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Marijuana Smoking, Pulmonary Function, and Lung Macrophage Oxidant Release

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SHERMAN, M. P., M. D. ROTH, H. GONG, JR. AND D. P. TASHKIN. *Marijuana smoking, pulmonary function, and lung macrophage oxidant release.* PHARMACOL BIOCHEM BEHAV **40**(3) 663–669, 1991.—Pulmonary alveolar macrophages lavaged from tobacco smokers release increased levels of oxidants and have been implicated in the pathophysiology of emphysema. It is unknown whether lung macrophages recovered from marijuana smokers also liberate excessive levels of oxidants. To evaluate this possibility, pulmonary alveolar macrophages were obtained by bronchoalveolar lavage from nonsmokers, smokers of marijuana only, smokers of tobacco only, and smokers of tobacco plus marijuana. Spontaneous and stimulated superoxide anion release was measured by the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. These findings were correlated with recent lung function tests. Superoxide anion production by macrophages, studies of small airway integrity (closing volume, closing capacity, and the slope of Phase III of the single-breath nitrogen washout curve), and evaluation of alveolar gas exchange (diffusing capacity of carbon monoxide) were similar in both nonsmokers and marijuana smokers. However, tobacco smoking was associated with both significantly higher levels of superoxide anion release by pulmonary alveolar macrophages and significant abnormalities of small airway function and alveolar diffusing capacity. Based on the results of this study, pulmonary alveolar macrophages of marijuana-only smokers do not produce increased amounts of oxidants when compared to macrophages of nonsmoking subjects. This observation may account for the absence of abnormalities in small airway function and alveolar diffusing capacity in marijuana-only smokers, in contrast to the presence of such findings in smokers of tobacco, regardless of marijuana use.

Smoking Marijuana Tobacco Macrophages Lung Oxidants Pulmonary function tests

DRUG abuse education is most likely responsible for the declining marijuana use among high school students in recent years (1). Because adolescents are probably unaware of recent reports linking marijuana smoking to abnormal pulmonary function (24) and cancer (3), they may also be reacting to widespread information relating tobacco smoking and its association with an increased risk of emphysema and pulmonary malignancy (17). In tobacco smokers, the pathogenesis of emphysema has been conceptually associated with excessive neutrophil elastase activity in the lung. The action of neutrophil elastase on the extracellular matrix proteins of the lung would ordinarily be counterbalanced by α-1-antitrypsin inhibitor; however, either oxidants present in tobacco smoke (21) or oxidants released from neutrophils (18) can inactivate α-1-antitrypsin. More recently, pulmonary alveolar macrophages of tobacco smokers have been implicated as a source of spontaneously released oxidants that can inactivate α-1-antitrypsin inhibitor (16). This is an attractive hypothesis, as these phagocytes constantly reside in the pulmonary airways and alveoli. It is unknown whether marijuana smoking also results in an increased oxidant release from pulmonary alveolar macrophages, and if it does, whether this is also associated with an increased risk of chronic obstructive lung disease. Pulmonary function tests show that marijuana smoking causes resistance and specific conductance abnormalities in the large airways, as opposed to small-airway disease that is noted in tobacco smokers (24). Based on these differences in pulmonary function studies, we postulated that macrophages of marijuana-only smokers may not produce the increased amounts of oxidants that have been observed in the macrophages of tobacco-only smokers. To test this hypothesis, we measured spontaneous and stimulated superoxide anion release by macrophages recovered from the lungs of nonsmokers and smokers of marijuana alone, smokers of tobacco alone, and smokers of marijuana plus tobacco. We correlated this information with concurrent pulmonary function testing to see if oxidant release by macrophages could predict physiologic evidence of small-airways disease and impaired alveolar diffusion in smokers of marijuana and/or tobacco.

METHOD

Subject Recruitment

Four groups of subjects were recruited for these studies. They included: 1) marijuana-only smokers (MS), 2) tobacco-only smokers (TS), 3) marijuana and tobacco smokers (MTS), and 4) nonsmokers (NS). Subjects were enrolled either as new subjects from flyers and newspaper advertisements or restudied as partici-

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pants from prior studies on smoking and pulmonary function (24). Potential subjects had to pass a screening questionnaire administered by telephone. Subjects were excluded from the study if they had a recent history (past 4 weeks) of respiratory symptoms or infection, hazardous occupational or environmental exposures, chronic respiratory disorders, immunologic diseases, or cardiovascular illnesses. Eligible subjects completed a modified version of the ATS/DLD respiratory questionnaire (12) that was administered by an experienced interviewer. The questionnaire includes questions concerning respiratory symptoms, general health, residence, occupational history, socioeconomic status, alcohol and tobacco use and illicit drug exposure. This questionnaire was modified for the present project to include a detailed history of past and present marijuana and tobacco use. Volunteers were required to have a normal physical examination and normal laboratory tests. After subjects gave their informed consent, they underwent pulmonary function testing and outpatient fiberoptic bronchoscopy. This study was approved by the Human Subject Protection Committee of the UCLA School of Medicine.

Pulmonary Function Studies

Spirometry was performed with the subject seated using a 13.5-liter water-sealed spirometer (Warren E. Collins, Incorporated). Forced vital capacity (FVC) and forced expired volume in one second (FEV_1) were calculated from the best of at least two reproducible vital-capacity efforts. FVC and FEV_1 were expressed as percent of the expected normal values. The prediction equation for normal values was derived from asymptomatic, never-smoking, 25- to 59-year-old participants in the UCLA Population Studies of Chronic Obstructive Respiratory Disease (10,11). Closing volume (CV/VC, %) was determined at least in duplicate using a standardized single-breath nitrogen washout technique (4). The subject was standing during the measurement, which was made using an electronic rolling-seal spirometer (Cardio-Pulmonary Instruments, Inc., model 220) with a rapid-responding nitrogen analyzer (Cardio-Pulmonary Instruments, Inc., model 410) that was connected to an X-Y recorder (Hewlett Packard, model 7041A). Closing capacity (CC/TLC, %) was calculated using the residual volume determined by helium dilution. The slope of Phase III of the single-breath nitrogen washout curve ($\Delta N_2/L$) was also determined (5). Diffusing capacity for carbon monoxide (DLCO) was determined by the single-breath technique (20). Peripheral venous blood was obtained anaerobically and analyzed for hemoglobin and carboxyhemoglobin using a CO-oximeter (Model 282; Instrument Laboratories, Lexington, MA) to correct DL_{CO} measurements for effects of anemia (8) and carboxyhemoglobinemia (19). Corrected DL_{CO} was expressed as a percent of the expected value based on the predictive equations of Cotes (7).

Bronchoalveolar Lavage

Fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) were performed in a standard manner, as previously reported (2). Briefly, subjects were fasted and premedicated intravenously with midazolam and meperidine. Topical anesthesia was achieved with a 4% lidocaine spray to the pharynx and by direct topical application of 2% lidocaine to the airways. A fiberoptic bronchoscope was introduced orally into the bronchial tree, and the tip was wedged under direct vision into a segmental or subsegmental bronchus of the right middle lobe. In most subjects, a total of 300 ml of sterile nonbacteriostatic, normal saline was instilled in 50-ml aliquots and immediately retrieved by gentle manual suction with a 60-ml plastic syringe. The lavage material recovered was filtered through 4 layers of sterile gauze into 50-ml plastic tubes each containing 15 ml of RPMI 1640 (GIBCO, Grand Island, NY) that was supplemented with 5% fetal calf serum (Whittaker M.A. Bioproducts, Inc., Walkersville, MD) and 500 U of preservative-free heparin.

Cell Recovery and Analysis

The material obtained by BAL was immediately centrifuged at $250 \times g$ for 10 min. The cell pellet was resuspended in a balanced salt-HEPES buffer (22). Total cell yield was ascertained by hematocytometer counts, at which time viability was determined by trypan blue exclusion. Cytocentrifuge slide preparations were made and stained by the Giemsa method (Diff-Quik, American Scientific Products, McGaw Park, IL). Specimens were excluded from analyses if cell viability was not $\geq 95\%$ and cellular differential counts did not have $\geq 90\%$ macrophages.

Superoxide Anion Production by Pulmonary Alveolar Macrophages

The kinetics of superoxide anion (O_2^-) release by PAM were measured at 37°C by the superoxide dismutase-inhibitable reduction of ferricytochrome *c*, which was monitored spectrophotometrically at 550 nm (22). The standard reaction mixture contained: 0.5×10^6 PAM, 50 μM ferricytochrome *c*, the stimulus, and buffer. Duplicate samples were tested, one having no superoxide dismutase and the other containing 30 μg of superoxide dismutase. The remainder of the final 1-ml volume was a balanced salt-HEPES buffer. Three stimuli were utilized as inducers of O_2^- production. One $\times 10^9$ zymosan particles was opsonized in 1 ml of the subject's serum by tumbling at 37°C for 30 min (designated STZ). After washing by centrifugation, the STZ was resuspended in saline and added to the reaction mixture at a particle:macrophage ratio of 100:1. In a second set of conditions, macrophages were preincubated with dihydrocytochalasin B (DHCB) at a concentration of 5 μg/ml for 10 min followed by the addition of STZ. The third stimulus was phorbol myristate acetate (PMA), which was added at a concentration of 100 ng/ml. All reagents for these studies were obtained from a single vendor (Sigma Chemical Co., St. Louis, MO). Basal or unstimulated O_2^- release was measured for 10 min before addition of a stimulus. The maximal rate of stimulated O_2^- generation was calculated from the 10-min interval which followed the lag period.

Data Analysis

Data was analyzed using the Statview 512+ statistical software package (BrainPower Inc., Calabasas, CA). Following data exploration, values were excluded if they were $\geq$ median ± 3 interquartile ranges. After determination of normality of distribution and variance homogeneity, the appropriate parametric (analysis of variance and two-tailed independent *t*-test) or nonparametric tests (Kruskal-Wallis and Mann-Whitney U-tests) were applied to continuous variables (15). Simple linear regression or Spearman rank correlation was used to assess the relationships between the following parameters: smoking history and superoxide anion production, smoking history and pulmonary function test results, and superoxide anion production and pulmonary function testing (14). Categorical variables were analyzed using the Yates corrected χ^2 test. All *p* values were adjusted for multiple comparisons. An adjusted *p* value of ≤ 0.05 was considered significant.

RESULTS

Subject data are presented in Table 1 by smoking history. The ages of the four groups were not significantly different. The

TABLE 1

CHARACTERISTICS OF THE STUDY SUBJECTS

	Nonsmokers (NS)	Marijuana Smokers (MS)	Tobacco Smokers (TS)	Marijuana and Tobacco Smokers (MTS)
Age				
Years	31.6 ± 1.7*	37.3 ± 1.9	37.9 ± 2.1	34.1 ± 2.2
Range	(24–48)	(24–55)	(25–52)	(21–47)
Sex				
Male	10	13	12	12
Female	7	6	2	1
Pack-years	N/A	N/A	25.6 ± 4.4	17.0 ± 4.9
Range			(4.1–44.6)	(1.0–63.5)
Joint-years	N/A	62.4 ± 15.7	N/A	50.9 ± 14.9
Range		(15–316)		(6.9–214)
Joints/day	N/A	3.0 ± 0.5		2.0 ± 0.5
Cigarettes/day	N/A	N/A	20 ± 4	17 ± 5
Time Last Smoked (Hours)	N/A	26.4 ± 9.5	7.5 ± 1.9	54.7† ± 26.6/ 5.5‡ ± 1.5

*Mean ± SEM; N/A = not applicable.
†Number of hours since last smoked marijuana.
‡Number of hours since last smoked tobacco.

ratio of male to female subjects was similar in the nonsmoking and marijuana-smoking groups; however, the tobacco-only and marijuana plus tobacco-smoking subjects had a proportionally larger number of male volunteers.

The results of lung function testing are summarized in Table 2. Although FEV_1/FVC and DL_{CO} in smokers of marijuana only were somewhat below the values seen in nonsmokers, they were not statistically different. Pulmonary function tests that evaluated small-airways disease ($\Delta N_2/L$, CV/VC and CC/TLC) were nearly identical in marijuana-only smokers and nonsmokers. For most of the lung function studies, tobacco-only smokers had poorer respiratory function than nonsmokers. Marijuana plus tobacco smoking appeared to have a combined adverse effect on forced expiratory flows and diffusing capacity, as they were significantly below values for nonsmokers, while values for marijuana-only smokers and tobacco-only smokers were in between the results of those two groups.

In all four groups of subjects, the cells recovered from BAL were >93% PAM. Trypan blue exclusion revealed that >96% of these macrophages were viable. The production of O_2^- by PAM among the four groups is shown in Fig. 1. Unstimulated O_2^- release was highest in the PAM of tobacco-only smokers and more than 4-fold above that observed in marijuana-only smokers' macrophages ($p<0.05$). When stimulated with serum-treated zymosan (STZ) alone, O_2^- production was not different among the four groups. If the macrophages were incubated with dihydrocytochalasin B (DHCB) for 10 min before STZ stimulation, there was a nearly 2-fold increase in O_2^- release by the PAM of tobacco-only smokers compared to the cells of marijuana-only smokers and nonsmokers ($p<0.02$). The soluble stimulus, phorbol myristate acetate (PMA), likewise induced a nearly 2-fold rise in O_2^- production by PAM of tobacco smokers ver-

TABLE 2

LUNG FUNCTION MEASUREMENTS BY SMOKING STATUS

	Type of Smoking Group			
Lung Function Measurements	Nonsmokers (NS)	Marijuana Smokers (MS)	Tobacco Smokers (TS)	Marijuana and Tobacco Smokers (MTS)
FEV_1/FVC, %	83.1 ± 1.2*	78.7 ± 1.8	81.7 ± 1.8	77.0 ± 2.1†
$\Delta N_2/L$, %	0.76 ± 0.08	0.76 ± 0.09	1.44 ± 0.21‡	1.36 ± 0.40
CV/VC, %	13.6 ± 1.61	13.9 ± 1.6	15.9 ± 2.1	14.8 ± 2.7
CC/TLC, %	37.5 ± 1.5	38.4 ± 1.3	43.2 ± 2.1§	39.6 ± 3.0
DL_{CO}, % predicted	95.7 ± 2.6	89.4 ± 3.4	83.3 ± 5.5¶	81.7 ± 4.6¶

*Mean ± SEM, the number of subjects studied is shown in Table 1.
†$p<0.05$ for NS vs. MTS.
‡$p<0.05$ for NS and MS vs. TS.
§$p<0.05$ for NS vs. TS.
¶$p<0.05$ for NS vs. TS and MTS.
All comparisons are by analysis of variance.

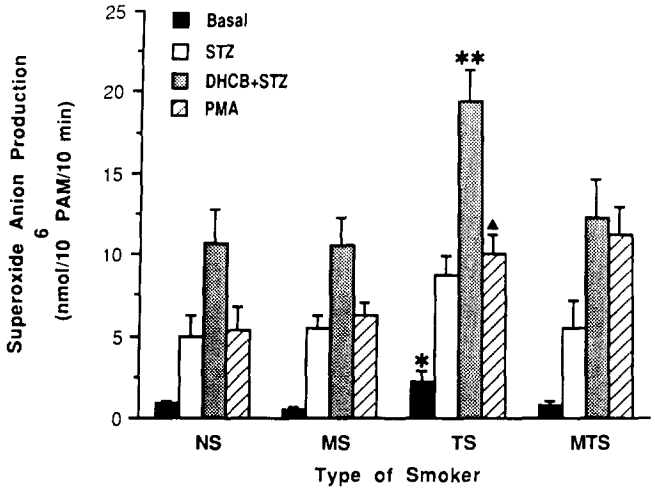

FIG. 1. Unstimulated and stimulated superoxide anion release by pulmonary alveolar macrophages of smokers and nonsmokers. The key in the graph delineates the type of stimulus. Abbreviations are as follows: Basal = no stimulus, STZ = serum treated zymosan, DHCB + STZ = 10-min preincubation with dihydrocytochalasin B followed by STZ stimulation, and PMA = phorbol myristate acetate. Smoker designations are: NS = nonsmokers, MS = marijuana smokers, TS = tobacco smokers, and MTS = marijuana and tobacco smokers. Data are presented as mean ± SEM. The number of subjects studied is summarized in Table 1. The single asterisk indicates a $p<0.05$ for TS compared to MS. The double asterisk signifies a $p<0.02$ for TS compared to NS and MS. The solid triangle symbolizes a $p<0.05$ for TS versus NS.

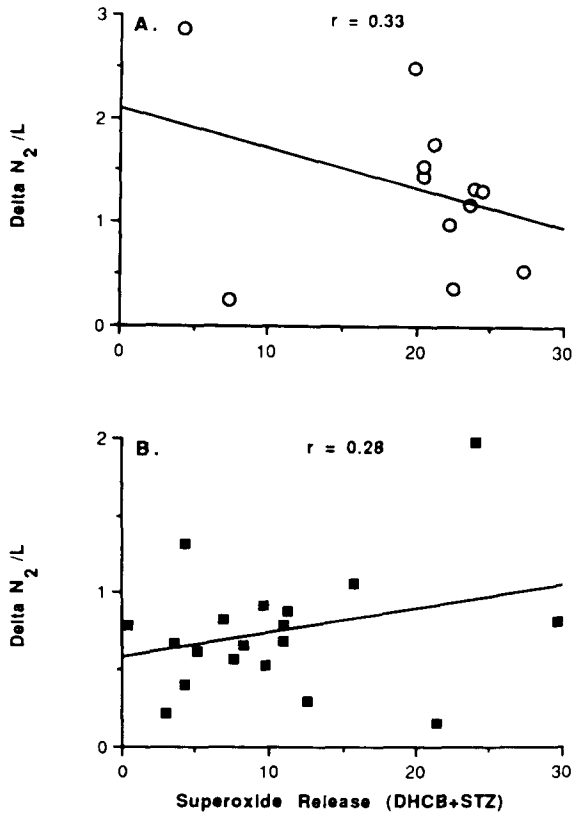

FIG. 2. Relationship between superoxide anion production by macrophages of marijuana-only and tobacco-only smokers and the slope of phase III of the single-breath nitrogen washout curve ($\Delta N_2/L$). The upper figure [(A), open circles] indicates tobacco-only macrophages, and the lower figure [(B), solid squares] represents macrophages of marijuana-only smokers. Superoxide anion production was stimulated by DHCB + STZ and is expressed as nmol/10^6 PAM/10 min.

sus those of marijuana smokers and nonsmokers ($p<0.05$). The PAM of the smokers of marijuana plus tobacco had a release of O_2^- in response to PMA which mirrored that seen in the cells of tobacco-only smokers, whereas their production of O_2^- when stimulated by DHCB plus STZ was more akin to the macrophages of marijuana-only smokers and nonsmokers.

When relationships were sought between elements of the smoking history and the respiratory burst by PAM, a strong correlation was found only for tobacco cigarettes smoked per day over the preceding month and PMA-stimulated O_2^- production ($r=.84$, $p<0.01$). Marijuana smoking had no relationship to O_2^- release induced by PMA. There was no association between any component of the smoking history and the significant O_2^- release seen in PAM of tobacco smokers following stimulation with DHCB and STZ. Cumulative lifetime smoking history (pack- or joint-equivalent-years) did not show a correlation with spontaneous or stimulated O_2^- production.

Figures 2A and B show the relationship between the most potent inducer of macrophage O_2^- release, DHCB + STZ, and $\Delta N_2/L$ in tobacco-only smokers and marijuana-only smokers, respectively. Rather than a rise in the slope of Phase III of the single-breath nitrogen washout curve with high levels of O_2^- production by PAM, tobacco smokers' cells had a slight decline in $\Delta N_2/L$ as O_2^- release increased with a combination of DHCB + STZ stimulation. There was also no clearcut association between $\Delta N_2/L$ and O_2^- production by macrophages recovered from subjects who smoked only marijuana. The relationship between the diffusing capacity for carbon monoxide and O_2^- release by PAM of tobacco-only smokers and marijuana-only smokers is depicted in Fig. 3A and B, respectively. For PAM of neither tobacco-only nor marijuana-only smokers was there a correlation between O_2^- generation with DHCB + STZ stimulation and diffusing capacity as a percent of the predicted value. In analyzing the macrophages from each group of subjects, we also could not find a relationship between spontaneous O_2^- release, stimulated O_2^- production by STZ, or O_2^- generation by PMA and any abnormality of pulmonary function (data not shown).

DISCUSSION

In this study, we extend our earlier observations that tobacco smokers have evidence of small-airway dysfunction based on CV, CC, and $\Delta N_2/L$ values and that smokers of marijuana only do not (24). The slope of Phase III of the single-breath nitrogen washout curve seemed to be the parameter with the greatest alteration, as smokers of tobacco with or without marijuana had a mean value that was nearly double that of nonsmokers or marijuana-only smokers. Marijuana smoking alone did not appear to alter small-airway function in any substantive way, nor was there any evidence that marijuana had an interactive effect on small airway dysfunction in smokers of tobacco plus marijuana. On the other hand, a synergistic adverse effect of marijuana plus tobacco smoking on alveolar gas diffusion was suggested by the DL_{CO} results, although not demonstrated statistically (Table 2).

Marijuana smokers, in addition to having minimal evidence of small-airways disease, also had a spontaneous and stimulated O_2^- release by their PAM that was comparable to that of non-

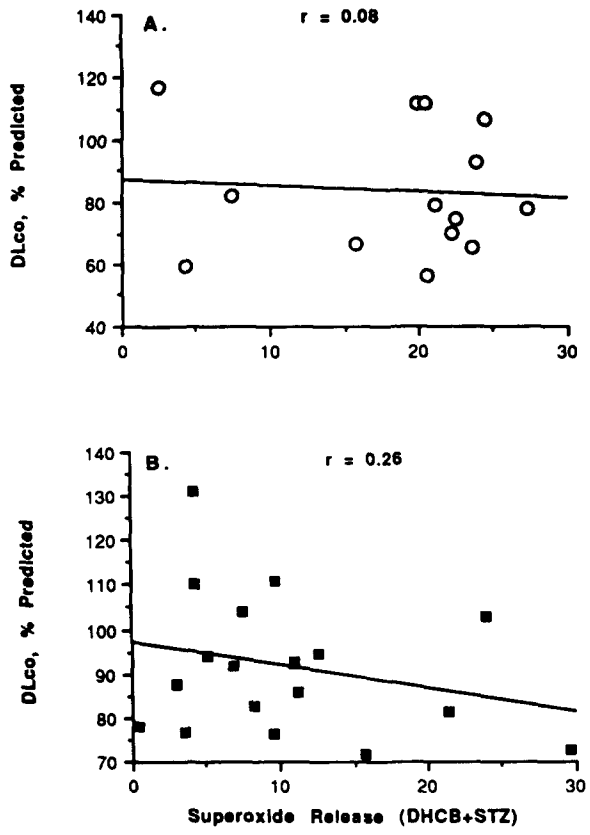

FIG. 3. Relationship between superoxide anion production by macrophages of smokers of marijuana only and tobacco only and carbon monoxide diffusing capacity (DL_{CO}). The upper graph [(A), open circles] depicts the macrophages of tobacco-only smokers, and the lower graph [(B), solid squares] shows cells of the marijuana-only smokers. The stimulus is the same as in Fig. 2, and results are expressed in the same manner.

smokers. Spontaneous release of O_2^- by PAM of marijuana-only smokers was one-half that seen in nonsmokers and 4-fold less than that of tobacco-only smokers. Looking at the macrophages of marijuana plus tobacco smokers in comparison to the other three groups gives some insight into the possible mitigating effects of marijuana on O_2^- production by PAM. There was less spontaneous O_2^- release by PAM recovered from smokers of marijuana plus tobacco compared to tobacco-only cells, although the difference was not statistically significant in this small sample. Superoxide anion release induced by STZ stimulation was also normalized in the MTS macrophages compared to PAM of tobacco-only smokers, although again the difference was not statistically significant. The smoking of marijuana plus tobacco also resulted in less O_2^- production initiated by DHCB + STZ when PAM of MTS subjects were compared to similar stimulation of TS macrophages. Whether this effect was due to marijuana or the fact that the MTS subjects used less tobacco is unknown (Table 1). The former possibility is supported by the observation that spontaneous O_2^- release by PAM of marijuana-only smokers was less than that by PAM of nonsmokers. Generation of O_2^- induced by PMA, however, remained unaffected in macrophages of marijuana plus tobacco smokers, and in fact, was slightly higher than that of the tobacco-only cells. This finding suggests that marijuana smoke affects events somewhere between receptor binding and activation of protein kinase C because PMA is thought to directly activate this enzyme (6). Whether marijuana alters superoxide generation via changes in oxidase electron transport chain assembly, cytoskeletal changes associated with oxidase activation, alterations in phosphoinositide breakdown, or several other potential mechanisms is untested (9,23). Lung macrophages from marijuana smokers may, however, prove to be probes that can answer some of the questions associated with the phagocytic respiratory burst. An alternate explanation is that marijuana stimulates the secretion of effectors that downregulate O_2^- release. Prostaglandin E_2 production may be a likely player in this scenario (13).

The differences in lung function and PAM respiratory burst characteristics noted between the tobacco versus the marijuana smokers whom we studied could be due to quantitative and/or qualitative differences in the dose of various smoke constituents delivered to the lung. Although our marijuana-smoking subjects were heavy, habitual smokers with a mean current smoking intensity of 3 joints per day, the tobacco smokers smoked a mean of 20 cigarettes per day (nearly 7 times the quantity of plant material smoked by the marijuana users). Moreover, while the marijuana smokers reported a lifetime smoking history of 62.5 joint-years (joints/day × number of years smoked), the tobacco smokers had an average lifetime history of 25.6 pack-years or 512 cigarette-years (tobacco cigarettes/day × number of years smoked), or nearly ten times the cumulative consumption of marijuana. However, it is not possible to extrapolate from the smoking history (e.g., number of cigarettes or joints smoked per day) to the actual "dose" to the lung of various constituents within the smoke. As we have emphasized elsewhere (2, 25, 26), the "dose" to the lung is affected by a number of variables in addition to the number of cigarettes smoked [e.g., the proportion of the cigarette or joint consumed (i.e., butt length), presence or absence of a filter, and smoking topography]. We have shown, for example, that marijuana smokers take larger puff volumes, inhale the smoke more deeply and retain the smoke in their lungs about four times longer than tobacco smokers (26). Moreover, the longer breathholding time characteristic of marijuana smoking, in comparison with tobacco smoking, is associated with a significantly larger amount of inhaled tar and a still larger amount of tar retained in the lung (25). On a weight-per-weight basis, we have also demonstrated that smoking one marijuana joint was associated with a four-fold greater deposition of tar in the lungs than that resulting from the smoking of a single tobacco cigarette (26). Thus the average of three joints of marijuana per day currently smoked by the subjects in the present study might be equivalent, in terms of pulmonary hazards, to smoking more than a half of a pack of tobacco cigarettes a day. It is also of interest that the number of tobacco (but not marijuana) cigarettes smoked per day was strongly correlated with PMA-stimulated O_2^- production by PAM, whereas the latter was not correlated with lifetime smoking history for either tobacco or marijuana. We believe, therefore, that the differences between PAM respiratory burst characteristics of marijuana and tobacco smokers are more likely due to qualitative differences between marijuana and tobacco smoke components than quantitative differences in the total "dose" of smoke constituents delivered to the lung.

Hubbard et al. (16) have suggested that O_2^- release by PAM of tobacco smokers is an important pathophysiologic event in emphysema via α-1-antitrypsin inactivation. The present study was unable to confirm this assumption as no relationships were found between spontaneous or stimulated O_2^- production by PAM and either small-airway or diffusion abnormalities in subjects who smoked either tobacco only or tobacco plus marijuana. Perhaps seeking a relationship between O_2^- generation by PAM and a pulmonary function variable is too insensitive a method to

establish pathophysiologic mechanisms. Such a comparison excludes the effects of oxidants within smoke and reactive oxygen intermediates released from interstitial and intravascular macrophages and neutrophils. Taken together, the combined effects of these oxidants may be sufficient to inactivate α-1-antitrypsin, thereby allowing protease action on the extracellular matrix to proceed at an accelerated rate. At present, there are no methods to quantitate the different phagocytes residing in varying compartments of the lung over a protracted period of time, and their combined release of oxidants over this same time interval also cannot be determined. Thus it is difficult to correlate intrapulmonary phagocyte numbers and their oxidant release with indices of small-airway physiology at one point in time. Somewhat reassuring are the respiratory burst studies of PAM and lung function measurements that were made in marijuana-only smokers. Based on near-normal O_2^- production by PAM under basal and stimulated conditions and the fact that tests of small-airways function are minimally altered compared to normals, marijuana-only smokers may be at some reduced risk of emphysema than their tobacco-smoking counterparts. However, this conclusion must be viewed with caution because neutrophils are also present in the alveoli of marijuana-only smokers in increased numbers (2). Like the smoking of tobacco alone, marijuana-only smoking may increase neutrophils in the submucosal regions of the airways, the pulmonary interstitium and capillaries of the lung, and their contribution to ultimate lung injury in this smoking population remains undefined at present. The latter point about neutrophils and other pathophysiologic mechanisms leading to pulmonary disease is emphasized by previously observed reductions in specific airway conductance in smokers of marijuana only compared to nonsmokers (24).

In summary, pulmonary alveolar macrophages recovered from marijuana-only smokers have a spontaneous and stimulated release of oxidants that is equivalent to nonsmoker's macrophages. This finding coincides with the absence of small-airways dysfunction or diffusion abnormality in both groups of subjects. In contrast, pulmonary alveolar macrophages from tobacco smokers generate more oxidants under basal and stimulated conditions, and tobacco smokers do have demonstrable evidence of small-airways disease. No relationship could be established, however, between the magnitude of oxidants released by tobacco smokers' macrophages and the severity of their airways dysfunction. Either our methods are too insensitive to detect such a relationship or other pathobiologic mechanisms predominate in the pathogenesis of small-airways disease.

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