the number of PFU produced by HSV2-infected Vero cell monolayers. Even at an E:T ratio of 1:1, less than 50% the number of plaques recorded for virus-infected Vero cell cultures was noted for cocultures containing P388D$_1$ cells treated with placebo or vehicle. THC exerted a dose-related increase in the percentage of virus plaques formed in cocultures with E:T ratios of 3:1 and 1:1 when compared with HSV2-infected Vero cell monolayers maintained in the absence of macrophages, indicating a decrease in macrophage extrinsic anti-HSV2 activity. Macrophages treated with 10^{-5} *M* or 10^{-6} *M* THC, and cocultured with HSV2-infected Vero cells at E:T ratios of 3:1 or 1:1, expressed greater than 70% the number of PFU expressed in HSV2-infected Vero cell cultures alone. However, at an E:T ratio of 5:1 THC exerted no major effect on macrophage extrinsic antiviral activity. For these latter cocultures, less than 10% the number of plaques noted for Vero cell monolayers was recorded.

Temporal Effect of THC Exposure on Macrophage Extrinsic Antiviral Activity. To assess whether macrophages treated *in vitro* with THC could recover their extrinsic antiviral activity following removal of the drug, an additional set of experiments was performed in which macrophages were maintained in THC-free medium for 48 hr or 120 hr following the 48-hr drug exposure period. The macrophages, then, were added to HSV2-infected Vero cell monolayers and were assessed for extrinsic antiviral activity. A time-dependent recovery of extrinsic anti-HSV2 activity by the macrophage-like cells was recorded. Results of the effect of drug removal on the recovery of extrinsic antiviral activity of J774A.1 macrophages at an E:T ratio of 3:1 are shown in Fig. 3. As expected, cocultures containing macrophages treated with vehicle elicited a minimal number of plaques at all time periods. These observations indicate that the J774A.1 cells exerted extrinsic antiviral activity throughout the experimental period. In agreement with previous experiments, cocultures containing macrophages treated with THC for 48 hr exhibited a dose-related increase in the percentage of plaques formed, indicating a dose-related decrease in extrinsic macrophage antiviral activity. Cocultures containing J774A.1 macrophages, regardless of the dose of drug exposure, maintained in drug-free medium for 48 hr yielded less than 40% the number of plaques that was recorded for HSV2-infected Vero cell monolayers cultured in the absence of macrophages. Cocultures

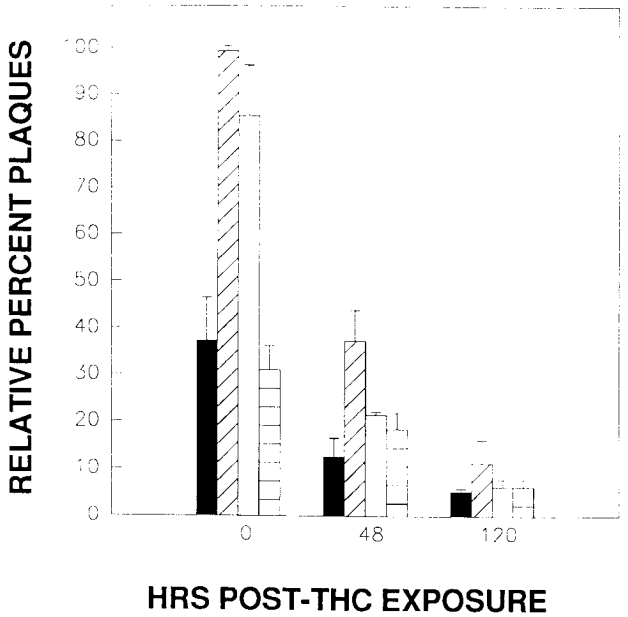

Figure 3. Recovery of extrinsic anti-HSV2 activity by murine J774A.1 macrophage-like cells. Macrophages were treated with THC (10^{-5}–10^{-7} *M*), vehicle, or placebo for 48 hr. The macrophages, then, were incubated in THC-free medium for either 48 hr or 120 hr. Approximately 60 PFU of HSV2 were added to each target Vero cell monolayer. The ordinate designates the percentage of plaques elicited by macrophage to Vero cell cocultures (E:T ratio, 3:1) when compared with plaques elicited in Vero cell monolayers not containing macrophages and inoculated with 60 PFU. A time-related decrease in plaque formation, indicative of a recovery of extrinsic anti-HSV2 activity, was observed for macrophages pretreated with 10^{-5}–10^{-7} *M* THC. The bars are representative of the following: ■, vehicle; ▨, 10^{-5} *M* THC; □, 10^{-6} *M* THC; ▤, 10^{-7} *M* THC.

containing macrophages maintained in drug-free medium for 120 hr exhibited less than 15% the number of plaques recorded for virus-infected Vero cell monolayers. These observations indicate a nearly total recovery of extrinsic antiviral activity by the THC-pretreated macrophages at all E:T ratios.

Discussion

Macrophages exert two important antiviral functions, intrinsic antiviral activity and extrinsic antiviral activity, in addition to their ability to bring about effector cell to target cell contact-dependent lysis of virus-infected cells (26-29). In intrinsic antiviral activity, macrophages engulf and degrade virus and thereby remain nonpermissive for virus replication (18–21, 30). In extrinsic antiviral activity, macrophages inhibit virus replication within infected target cells in a cell contact-dependent, interferon-independent manner (22, 31).

Macrophage cell lines were employed in this study, since they are free of contaminating other cell types and can be cultivated to relatively high numbers. These cell lines differ in degree of macrophage maturity and functional properties. The J774A.1, P338D_1, and RAW264.7 cells represent relatively "mature" macrophage-like cells and exhibit many of the macrophage-virus interactions attributable to primary macrophages. For example, J774A.1 cells characteristically produce lysozyme and superoxide radicals, which are important components of the intrinsic antiviral process (32). P388D_1 cells express Fc receptors and support growth of Influenza A NWS (H1N1) Strain (33) and West Nile virus (34) consequent to virus-antibody complex interaction with these receptors. Both J774A.1 and P388D_1 cells have been shown to produce acid-stable interferon when inoculated with Newcastle disease virus (35).

In the present investigation, THC was shown to have no effect on macrophage intrinsic antiviral activity. An overall reduction in infectious virus in both culture supernatants and cell lysates was recorded for macrophages pretreated with doses of THC as high as 10^{-5} *M*. In addition, there was no significant difference in virus uptake by macrophages treated with THC, as compared with macrophages treated with vehicle or placebo. These results indicate that THC causes neither a quantitative nor a temporal effect on HSV2 uptake by macrophages. Furthermore, the J774A.1 cells did not support productive virus infection, regardless of the drug treatment regimen. Thus, THC exposure did not alter or inhibit those cellular compartments that effect macrophage nonpermissiveness for virus replication. Sarmiento (30) has shown that macrophage intrinsic resistance to HSV1 infection is due to restriction of viral macromolecular synthesis. HSV replication was found to be inhibited in macrophages at multiple points in the virus growth cycle before the onset of virus DNA synthesis. The results of this study indicate that THC does not target those macrophage factors that are involved in restriction of HSV macromolecular synthesis. Similar results were obtained when P388D_1 cells were employed in the HSV2 uptake experiments, indicating that the failure of THC to alter intrinsic macrophage antiviral activity was not limited to the J774A.1 cells.

In contrast, THC affected the capacity of macrophages to exert extrinsic antiviral activity. The mechanism by which macrophages exert this activity remains unresolved, although some aspects have been defined. Morahan *et al.* (31) indicated that the extrinsic antiviral mechanism does not involve direct virus inactivation by extrinsic factors. Neither macrophage supernatants nor lysates alone inactivated virus infection, suggesting an effector cell to target cell contact-dependent mechanism. Furthermore, the extrinsic antiviral activity was unlike interferon-mediated inhibition, since it was exerted against virus-infected xenogeneic cells (36). Furthermore, Vero cells, which were used as targets in these experiments, have been shown not to produce interferon (37). Thus, the macrophage extrinsic antiviral activity was not species specific, in that the macrophages serving as effectors were of murine origin, while the virus-infected target cells (i.e., Vero cells) were of primate origin. Other secretory products of macro-

phages, such as interleukin 1 (38–41) and tumor necrosis factor (42–45), have been shown to possess antiviral properties. However, the action of these molecules is neither dependent on effector cell to target cell conjugation nor on cell contact, as demonstrated for extrinsic antiviral activity (36). Thus, it seems unlikely that interferon, interleukin 1, or tumor necrosis factor accounts for the majority, if any, of the extrinsic antiviral activities attributed to macrophages. Nevertheless, other secretory products, such as neutral proteases and prostaglandins, could be responsible for nonspecific antiviral effects of macrophages on virus or virus-infected cells.

THC did not prevent any of the macrophage cell types from attaching to the virus-infected xenogeneic Vero cells. These results are in agreement with our previous studies, which demonstrated that THC, injected intraperitoneally into mice that were administered *Propionibacterium acnes,* did not prevent peritoneal macrophages from attaching to virus-infected cells or to tumor cells (46). Similarly, macrophages treated *in vitro* with 10^{-5} *M* to 10^{-7} *M* THC remained unaffected in their ability to attach to target cells (46). Thus, these observations indicate that THC affects macrophage extrinsic antiviral activity at a step other than effector cell to target cell attachment. The mechanism by which THC effects this inhibition, however, remains unresolved. THC has been shown to affect macromolecular synthesis in a variety of cellular systems (46–50). We have shown that the cannabinoid inhibits differential expression of macrophage proteins in response to external stimuli, such as bacterial lipopolysaccharide and *P. acnes* (10). These observations suggest that THC may alter extrinsic antiviral activity by suppressing macromolecular synthesis or expression by macrophages. Alternatively, THC may bring about a morphological disruption of cellular membranes (51, 52) or of cytoskeletal elements (53). Perturbations of cellular membranes could alter cellular compartments of protein synthesis or posttranslational events and thereby suppress the synthesis of these macrophage factors, which block virus replication at the level of expression of the early to delayed-early virus genes. It has been proposed that an intact cytoskeletal system is requisite for natural killer cell and for cytotoxic T lymphocyte cell contact-dependent killing of target cells. Following conjugation of the effector cell to the target cell, a rapid reorientation of the microtubular organizing center and Golgi apparatus complex occurs within the target cell. This orientation is effected toward the target cell and results in the direction of secretory vesicles containing cytolytic components along the cytoskeletal tracts to the target cell (54, 55). Thus, THC, in addition to suppressing protein synthesis, could bring about a disruption of cytoskeletal elements within the effector cell with the consequent failure in transport of effector molecules to the virus-infected cell. We have shown that THC administered *in vitro* elicits membrane perturbation of cell surface and cytoplasmic membranes in rat neuroblastoma cells (53).

The THC-induced inhibition of macrophage extrinsic antiviral activity was shown to be reversible. J774A.1 and P388D_1 macrophages treated with THC for 48 hr, and then incubated in drug-free medium for 48 or 120 hr, regained extrinsic antiviral activity in a time-dependent fashion. These observations are indicative of an ongoing "repair" process by which macrophages gradually regain their functional competence against virally infected cells following removal of THC from the environment. The results showing the reversible effects of THC on macrophage extrinsic antiviral activity may be attributed to macrophage regeneration and the "sloughing off" of putatively drug-damaged cell membranes. A similar process has been shown to occur in rat neuroblastoma cells (53). Ejection of membranous structures was most evident when cells were treated *in vitro* with 10^{-5} *M* and 10^{-6} *M* THC. The rat B103 neuroblastoma cells were shown to express surface blebs following exposure to drug. Scanning electron microscopy revealed extracellular globular membranous bodies surrounding the drug-exposed cells. In addition, transmission electron microscopy demonstrated cytoplasmic membranous inclusions seemingly in the process of extrusion from the drug-treated cells. Similar extruded globular structures and surface blebs were observed by Nomarski optics microscopy for macrophages treated *in vitro* with THC, especially at higher drug doses (i.e., 10^{-5} *M*). While these effects elicited by 10^{-5} *M* THC could represent the outcome of exposure to a relatively high drug concentration, they nevertheless may depict the upper limit of a continuum of THC-induced effects that bring about repair of drug-damaged macrophage membranes. The elimination of damaged membranes following removal of the drug from the extracellular environment may allow for cells to regenerate cellular membranes with a consequent temporal-dependent reestablishment of extrinsic antiviral activity.

The THC doses that were employed in this *in vitro* study are achievable in humans. Agurell *et al.* (56) have shown that serum THC peaks at concentrations as high as 500 ng/ml within 30 min of smoking marijuana. This peak period is followed by an equilibrium phase, during which concentrations of THC may range from 0.5 ng/ml to 0.25 ng/ml in 1–2 days. Wall *et al.* (57) measured baseline levels of THC of approximately 1 ng/ml in regular marijuana users. The rapid early decline of THC in plasma is a consequence of extensive metabolism of the drug (58) and of uptake by tissues due to its highly lipophilic nature (59). For example, relatively high concentrations of THC could be antici-

pated in lung alveolar macrophages as a consequence of direct exposure to marijuana smoke.

The authors thank Dr. Louis Harris and Dr. Billy Martin of the Department of Pharmacology and Toxicology for kindly providing Δ^9-tetrahydrocannabinol. This research was supported by Grants R01 DA03647 and R01 DA05832 from the National Institute on Drug Abuse.

1. Nahas GG, Sucia-Foca N, Armand SP, Morishima A. Inhibition of cellular mediated immunity in marihuana smokers. Science **183**:419–420, 1974.
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. Gupta G, Grieco M, Cushman P. Impairment of rosette-forming T-lymphocytes in chronic marihuana smokers. N Engl J Med **291**:874–876, 1974.
4. Cushman P, Khurana R. Marijuana and T-lymphocyte rosettes. Clin Pharmacol Ther **19**:310–317, 1976.
5. Specter SC, Klein TW, Newton C, Mondragon M, Widen R, Friedman H. Marijuana effects on immunity: Suppression of human natural killer cell activity by delta-9-tetrahydrocannabinol. Int Soc Immunopharmacol **8**:741–745, 1986.
6. Klein TW, Newton C, Friedman H. Inhibition of natural killer cell function by marijuana components. J Toxicol Environ Health **20**:321–332, 1987.
7. Schwartzfarb L, Needle N, Chavez-Chase M. Dose related inhibition of leukocyte migration by marihuana and delta-9-tetrahydrocannabinol. J Clin Pharmacol **14**:35–41, 1974.
8. Mann PEG, Cohen AB, Finley TN, Ladman AJ. Alveolar macrophages. Structural and functional differences between nonsmokers and smokers of marijuana and tobacco. Lab Invest **25**:111–120, 1971.
9. Lopez-Cepero M, Friedman M, Klein T, Friedman J. Tetrahydrocannabinol-induced suppression of macrophage spreading and phagocytic activity *in vitro*. J Leukocyte Biol **39**:679–686, 1986.
10. Cabral GA, Mishkin EM. Delta-9-tetrahydrocannabinol inhibits macrophage protein expression in response to bacterial immunomodulators. J Toxicol Environ Health **26**:175–182, 1989.
11. 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.
12. Cabral GA, Mishkin EM, Marciano-Cabral F, Coleman P, Harris L, Munson AE. Effect of delta-9-tetrahydrocannabinol on herpes simplex virus type 2 vaginal infection in the guinea pig. Proc Soc Exp Biol Med **182**:181–186, 1986.
13. Mackaness GB. The immunological basis of acquired cellular resistance. J Exp Med **120**:105–112, 1964.
14. Unanue ER. The regulatory role of macrophages in antigenic stimulation. Part 2: Symbiotic relationship between lymphocytes and macrophages. Adv Immunol **31**:1–136, 1981.
15. Howie S, MacBride WH. Cellular interactions and thymus-dependent antibody responses. Immunol Today **3**:273–278, 1982.
16. Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells. In: Nelson DS, Ed. Immunobiology of the Macrophage. New York: Academic Press, p535, 1976.
17. Probert M, Stott EJ, Thomas LH. Interactions between calf alveolar macrophages and parainfluenza-3 virus. Infect Immun **15**:576–585, 1977.
18. Johnson RT. The pathogenesis of herpesvirus encephalitis. II: A cellular basis for the development of resistance with age. J Exp Med **120**:359–374, 1964.
19. Stevens JG, Cook ML. Restriction of herpes simplex virus by macrophages. An analysis of the cell-virus interaction. J Exp Med **133**:19–38, 1971.
20. Selgrade MK, Osborn JE. Role of macrophages in resistance to murine cytomegalovirus. Infect Immun **10**:1383–1390, 1974.
21. Mims GA, Gould J. The role of macrophages in mice infected with murine cytomegalovirus. J Gen Virol **41**:143–153, 1978.
22. Morahan PS, Glasgow LA, Crane JL, Kern ER. Comparison of antiviral and antitumor activity of activated macrophages. Cell Immunol **18**:404–415, 1977.
23. Hamelin C, Chagnon A, Farwell M. Identification of atypical strains of herpes simplex virus using a simplified DNA finger printing method. Microbiol Immunol **28**:723–729, 1984.
24. Rapp F. Variants of herpes simplex virus: Isolation, characterization, and factors influencing plaque formation. J Bacteriol **86**:985–991, 1963.
25. Snedecor GW, Cochran WG. Statistical Methods (6th Ed.). Ames, IA: Iowa State University Press, chap. 4, 1967.
26. Stanwick TL, Campbell DE, Nahmias AJ. Spontaneous cytotoxicity mediated by human monocyte-macrophages against human fibroblasts infected with herpes simplex virus-augmentation by interferon. Cell Immunol **53**:413–416, 1980.
27. Koff WC, Showalter SD, Seniff DA, Hampar B. Lysis of herpesvirus-infected cells by macrophages activated with free or liposome-encapsulated lymphokine produced by a murine T cell hybridoma. Infect Immun **42**:1067–1072, 1983.
28. Leblanc PA. Macrophage activation for cytolysis of virally infected target cells. J Leukocyte Biol **45**:345–352, 1989.
29. Leblanc PA, Heath LS, Um H. Activated macrophages use different cytolytic mechanisms to lyse a virally infected or a tumor target. J Leukocyte Biol **48**:1–6, 1990.
30. Sarmiento M. Intrinsic resistance to viral infection. Mouse macrophage restriction of herpes simplex virus replication. J Immunol **141**:2740–2748, 1988.
31. Morahan PS, Morse SS, McGeorge MB. Macrophage extrinsic antiviral activity during herpes simplex virus infection. J Gen Virol **46**:291–300, 1980.
32. Damiani G, Kiyotaki C, Soeller W, Sasada M, Peisach J, Bloom BR. Macrophage variants in oxygen metabolism. J Exp Med **152**:808–822, 1980.
33. Ochiai H, Kurokawa M, Hayashi K, Niwayama S. Antibody-mediated growth of influenza A NWS virus in macrophagelike cell line P388D1. J Virol **62**:20–26, 1988.
34. Cardosa MJ, Gordon S, Hirsch S, Springer TA, Porterfield JS. Interaction of West Nile virus with primary murine macrophages: Role of cell activation and receptors for antibody and complement. J Virol **57**:952–959, 1986.
35. Salo RJ, Bleam DK, Greer VL, Ortega AP. Interferon production in murine macrophage-like cell lines. J Leukocyte Biol **37**:395–406, 1985.
36. Morse SS, Morahan PS. Activated macrophages mediate interferon-independent inhibition of herpes simplex virus. Cell Immunol **58**:72–84, 1981.
37. Desmyter J, Melnick JL, Rawls WE. Defectiveness of interferon production and of rubella virus interference in a line of African green monkey kidney cells (Vero). J Virol **2**:955–961, 1968.
38. Van Damme J, De Ley M, Van Snick J, Dinarello CA, Billiau A. The role of interferon-beta 1 and the 26-kDa protein (interferon-beta 2) as mediators of the antiviral effect of interleukin 1 and tumor necrosis factor. J Immunol **139**:1867–1872, 1987.
39. Keller F, Schmitt C, Kerin A. Interaction of mouse hepatitis virus 3 with Kupffer cells explanted from susceptible and resistant mouse strains. Antiviral activity, interleukin-1 synthesis. Microbiol Immunol **1**:87–95, 1988.
40. Van der Meer JW, Rubin RH, Pasternack M, Medearis DN,

Lynch P, Dinarello CA. The *in vivo* and *in vitro* effects of interleukin-1 and tumor necrosis factor on murine cytomegalorivus infection. Biotherapy **1**:227–231, 1989.
41. Ruggiero V, Antonelli G, Gentile M, Conciatori G, Dianzani F. Comparative study on the antiviral activity of tumor necrosis factor (TNF)-alpha, lymphotoxin/TNF-beta, and IL-1 in WISH cells. Immunol Lett **21**:165–169, 1989.
42. Mestan J, Digel W, Mittnacht S, Hillen H, Blohm D, Moller A, Jacobsen H, Kirchner H. Antiviral effects of recombinant tumour necrosis factor *in vitro*. Nature **323**:816–819, 1986.
43. Koff WC, Fann AV. Human tumor necrosis factor alpha kills herpes virus-infected normal cells. Lymphokine Res **5**:215–221, 1986.
44. Paya CV, Kenmotsie N, Schoon RA, Leibson PJ. Tumor necrosis factor and lymphotoxin secretion by human natural killer cells to antiviral cytotoxicity. J Immunol **141**:1989–1995, 1988.
45. Duerksen-Hughes P, Wold WS, Gooding LR. Adenovirus E1A renders infected cells sensitive to cytolysis by tumor necrosis factor. J Immunol **143**:4193–4200, 1989.
46. Cabral GA, Vasquez R. Effects of marihuana on macrophage function. In: Friedman H, Specter S, Klein TW, Eds. Advances in Experimental Medicine and Biology: Vol. 288, Drugs of Abuse, Immunity, and Immunodeficiency. New York: Plenum Press, pp93–105, 1991.
47. Blevins RD, Regan JD. Delta-9-tetrahydrocannabinol: Effect on macromolecular synthesis in human and other mammalian cells. Arch Toxicol **35**:127–135, 1976.
48. Nahas GG, Morishima A, Desoize B. Effect of cannabinoids on macromolecular synthesis and replication of cultured lymphocytes Fed Proc **36**:1748–1752, 1977.
49. Mon JJ, Jansing RL, Doggett S, Stein JL, Stein GS. Influence of delta-9-tetrahydrocannabinol on cell proliferation and macromolecular synthesis in human cells. Biochem Pharmacol **21**:1759–1765, 1978.
50. Desoize B, Leger C, Nahas GG. Plasma membrane inhibition of macromolecular precursor transport by THC. Biochem Pharmacol **28**:1113–1118, 1979.
51. Poddar MK, Mittra G, Ghosh JJ. Delta-9-tetrahydrocannabinol-induced changes in brain ribosomes. Toxicol Appl Pharmacol **46**:737–757, 1978.
52. Meyers WA, Heath RG. *Cannabis sativa*: Ultrastructural changes in organelles and neurons in brain septal region of monkeys. J Neurosci Res **4**:9–17, 1979.
53. Cabral GA, McNerney PJ, Mishkin EM. Interaction of delta-9-tetrahydrocannabinol with rat B103 neuroblastoma cells. Arch Toxicol **60**:438–449, 1987.
54. Kupfer A, Dennert G. Reorientation of the microtubule-organizing center and the Golgi apparatus in cloned cytotoxic lymphocytes triggered by binding to lysable target cells. J Immunol **133**:2762–2766, 1984.
55. Kupfer A, Dennert G, Singer SJ. The reorientation of the Golgi apparatus and the microtubule-organizing center in the cytotoxic effector cell is a prerequisite in the lysis of bound target cells. J Mol Cell Immunol **2**:37–49, 1985.
56. Agurell S, Lindgre JE, Ohlsson A, Gillespie HK, Hollister LE. Recent studies on the pharmacokinetics of Δ^1-tetrahydrocannabinol in man. In: Agurell DJ, Dewey WL, Willette RE, Eds. The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects. Orlando, FL: Academic Press, 1984.
57. Wall ME, Brine D, Bursey JT, Rosenthall D. Detection and quantitation of tetrahydrocannabinol in physiological fluids. In: Vinson JA, Ed. Cannabinoid Analysis in Physiological Fluids. ACS Symposium Series 98, Washington, DC: American Chemical Society, pp39–58, 1979.
58. Mechoulam R, McCallum NK, Burstein S. Recent advances in the chemistry and biochemistry of cannabis. Chem Rev **76**:75–112, 1976.
59. Wing DR, Leuschner JTA, Brent GA, Harvey DJ, Paton WDM. Quantification of *in vivo* membrane associated delta-1-tetrahydrocannabinol and its effect on membrane fluidity. In: Harvey DJ, Ed. Proceedings of the Ninth International Congress of Pharmacology Third Satellite Symposium on Cannabis. Oxford: IRL Press, pp411–418, 1985.