Pharmacology Biochemistry & Behavior, Vol. 40, pp. 643–649. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Chronic Marijuana Smoke Alters Alveolar Macrophage Morphology and Protein Expression

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CABRAL, G. A., A. L. STINNETT, J. BAILEY, S. F. ALI, M. G. PAULE, A. C. SCALLET AND W. SLIKKER, JR. *Chronic marijuana smoke alters alveolar macrophage morphology and protein expression.* PHARMACOL BIOCHEM BEHAV **40**(3) 643–649, 1991.—Male rhesus monkeys were subjected to chronic exposure to marijuana smoke. High dose animals (HI) were exposed 7 days/week to 1 MJ cigarette/day; low dose animals (LO) were exposed on 2 consecutive weekend days to 1 MJ cigarette/day; placebo animals (EM) were exposed to 1 ethanol-extracted MJ cigarette/day for 7 days/week; sham animals (SH) were exposed to sham smoking conditions 7 days/week. This regimen was maintained for 1 year and was followed by a 7 month rest period. Alveolar macrophages of animals exposed to the LO and HI dose smoking regimens exhibited irregular cell surface morphology, increased vacuolization, and a spherical conformation upon adherence to plastic. Gel protein profiles of purified macrophages from HI and LO animals showed marked differences in both constitutive and bacterial lipopolysaccharide-elicited protein expression when compared with those of macrophages from the EM or SH animals. These results indicate that chronic THC exposure alters macrophage morphology and protein expression to external stimuli even after a 7 month rest period.

Chronic marijuana smoke Delta-9-tetrahydrocannabinol Alveolar macrophages Macrophage morphology Macrophage protein

MARIJUANA is the major illicit drug of abuse in the United States. In addition to a host of deleterious pharmacological and psychological effects, this substance has been shown to be injurious to the lungs. Habitual marijuana smoking has been associated with chronic respiratory tract symptoms, increased frequency of acute bronchitis, extensive tracheobronchial epithelial disease, and abnormalities in alveolar macrophage structure and function (2,32). Marijuana smoke, in addition, has been shown to be immune suppressive in vivo and in vitro (22, 23, 28). Delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana has been shown to account for the majority, if not all, of the immunosuppressive properties of marijuana.

THC has been shown to alter macrophage form, function, and motility (14,19). Macrophages play a central role in host resistance to infection. These cells degrade exogenous particulate antigens (33), present processed antigen to helper T-lymphocytes (33), and secrete immunoregulatory signals (17,33). In executing these diverse activities, macrophages undergo a multistep process to "full" activation, each step characterized by defined functions and associated with restructuring of protein profiles. The association of protein expression with each of the macrophage activation states suggests that newly expressed proteins participate in the activities attributed to that macrophage state.

Although there is accumulating data which indicate that acute exposure to THC in vivo and in vitro alters macrophage function (14, 19, 32), little is known of the chronic effects of THC on macrophage protein expression and function. Direct exposure of alveolar macrophages to marijuana smoke via the air pathway could affect the capacity of these cells to execute antibacterial and antiviral functions. Thus the purpose of the present investigation was to define the effect of in vivo chronic marijuana smoke exposure on alveolar macrophage morphology and protein expression.

METHOD

Animals and Drug Exposure

Male rhesus monkeys (*Macaca mulatta*) were subjected to smoke inhalation using a smoke generator and masks in order to match typical human route of exposure. Marijuana (MJ) cigarettes containing 2.6% THC were characterized and provided by the National Institute on Drug Abuse. Animals (1.5–3.5 years of age) were assigned to 4 groups (N = 6/group). High dose (HI) animals were exposed to the smoke of 1 standard MJ cigarette/day for 7 days/week; low dose (LO) animals were exposed to the smoke of one standard MJ cigarette/day on weekends for two consecutive days; placebo (EM) animals were exposed to the smoke of one ethanol-extracted MJ cigarette/day for 7 days/week; and sham (SH) animals were exposed 7 days/week to

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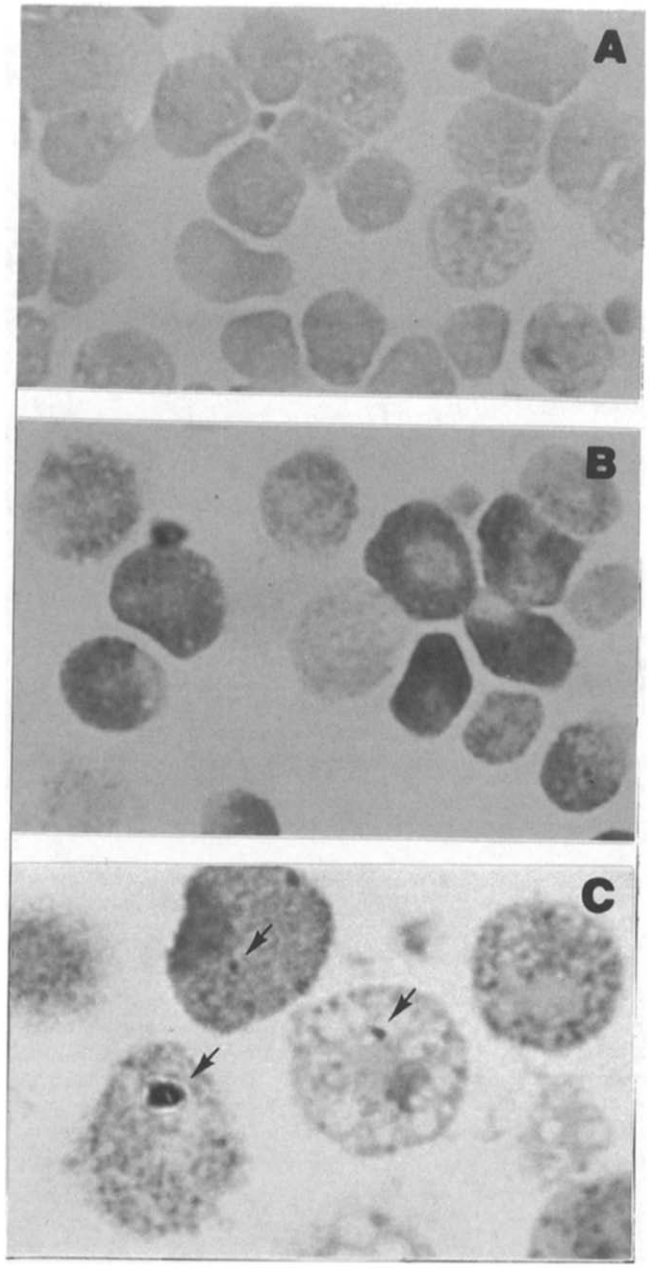

FIG. 1. Light micrographs of alveolar macrophages of rhesus monkeys. (A) Macrophages in alveolar exudates of animals subjected to sham smoking conditions (SH) exhibit a low intensity staining pattern for nonspecific esterase. (×400). (B) Macrophages in alveolar exudates of animals subjected to HI dose chronic marijuana smoking conditions exhibit intense staining for nonspecific esterase (×400). (C) Alveolar macrophages from animals subjected to the HI dose chronic marijuana smoking protocol exhibit irregularity of cell conformation and increased vacuolization. Also note the particulates resident within the cytoplasm (arrows) (×1200).

sham smoking conditions. Following 1 year of this regimen, animals were subjected to a 7 month rest period.

Processing of Alveolar Macrophages

Animals were sacrificed by the administration of pentobarbital overdose. Lung lavages were performed on each animal and the alveolar exudates were placed immediately on ice. Cytospins were prepared from each exudate for cytological analysis using a Shandon Cytospin 2 (Shandon Southern Products, Cheshire, England). A second aliquot of each exudate was fixed in cold (4°C) 0.025 Sorensen's phosphate buffer (pH 7.2) containing 0.25 M sucrose, 2% paraformaldehyde, and 0.05% glutaraldehyde, dehydrated through a graded series of ethanol, passed through propylene oxide, and embedded in Epon-Araldite (Polysciences, Warrington, PA). The remainder of the alveolar exudates was purified for macrophages by adherence to plastic (13), and 10^7 cells/culture were maintained for 24 h in RPMI1640 medium in the presence or absence of 1 μg/ml bacterial lipopolysaccharide (LPS; *E. coli* 055; BJ; Sigma Chem. Co., St. Louis, MO). Cells, then, were rinsed in balanced salt solution, suspended in lysis buffer containing 0.2 U aprotinin trypsin inhibitor/ml and 1 mM phenylmethylsulfonyl fluoride (PMSF) to minimize proteolysis, and stored at −80°C until subjected to polyacrylamide gel electrophoresis (PAGE). Cell differentials were performed on May-Grunwald-stained cytospins. Replicate cytospins then were stained for nonspecific esterase (36). Briefly, each slide was placed for 30 s in cold (4°C) phosphate-buffered (pH 6.6) fixative containing 45% acetone and 7.5% formalin, rinsed in distilled water, and allowed to air dry. The slides, then, were stained with a mixture composed of hexazotized parosaniline and α-naphthyl butyrate in M/15 Sorensen's buffer (pH 6.3) and counterstained with 0.5% methyl green. Using this staining protocol, macrophages stain pink due to the presence of nonspecific esterase activity, while other cells stain blue. Alveolar exudate cells embedded in plastic were sectioned with a LKB ultramicrotome (LKB Produkter, Bromma, Sweden), placed on glass slides, and stained with 2% Toluidine blue O. Cytospin preparations and plastic sections were examined in a Nikon Labophot light microscope (Nikon, Tokyo, Japan).

Electrophoretic Analysis

Macrophage lysates were subjected to microfuge centrifugation (Brinkmann, Westbury, NY) in order to pellet marijuana particulates which were resident within alveolar macrophages of animals exposed to marijuana smoke. Protein concentrations, then, were determined by the method of Bradford (3), and a constant amount of protein was employed for electrophoresis of each sample. The clarified macrophage lysates were subjected to 10% one-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) at 30 mA/gel as previously described (9,26). Molecular weight protein standards (LKB Produkter, Bromma, Sweden) were coelectrophoresed with each set of samples. Gels were either stained with Gelcode color silver stain (Pierce Chemical Co., Rockland, IL) or double-stained with silver (Biorad Laboratories, Richmond, CA) and Coomassie brilliant blue R250 after the method of Dzandu et al. (11,12). Gel profiles were analyzed using a Hoefer GS-300 Scanning Densitometer (Hoefer Scientific Instruments, San Francisco, CA) interfaced to an Apple 2e computer using GS-340 Data System software (Hoefer). Select samples, also, were subjected to two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Alveolar macrophage lysates were concentrated by lyophilization and reconstituted in a solubilization solution containing 2% SDS, 0.05 M cyclohexylaminoethane sulfonic acid (CHES), 10% glycerol, 2% β-mercaptoethanol, 1.3% pH 3.5–10 ampholines (LKB Produkter), and 0.7% pH 3–10 Pharmalytes (Pharmacia, Uppsala, Sweden). Solubilized proteins, then, were subjected to analytical 2D-

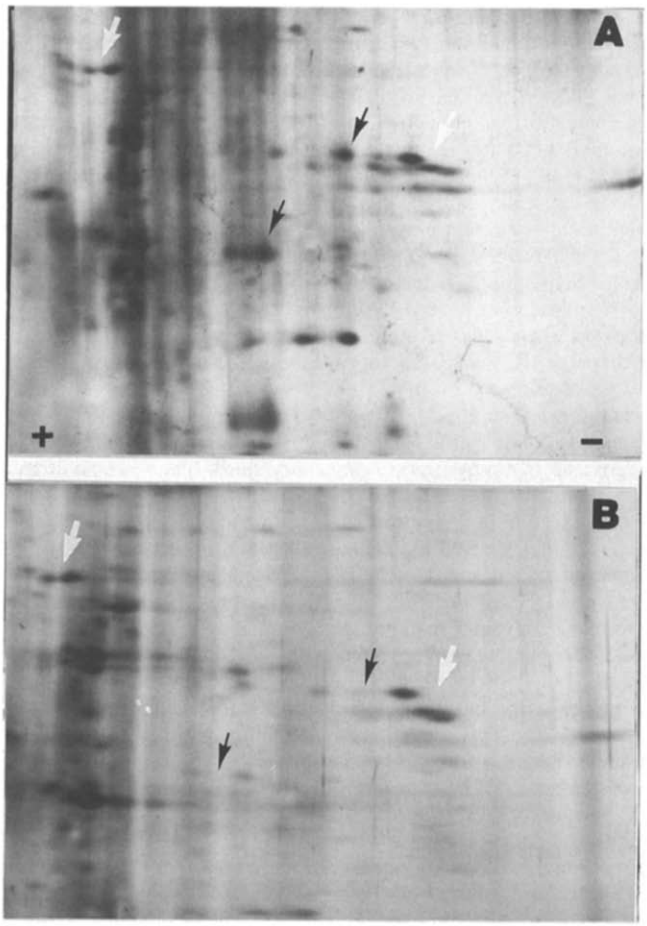

FIG. 2. Two-dimensional polyacrylamide gel of alveolar macrophages of rhesus monkeys subjected to chronic marijuana (2.6% THC) exposure under LO or HI smoking conditions. Solubilized macrophages were focused in a pH gradient of 4.0 to 8.3 in the first dimension and subjected to 10% SDS-PAGE in the second dimension at 30 mA/gel. Resultant gels were stained with silver. (A) Gel protein profile of alveolar macrophages of animals subjected to chronic LO dose smoking conditions. The pH gradient runs from 4.0 on the left (anode) to 8.3 on the right (cathode). (B) Gel protein profile of alveolar macrophages of animals subjected to chronic HI dose smoking conditions. The white arrows designate representative protein species which are unaffected by either LO dose or HI dose chronic smoking conditions. The solid arrows designate representative protein species which are depleted in the HI dose profiles when compared with those of the LO dose profiles. A generalized depletion of certain protein species, especially for proteins with relative molecular weights less than 68 kD, was noted for HI dose macrophage profiles when compared with LO dose profiles.

PAGE after the method of O'Farrell (25). Briefly, proteins were subjected to isoelectric focusing (pH gradient 4.0–8.3) in the first dimension and to 10% SDS-PAGE in the second dimension (30 mA/gel). Gels were stained with silver using Gelcode Color Stain (Pierce Chemical Co.) and photographed using Ektachrome HC color slide film (Eastman Kodak Co., Rochester, NY). Carbamylated creatinine phosphokinase (Pharmacia-LKB Biotechnology, Piscataway, NJ) was included as an internal marker for isoelectric points (pI). In addition, molecular weight (mol. wt.) standards (Pharmacia-LKB) were coelectrophoresed with each gel during its second dimension. The application of pI and mol. wt. standards allowed for the establishment of pI:mol. wt. coordinates for comparison and identification of proteins of different gels.

RESULTS

Effect of Marijuana on Alveolar Macrophage Morphology

Alveolar exudate cell counts were expressed as total number of cells recovered and as the number of cells/ml in each bronchoalveolar lavage. Typical lavages yielded approximately 5×10^7 total cells at a concentration of approximately 5×10^6 cells/ml. No significant differences in either total cell number or cell concentration were noted for any of the animal treatment groups.

In order to determine the effect of marijuana pulmonary exposure on macrophage morphology, cytospins and plastic-embedded sections of alveolar exudates were examined by light microscopy. Typical results are illustrated in Fig. 1. Alveolar exudates from animals of all treatment groups contained cellular populations which were composed of greater than 85% macrophages, based on positive nonspecific esterase (NSE+) staining. The NSE+ cells were readily distinguished by the presence of red-stained granules within the cytoplasm versus the green counterstained nonspecific esterase-negative (NSE−) cells. Macrophages from sham (SH) control animals, while NSE+, showed a relatively low intensity staining pattern. Less than 5% of macrophages of SH animals exhibited intense staining for NSE within the cytoplasm. In contrast, a gradient of intense NSE+ staining was seen for macrophages in exudates of animals subjected to smoking conditions. Greatest intensity of staining was noted for cells from HI animals, followed by those from EM animals and LO animals, in that order. In the case of alveolar exudates obtained from animals subjected to the HI dose regimen, greater than 75% of cells were intensely stained for NSE.

Macrophages from animals exposed to marijuana smoke 7 days/week (i.e., EM and HI dose groups) contained an increased content of particulate matter, as evidenced by the presence of numerous inclusion bodies in the cytoplasm when compared with those of SH controls (Fig. 1C). Particulate inclusions also were seen in the cytoplasm of macrophages from the LO dose animal group. Indeed, centrifugation ($1000 \times g$) of alveolar macrophages from the EM, LO, and HI smoke exposure animals yielded cell pellets which were pigmented macroscopically. These results indicated that pigmented particulates were associated with the cellular fraction. Light microscopy demonstrated that the cells from the HI and EM groups had comparable amounts of cytoplasmic particulate matter, while cells from the LO dose group exhibited slightly fewer. Relatively few intracytoplasmic particulate inclusions were seen in macrophages of the SH exposure group. Macrophages from SH animals exhibited the "capability" to spread, as evidenced by their flattened appearance upon adherence to plastic and by the presence of pseudopodia extending from the surface. Approximately 50% of the SH macrophages exhibited these features. In contrast, less than 5% of the macrophages from the HI dose smoke exposure animals showed evidence of spreading, with the majority of cells exhibiting a spherical conformation. Cells from the LO dose animals also showed a lower frequency of flattened cells when compared with those from SH animals but to a lesser extent than the HI dose exposure macrophages.

Large cytoplasmic vacuoles were seen in alveolar macrophages from animals which were subjected to smoke inhalation

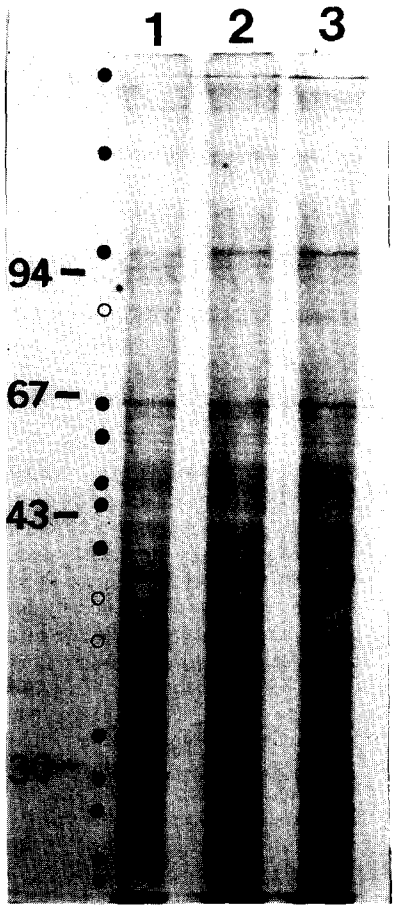

FIG. 3. Analytical one-dimensional SDS-polyacrylamide gel of rhesus monkey alveolar macrophages subjected to in vitro activation with bacterial lipopolysaccharide (LPS). Macrophages purified from alveolar exudates were cultured for 24 h with 1 μg/ml LPS, solubilized, and subjected to SDS-PAGE. The numbers along the left border represent relative molecular weights (kD). Lanes 1, 2, and 3 represent protein profiles of alveolar macrophages of animals subjected to HI, LO or EM smoking conditions. The solid dots represent protein species in the HI dose profile which are decreased when compared with the EM profile. The open dots represent species in the HI dose profile which are increased when compared with the EM profile. THC elicited differential expression to LPS in the alveolar macrophages of HI dose animals when compared with those of EM dose animals.

when compared with SH controls. This effect was most pronounced for alveolar macrophages of the HI dose animals. Large vacuoles also were seen, but to a lesser extent, in macrophages of the LO and EM dose animals. Also apparent for macrophages from HI dose animals was the irregular outline of the outer membrane when compared with SH controls. Alveolar macrophages exposed to the HI dose marijuana smoke regimen exhibited a rougher, more irregular surface and the presence of surface "blebs."

Effect of Marijuana Smoke on Alveolar Macrophage Protein Expression

Macrophages purified from alveolar exudates were subjected to PAGE in order to determine the effect of marijuana smoke exposure on constitutive and LPS-induced protein expression. One-dimensional and two-dimensional SDS-PAGE revealed that protein profiles of macrophages from EM, LO, and HI dose animals showed marked differences in constitutive protein expression when compared with the profiles of SH macrophage controls. Figure 2 illustrates comparative results of two-dimensional polyacrylamide gel electrophoresis of LO versus HI alveolar macrophage proteins. Macrophages from the EM animals showed alterations in protein profiles, especially for species with relative molecular weights less than 68 kDa, when compared with those from SH animals. Macrophage samples from animals which were exposed to the LO dose marijuana regimen, in turn, showed alterations in protein expression when compared with either the SH or EM macrophages. Again, the majority of protein alterations were noted for species of relative molecular weight less than 68 kDa. Restructuring of protein expression included decreases in the apparent concentration of some proteins accompanied by hyperelevated expression of others. In addition, some protein species remained unaffected when compared with either the SH or EM profiles. The macrophages from the HI dose animals showed the greatest alteration in protein expression when compared with either SH or EM macrophages. A generalized dose-related depletion in protein expression was noted for proteins of alveolar macrophages of animals subjected to the HI dose regimen, when compared with those of animals subjected to the LO dose regimen. Depletion in protein expression was noted most for those species of relative molecular weights less than 68 kDa. This depletion of proteins was noted, also, when protein profiles of alveolar macrophages of the HI dose animals were compared with those from the SH or EM animals.

The effect of chronic exposure to marijuana smoke on the capacity of alveolar macrophages to undergo protein profile restructuring in response to the bacterial "activator" LPS was evaluated by analytical one-dimensional SDS-PAGE. Macrophages from the SH animals, as expected, exhibited a restructuring of protein profiles following in vitro exposure to LPS when compared with SH macrophages not treated with LPS (data not shown). Figure 3 illustrates typical results of in vitro LPS treatment on protein expression of alveolar macrophages from animals subjected to the EM, LO, or HI dose smoking regimens. Macrophages from the animals exposed to EM marijuana smoke exhibited a restructuring of protein profiles following treatment with LPS. The EM profiles showed changes at relative molecular weights of 90 kDa and 70 kDa when compared with profiles of LPS-treated SH macrophages, indicating that ethanol-extracted marijuana had some effect on protein restructuring. No major changes, however, were seen between the SH and EM profiles for other proteins. In contrast, alveolar macrophages from animals subjected to the THC-containing marijuana applied in the LO and HI dose regimens showed differential expression in LPS-elicited proteins when compared with LPS-treated EM samples. The most pronounced alterations were seen in LPS-elicited protein expression for alveolar macrophages from the HI dose animals. Decreases in protein expression were seen for proteins ranging in relative molecular weights from 110 kDa to 20 kDa when compared with the protein expression profiles from macrophages of EM animals. These results are indicative of a generalized suppression of LPS-elicited protein expression. In addition, the expression of several protein species was apparently elevated (e.g., 80 kDa, 38 kDa, and 36 kDa) when compared with both the LO dose and EM dose macrophage expression profiles. Thus macrophages from the HI and LO animals responded differentially to LPS in vitro "activation" when compared with macro-

phages obtained from the EM or SH animals.

DISCUSSION

The results of this study indicate that chronic exposure to marijuana smoke affects the morphology and protein expression of alveolar macrophages of rhesus monkeys even after a 7 month rest period. The rest period was included in the experimental protocol since various behavioral and cognitive studies were carried out following the cessation of smoke exposure (27,31). First, as determined by light microscopy, exposure to marijuana smoke effected a hyperelevation in nonspecific esterase activity in macrophages of EM, LO, and HI dose animals. The elevated nonspecific esterase activity appeared to be directly related to chronic smoke exposure in that macrophages of animals subjected to the EM and HI regimens were more intensely positive than those from animals exposed to the LO dose protocol. Macrophages from the SH animals, in turn, exhibited the lowest intensity in staining for nonspecific esterase. Thus nonspecific esterase intensity may be indicative of chronic smoking conditions and may be a consequence of persistent uptake of exogenous particulate matter. These observations are supported further by light microscopy which indicated that macrophages from all of the animals exposed to marijuana smoke (i.e., EM, LO, and HI dose animals) contained numerous intracytoplasmic particulate inclusions. Thus even moderate exposure to marijuana smoke over a prolonged period of time may result in accumulation of exogenous particulate matter within alveolar macrophages. The observation of these inclusions within macrophages of animals chronically exposed to marijuana smoke is in agreement with that of Mann et al. (20) who noted that electron dense and lucent inclusions were present in marijuana smokers' macrophages, and that these inclusions were similar to those of tobacco smokers. Thus the observation of high nonspecific esterase expression in macrophages of animals subjected to EM, LO, and HI dose marijuana smoke may represent a response to the continuous uptake of exogenous particulates and to the persistence of particulate matter within the cytoplasm of alveolar macrophages, regardless of whether THC is present.

Intracytoplasmic particulate inclusions were seen within alveolar macrophages of animals subjected to the EM, LO, and HI marijuana smoke dose regimens even after a 7 month rest period. These observations indicate that marijuana particulates recycle within macrophage populations in the lung. It has been suggested that the half-life of alveolar macrophages averages approximately 27 days (34). Thus intracytoplasmic particulates within macrophages may be taken up by surrounding macrophages during macrophage turnover. The recycling of marijuana particulates from macrophage to macrophage could constitute a means by which cells would remain in a persistent state of exposure to THC, the major psychoactive component of marijuana. This compound is highly lipophilic (35) and has been shown to elicit morphological alterations of both intracytoplasmic and cell surface membranes (5, 21, 29). In the present study, alveolar macrophages exposed to LO and HI marijuana smoke exhibited an increase in the number and size of cytoplasmic vacuoles, irregularity in cell surface outline, and the presence of "blebs" extending from the cell surface. These membranous alterations are similar to those recorded for rat B103 neuroblastoma cells exposed in vitro to THC (5). Davis et al. (10) also have reported that large globular projections, or "blebs" extended from the surfaces of alveolar macrophages of marijuana smokers.

The majority (i.e., >75%) of macrophages from animals subjected to the HI dose regimen exhibited a spherical conformation following adherence to plastic. Davis et al. (10) have indicated that alveolar macrophages of human nonsmokers assume two different shapes following adherence to plastic. Flat cells (45%) appeared to be the more active phagocytes when compared with rounded cells (55%) which appeared more quiescent. In contrast to our observations, no differences were noted in the proportion of flat versus rounded cells in alveolar exudate preparations of human marijuana smokers. However, in agreement with our observations, Lopez-Cepero et al. (19) noted that THC administered in vitro inhibited in a dose-related manner the spreading activity of mouse peritoneal macrophages placed on glass surfaces. Furthermore, these investigators reported that inhibition of spreading activity was correlated with a decrease in macrophage capacity to phagocytose yeast particles. The expression of cell surface blebs and the assumption of a spherical shape by alveolar macrophages of animals subjected to the HI dose regimen are features which have been noted for cells exposed to toxins. Leopardi et al. (18) reported that α-lymphotoxin and cytotoxic T-lymphocytes elicited the formation of blebs on mouse L929 cells. The formation of blebs on these cells was consistent with a state of drug-induced direct injury to cell surface membranes with the consequence of altered selective permeability of the plasma membrane. Leopardi et al. (18) postulated that enhanced calcium flux due to lymphotoxin-induced membrane damage, as evidenced by the formation of blebs on the cell surface, resulted in an altered arrangement of filamentous actin. This alteration, in turn, effected rearrangement of mouse L929 cytoskeletal stress fibers which normally connect cellular processes with the central region of the cell. The resultant loss of plasma membrane, with the attendant abnormality in calcium regulation, could account for a rearrangement of L929 cell conformation and lead to a spherical shape. A similar mechanism may be operative for alveolar macrophages directly exposed to marijuana smoke. That is, THC interaction with macrophage membranes could effect cell injury in a manner similar to that induced by a variety of toxins (30). The rounding of alveolar macrophages of animals exposed to marijuana smoke containing THC (i.e., LO and HI smoking dose regimens) could be indicative of a decreased capacity of these cells to execute certain functional activities such as phagocytosis as reported by Lopez-Cepero et al. (19) for murine peritoneal macrophages exposed in vitro to THC. Thus particulates newly introduced in marijuana smoke, or intracellular marijuana particulates recycled into surrounding macrophages due to macrophage turnover, could be ingested by alveolar macrophages. Subsequent intracellular release of THC resident in marijuana particulates, and the interaction of the THC with intracellular and plasma membranes, could lead to macrophage dysfunction.

Alveolar macrophages of animals exposed to marijuana smoke containing THC (i.e., LO and HI dose regimens) exhibited an inhibition in both constitutive and LPS-elicited protein synthesis. These observations are in agreement with our previous results which indicated that in vivo administration of the cannabinoid inhibited protein synthesis of peritoneal macrophages of mice receiving *P. acnes* (6). Inhibition of macromolecular synthesis by THC also has been reported for a diverse array of cell types in addition to macrophages (1, 7, 24). However, the identity of the macrophage proteins affected by THC remains unresolved. THC has been shown to suppress the induction of alpha/beta interferons in mice (5) and to inhibit the expression of herpes simplex virus-specified glycoproteins in infected cells (22). Thus it is likely that the cannabinoid effects a generalized inhibition of

macrophage protein expression in response to external stimuli.

The manner in which THC inhibits protein expression has not been determined. However, since THC is a highly lipophilic molecule, its interaction with cellular membranes leading to altered membrane fluidity and selective permeability changes (35) may account for the suppression of protein synthesis. Indeed, alterations in cell surface membrane selective permeability, with the attendant increase in intracellular sodium, have been proposed as the mode by which certain viruses effect shutdown of host cell macromolecular synthesis (8, 15, 16). THC may have a similar effect on cells. Alternatively, THC-induced morphological disruption of cellular membranes (21,29) could contribute to suppression of protein expression. That is, THC-induced membrane disruption could alter cellular compartments of protein synthesis and/or posttranslational events. We have shown that in vitro exposure to THC elicits membrane perturbation of both cell surface and cytoplasmic membranes in rat B103 neuroblastoma cells (5).

The results of this investigation indicate that chronic exposure to marijuana smoke elicits a spectrum of effects on alveolar macrophages even after cessation of smoking for 7 months. Some of the effects, such as elevated nonspecific esterase activity may be attributed to marijuana components other than THC. Others, such as membrane perturbation and suppression of protein synthesis may be resultant of direct interaction of the THC within marijuana smoke. The persistence of marijuana particulates within macrophage populations suggests that THC-elicited alterations on alveolar macrophages may be effected for extended periods even after cessation of marijuana smoking. The failure of alveolar macrophages chronically exposed to THC to elicit protein expression in response to bacterial LPS suggests that these cells have a decreased capacity to respond to external stimuli. Hosts thus affected could be at increased risk of infection with virus or bacterial pathogens introduced via the air pathway.

ACKNOWLEDGEMENTS

This research was supported by grant RO1 DA03647 and by IAG 224-83-0005 from the National Institute on Drug Abuse.

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