

Airway Inflammation in Young Marijuana and Tobacco Smokers

MICHAEL D. ROTH, ASHIM ARORA, SANFORD H. BARSKY, ERIC C. KLEERUP, MICHAEL SIMMONS, and DONALD P. TASHKIN

Division of Pulmonary and Critical Care Medicine, Department of Medicine; Department of Pathology; and the Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, California

Forty healthy young subjects, ages 20 to 49 yr, underwent videobronchoscopy, mucosal biopsy, and bronchial lavage to evaluate the airway inflammation produced by habitual smoking of marijuana and/or tobacco. Videotapes were graded in a blinded manner for central airway erythema, edema, and airway secretions using a modified visual bronchitis index. The bronchitis index scores were significantly higher in marijuana smokers (MS), tobacco smokers (TS), and in combined marijuana/tobacco smokers (MTS), than in nonsmokers (NS). As a pathologic correlate, mucosal biopsies were evaluated for the presence of vascular hyperplasia, submucosal edema, inflammatory cell infiltrates, and goblet cell hyperplasia. Biopsies were positive for two of these criteria in 97% of all smokers and for three criteria in 72%. By contrast, none of the biopsies from NS exhibited greater than one positive finding. Finally, as a measure of distal airway inflammation, neutrophil counts and interleukin-8 (IL-8) concentrations were determined in bronchial lavage fluid. The percentage of neutrophils correlated with IL-8 levels and exceeded 20% in 0 of 10 NS, 1 of 9 MS, 2 of 9 TS, and 5 of 10 MTS. We conclude that regular smoking of marijuana by young adults is associated with significant airway inflammation that is similar in frequency, type, and magnitude to that observed in the lungs of tobacco smokers. Roth MD, Arora A, Barsky SH, Kleerup EC, Simmons M, Tashkin DP. Airway inflammation in young marijuana and tobacco smokers.

AM J RESPIR CRIT CARE MED 1998;157:928-937.

Tobacco and marijuana are the two most commonly smoked substances in our society, and the use of marijuana by younger individuals has increased significantly in recent years (1, 2). In 1993, 13% of high school students and college-age adults (16% of young males) reported active marijuana use and 2.5% (3.5% of males) admitted to daily use. In the same surveys, 21% of these young adults also admitted to daily tobacco smoking. Paralleling this rising trend in smoking among young persons is the increasing perception that smoking is not harmful. In 1993, fewer young adults perceived regular smoking of marijuana to be harmful than at any time since 1987 (2). Similarly, despite significant adverse publicity, 30% of high school seniors did not consider tobacco smoking as a serious health risk (2). This latter attitude may reflect a perception that the consequences of smoking (cardiovascular disease, emphysema, chronic bronchitis, and cancer) only affect the elderly.

We previously reported that while 20 to 26% of young smokers of marijuana and/or tobacco have some symptoms of chronic bronchitis (3), as many as 80% have evidence of cellular atypia and mucosal metaplasia when their major bronchi

are biopsied and analyzed (4-6). These observations suggest that even "asymptomatic" smoking is associated with significant mucosal damage. To further address the early effects of marijuana and tobacco smoking on the lung, we performed bronchoscopy on a small cohort of nonsmokers (NS) and relatively asymptomatic young smokers of marijuana alone (MS), tobacco alone (TS), or both marijuana and tobacco (MTS). We adapted the visual bronchitis index developed by Thompson and associates (7, 8) as a semiquantitative tool to determine the presence and extent of airway erythema, edema, and hypersecretion. We also performed endobronchial biopsies on the same subjects in order to correlate our visual observations with histopathologic evidence of airway inflammation. Finally, bronchial lavage was performed to evaluate the distal airways for evidence of neutrophilia and/or elevations in interleukin-8 (IL-8). Our findings indicate that endobronchial inflammation occurs in a large proportion of young marijuana and/or tobacco smokers. These inflammatory changes may be extensive and occur even in the absence of any symptoms or abnormalities on routine spirometry. A greater awareness of the injurious effects of smoking in young individuals is needed and could be an important tool in stemming the tide of increased use of marijuana and tobacco.

METHODS

Subjects

Endobronchial inflammation was evaluated in four groups of healthy volunteers who were part of ongoing studies to evaluate the effects of inhaled substance abuse on the lung: 10 NS, 10 MS, 10 TS, and 10

(Received in original form January 9, 1997 and in revised form September 16, 1997)

Supported by National Institutes of Health/National Institute on Drug Abuse (NIH/NIDA) Grant No. R01 DA03018.

Correspondence and requests for reprints should be addressed to Michael D. Roth, M.D., Division of Pulmonary and Critical Care, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90095-1690. E-mail: mroth@med1.medsch.ucla.edu

Am J Respir Crit Care Med Vol 157. pp 928-937, 1998

MTS. Subjects all resided within the Los Angeles metropolitan area and were recruited by both personal referral and media advertisements as previously described (3, 9). Their candidacy and smoking status were confirmed by detailed questionnaire at the time of entry into the study and again at the time of bronchoscopy. Inclusion criteria included an age between 21 and 50 yr and an appropriate smoking status. Nonsmokers had never smoked marijuana or tobacco regularly and had no smoking exposure in the preceding 5 yr. Marijuana smokers had a minimum history of 2 joint-years, were currently smoking a minimum of 5 joints/week, never routinely smoked tobacco (except for one subject who stopped smoking tobacco in 1980), and had no tobacco exposure within the past 2 yr. Tobacco smokers had a minimum history of 5 pack-years, were currently smoking at least a half pack/day for the last 1 yr, had no history of regular marijuana use (except for one subject who stopped smoking marijuana in 1975) and no marijuana exposure within the last 6 mo prior to study. Combined marijuana and tobacco smokers met the minimum criteria for both substances. Exclusion criteria included: upper respiratory tract infection in the 4 wk preceding bronchoscopy, use of any other illicit substances in the past 6 mo (phencyclidine, freebase cocaine, any intravenous drug), use of any prescription medication, or history of chronic bronchitis, emphysema, asthma, sinusitis, or any significant coexisting cardiopulmonary condition. All subjects completed a screening physical examination, demonstrated normal findings on a 12-lead electrocardiogram, were HIV-negative by serology, and had normal coagulation as measured by platelet count, prothrombin time, and partial thromboplastin time.

Spirometry

All subjects underwent spirometry with determination of FVC, FEV₁, and FEF_{25-75%} according to standard American Thoracic Society criteria (10). Percent predicted values were calculated using the equations of Morris and coworkers (11).

Bronchoscopy

Written informed consent was obtained from all subjects in accordance with requirements established by the University of California, Los Angeles (UCLA) Human Subject Protection Committee. All bronchoscopies were performed in the outpatient bronchoscopy suite of the UCLA Medical Center. In preparation for the study, an intravenous catheter was inserted, the subjects were premedicated with 0.5 mg of intravenous atropine, and the upper airway was anesthetized with nebulized 4% lidocaine and topical 20% benzocaine spray. All subjects were sedated with intravenous midazolam (in increments of 0.5 mg) and meperidine (in 25-mg increments) to achieve conscious sedation according to established UCLA guidelines. An Olympus 20D bronchoscope (Olympus Corporation of America, Hyde Park,

NY) attached to a LIFE videorecording system (Xillix Technologies Corp., Richmond, British Columbia) was used for all procedures, and the sessions were recorded on superVHS videotape (12, 13). The imaging system was color-calibrated before each procedure. The bronchoscope was introduced transorally, and the vocal cords, trachea, carina, right and left mainstem bronchi, lobar bronchi and their immediate branches (up to the third order) were inspected in a methodical manner. In order to reduce coughing and mucosal trauma, 2-ml aliquots of 2% lidocaine were applied locally before entering each major bronchial subdivision. Bronchial lavages were performed by wedging the tip of the bronchoscope into first the medial, and then the lateral, subsegment of the right middle lobe (RML), instilling 20 ml of room temperature normal saline into each segment, and then manually aspirating with a syringe. Endobronchial biopsies were taken at five different carinae using a 5-French cupped biopsy forceps (Mill-Rose Laboratories, Inc., Mentor, OH): medial-basal segment of the right lower lobe (RLL); right upper lobe (RUL); anterior-basal segment of the left lower lobe (LLL); bifurcation between the left upper lobe (LUL) and lingula; and the main carina (Figure 1). Biopsy specimens were immediately placed in either 10% formalin or 95% alcohol fixative.

Visual Bronchitis Index

The video recordings from all bronchoscopies were reviewed at the completion of the study by an experienced bronchoscopist (MDR) who was blinded with respect to the identity and smoking status of the subjects. This strategy was designed to eliminate the interobserver and temporal variabilities that occur when different bronchoscopists score the airways at the time of each individual study. The central airways were evaluated according to a modified visual bronchitis index (BI) (7, 8) in order to determine the presence and degree of mucosal erythema, edema, and secretions. Each parameter was scored on a scale of 0 to 3 as defined in Figure 1. Results were recorded as the worst abnormality observed within each of three tracheobronchial zones as shown in Figure 1, upper zone (distal trachea, proximal mainstem bronchi, and RUL), middle zone (bronchus intermedius, distal left mainstem, and LUL/lingula) and lower zone (RLL and LLL). The total score for each parameter was the sum of scores from each of these three zones (maximum score of 9 points). The cumulative BI for each subject was determined by adding the total scores for all three parameters (maximum score of 27 points). Mucosal friability, a criterion included in the BI described by Thompson and associates (7), was not included in this study.

Histopathology

Bronchial biopsies were paraffin-embedded, oriented with the help of a dissecting microscope, and processed for routine light microscopy. Alcian blue staining was performed in addition to routine hematoxy-

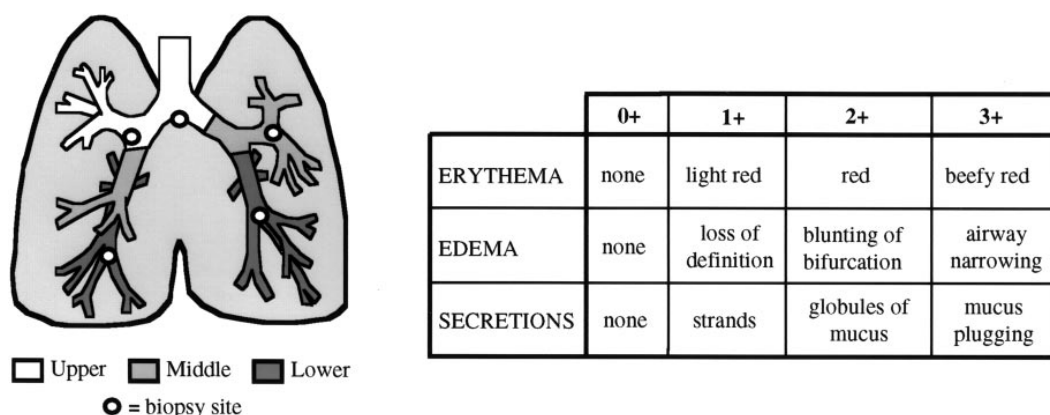


Figure 1. The bronchitis index. The tracheobronchial tree was divided into three zones: upper (distal trachea, right and proximal left mainstems, and RUL); middle (bronchus intermedius, RML, distal left mainstem, lingula, and LUL); and lower (RLL and LLL). Each zone was evaluated for the presence and degree of erythema, edema, and secretions using the 0 to 3+ scale as shown. Endobronchial biopsies were obtained at five different sites (open circles).

lin-eosin in order to better delineate the extracellular matrix of the submucosa. Biopsies for each subject were scored in a blinded manner by a lung pathologist (SHB) for the presence (in any section) and severity of four criteria: vascular hyperplasia as a correlate of mucosal erythema; submucosal edema and inflammatory cell infiltration as two correlates of airway edema; and goblet cell hyperplasia as a correlate of increased airway secretions. Vascular hyperplasia was measured semiquantitatively on a scale of 0 to 2+, with 0 representing a normal mucosa with scant capillaries; 1+ representing a moderate increase in submucosal capillaries; and 2+ representing a marked proliferation of capillaries throughout the submucosa with associated vascular ectasia. Submucosal edema was also evaluated semiquantitatively on a 0 to 2+ scale by observing the degree of separation between collagen fibers on sections stained with Alcian blue. Specimens with a normal submucosa were scored as 0; biopsies demonstrating focal deposits of edema were scored as 1+; and samples with large pools of submucosal fluid that completely disrupted collagen structures were scored as 2+. Submucosal infiltrates were identified by the presence of nests of inflammatory cells in the subepithelium. Goblet cells are normally present on bronchial biopsies but are interspersed among reserve cells and ciliated columnar cells, and usually represent less than 25% of the epithelial surface layer. Goblet cell hyperplasia was defined as an overall increase in goblet cell number and density. The increase could manifest itself as either lateral spread across a field, or as a vertical proliferation of goblet cells layered on top of each other.

Bronchial Lavage Analysis

Lavage samples from the two segments were passed through a 100- μ m sterile nylon filter (Becton Dickinson, San Jose, CA) to remove mucus and particulates, pooled, and centrifuged at $200 \times g$ for 8 min at 4°C. Supernatants were collected and immediately stored at -80°C. Cell pellets were washed in RPMI-1640 culture media (Bio-Whittaker; Walkersville, MD) and cytocentrifuge slides prepared as previously described (14). IL-8 concentrations in bronchial lavage fluid were determined with an IL-8-specific ELISA kit (Genzyme Corp., Cambridge, MA) according to the manufacturer's protocol. Samples in aliquots of 100 μ l were analyzed in duplicate with cytokine standards and measured on a microplate reader (Spectra/SLT Lab Instruments, Salzburg, Austria). A standard curve was constructed and sample values determined using automated regression software (winSeLect; Tecan U.S. Inc., Research Triangle Park, NC). This assay measured IL-8 concentrations in the range of 8 to 512 pg/ml with an average coefficient of variability of < 8%. Cytocentrifuge slides were stained with Wright-Giemsa and 400 cells counted in order to determine the percentage of neutrophils, lymphocytes, and alveolar macrophages.

Statistical Analysis

Results are presented as mean values $\pm$ SD unless otherwise stated. The age and spirometric values for all groups were compared using a one-way analysis of variance (ANOVA) with multiple comparisons testing by the method of Scheffé. Current and lifetime smoking amounts were compared between smokers of each substance alone and smokers of both (e.g., TS versus MTS for tobacco smoking amounts) using Student's *t* test. Bronchitis index scores were compared between groups using several different approaches, including *t* tests when comparing the scores as continuous measures, and chi-square and Fisher exact tests when representing and comparing the scores as either normal or abnormal. The total scores for the different lung zones were compared using two-way ANOVA blocking on subjects and Scheffé multiple comparison testing. Both Pearson and Spearman correlations were performed between BI and age for NS and for all smokers of any substance, and for current and lifetime smoking amounts among appropriate smoking groups (e.g., tobacco smoking amounts among TS). The same approach was used to identify correlations between smoking history (both current and lifetime) and percent neutrophils in the bronchial lavage; between spirometry results and BI scores; and between BI scores and histopathologic findings. Histopathology results were compared between NS and each smoking group, and between NS and all smokers combined, using chi-square and Fisher exact tests. A *p* value $\leq$ 0.05 was considered statistically significant for all tests.

RESULTS

Subject Demographics

Of the 40 subjects evaluated in this study, 77% were men and 23% were women, 57% were Caucasian, 28% were African American, 7.5% were Hispanic, and 7.5% were Asian. The male predominance of these subjects is characteristic of the smoking population in general (2). All of the smoking groups were, on average, somewhat older than the NS population (see Table 1). However, within the age ranges studied, there were no direct correlations between the different outcomes and increasing age. Marijuana smoking habits were quite variable, ranged from 0.5 to 13 joints/day and from 2 to 202 joint-years (joints/day $\times$ number of years), but current use was similar for both MS and MTS. Tobacco smoking also varied between subjects, from 7 to 40 cigarettes/day and from 6 to 75 pack-years (packs/day $\times$ years), but current usage of tobacco was significantly greater in TS than MTS (26 ± 9.6 versus 15 ± 7.6 cigarettes/day). On average, the bronchoscopy was performed 4.4 ± 4.3 h after smoking the last cigarette and 9.8 ± 6.9 h after smoking the last marijuana cigarette (excluding two subjects from the MTS group who had not smoked marijuana for 8 or 30 d).

Symptoms and Pulmonary Function

Subjects were queried regarding the presence of cough, sputum, and/or wheezing. Whereas none of the NS described any of these respiratory symptoms, 20% of smokers from each category noted cough, 20% from each smoking group noted sputum production, and 10 to 20% of smokers from each group noted wheezing (Table 1). On average, screening pulmonary function tests were within normal limits for all smoking and nonsmoking categories. However, one MTS had a low FEF_{25-75%} (43% of predicted) and a borderline low FEV₁ (72% of predicted). This subject noted a chronic cough but denied sputum production or wheezing. No correlation was found between the presence of symptoms, or abnormal spirometry results, and the objective outcomes of this study (BI, histopathology, or neutrophil scores).

Bronchitis Index

Visual inspection of the central airways was performed to detect mucosal changes induced by chronic smoking of marijuana and/or tobacco. Each component of the BI (erythema, edema, and sections) was evaluated separately. Mucosal erythema was a very frequent finding, occurring to some degree in 95% of our

TABLE 1
SUBJECT DEMOGRAPHICS

	NS (n = 10)	MS (n = 10)	TS (n = 10)	MTS (n = 10)
Sex	6 M, 4 F	9 M, 1 F	8 M, 2 F	8 M, 2 F
Age, yr	27 ± 6.8	33 ± 10.3	$40 \pm 7.2^{\dagger}$	36 ± 9.6
Pack-years	—	1.4 ± 4.4	22 ± 10	22 ± 22.8
Cigarettes/day*	—	—	$26 \pm 9.6^{\S}$	15 ± 7.6
Joint-years	—	71 ± 81.8	0.8 ± 2.2	52 ± 68
Joints/day*	—	5 ± 5.2	—	3 ± 3.4
Cough, % [†]	0	20	20	10
Sputum, %	0	20	20	20
Wheeze, %	0	20	20	10
FVC, % pred	104 ± 13	109 ± 9	101 ± 17	101 ± 17
FEV ₁ , % pred	109 ± 9	113 ± 6	107 ± 15	100 ± 15
FEF _{25-75%} , % pred	115 ± 23	107 ± 19	103 ± 30	93 ± 29

* Smoking use in the week preceding bronchoscopy.

[†] Percentage of subjects describing symptoms.

[‡] *p* $\leq$ 0.05 compared with NS.

[§] *p* $\leq$ 0.05 compared with MTS.

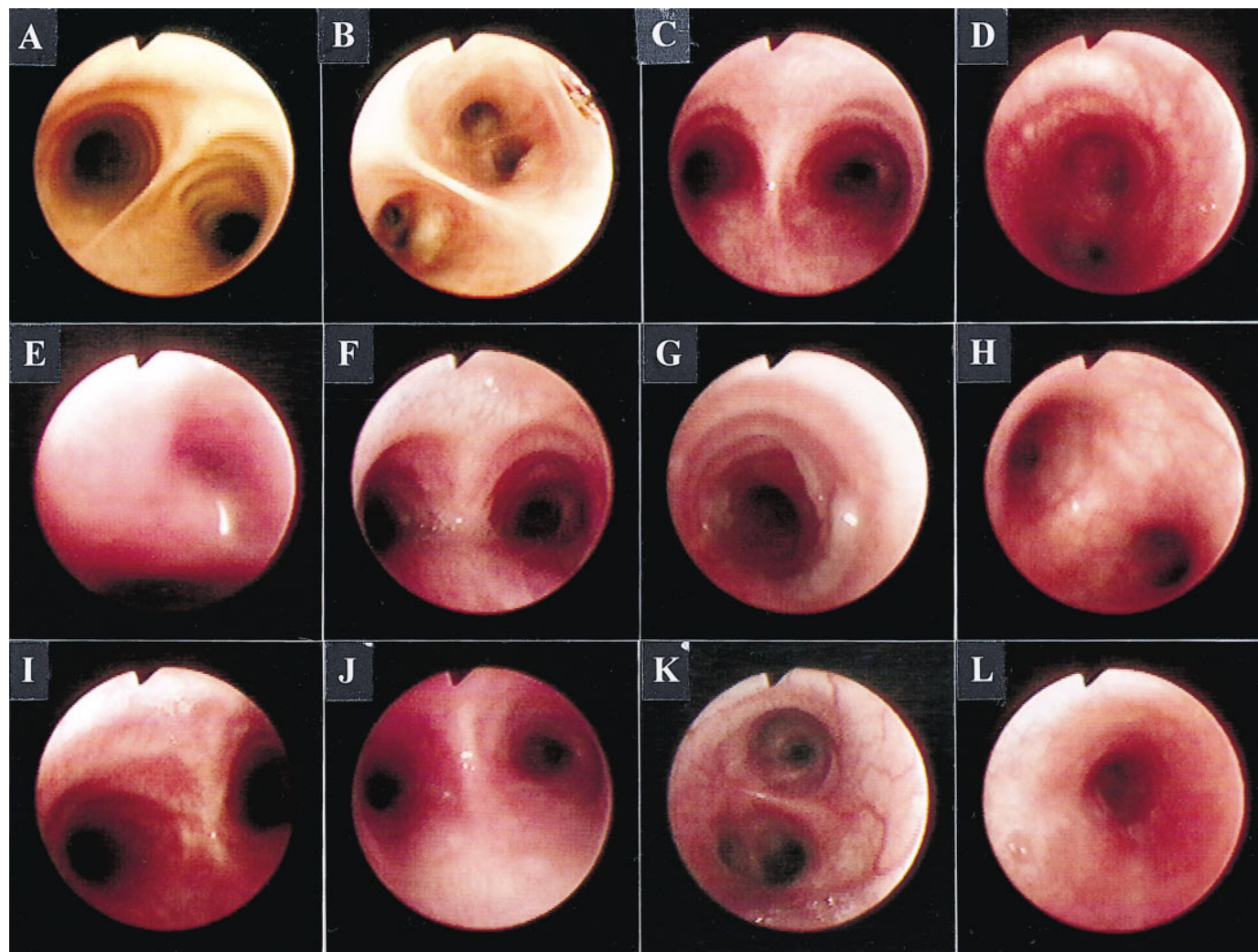


Figure 2. Representative photographs of the bronchial mucosa in our NS (A–B), MS (C–E), TS (F–H), and MTS (I–L) subjects. Pictures were reproduced from the SuperVHS recording performed during bronchoscopy. Each picture is from a different subject and was scored for the presence and severity of erythema (ery), edema (ed), and secretions (sec) according to the visual BI. (A) NS, main carina, ery 1+. (B) NS, carina between LUL/LLL, normal. (C) MS, main carina, ery 2+, ed 2+. (D) MS, distal left mainstem, ery 3+, ed 1+. (E) MS, orifice superior segment of RLL, ery 2+, ed 3+. (F) TS, main carina, ery 2+, ed 2+, sec 1+. (G) TS, left mainstem, ery 2+, ed 1+, sec 2+. (H) TS, carina between LUL/lingula, ery 2+, ed 2+. (I) MTS, main carina, ery 3+, ed 2+. (J) MTS, main carina, ery 2+, ed 3+. (K) MTS, carina between LUL/LLL, ery 3+, ed 1+. (L) MTS, left mainstem, ery 2+, ed 3+.

subjects, both smoking and nonsmoking. On close inspection, 1+ erythema was primarily due to diffuse hyperemia of mucosal capillaries, while 2–3+ erythema was a combination of microvascular hyperemia, engorgement of larger-appearing vessels, and diffuse mucosal color changes associated with airway edema (Figure 2). By setting a total erythema score of > 4 as abnormal, no NS were categorized as having abnormal erythema compared with 50% of MS, 50% of TS, and 60% of MTS (Figure 3). Whereas the erythema scores for many smokers were in the normal range (14 of 30), the average erythema score was statistically different between TS and NS, and between MTS and NS. The average erythema score in the MS group was the highest, yet the difference between MS and NS did not quite reach statistical significance ($p = 0.07$) due to considerable intersubject variability.

Airway edema in smokers, as a group, was statistically greater than that observed in NS. However, there was considerable overlap and only 2 MS, 1 TS, and 3 MTS were rated with cumulative edema scores greater than the highest score

assigned to a NS (Figure 3). Of these smokers, both of the MS and two of the three MTS demonstrated frank airway narrowing from edema (categorized as 3+ edema), including narrowing of the mainstem bronchi (Figure 2J, 2L) and complete obstruction of the RLL superior segment orifice in one subject (Figure 2E).

Other than mucoid stranding ($> 1+$), airway secretions were relatively infrequent in our study. However, only 2 of 10 NS exhibited any secretions at all, while 40% of MS, 70% of TS, and 50% of MTS demonstrated at least 1+ secretions. As a group, mucoid secretions were significantly more common in smokers than NS.

When the individual parameters of erythema, edema, and secretions were combined into a cumulative BI score, a clear difference was observed between NS and each of the smoking groups. The mean BI score for NS was 4.4 ± 1.6 , while that for MS was 8.2 ± 5.4 , that for TS was 8.0 ± 2.5 , and that for MTS was 8.5 ± 2.9 (Figure 4). Analyzed in a different manner, none of the NS had a BI score > 7 , whereas 50% of MS, 50% of TS,

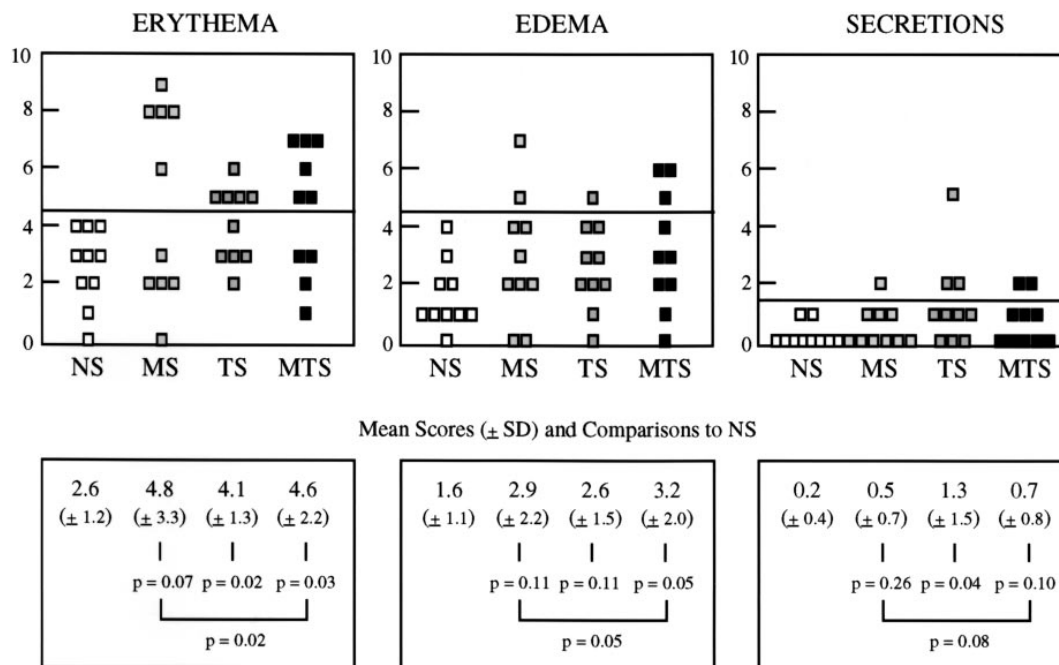
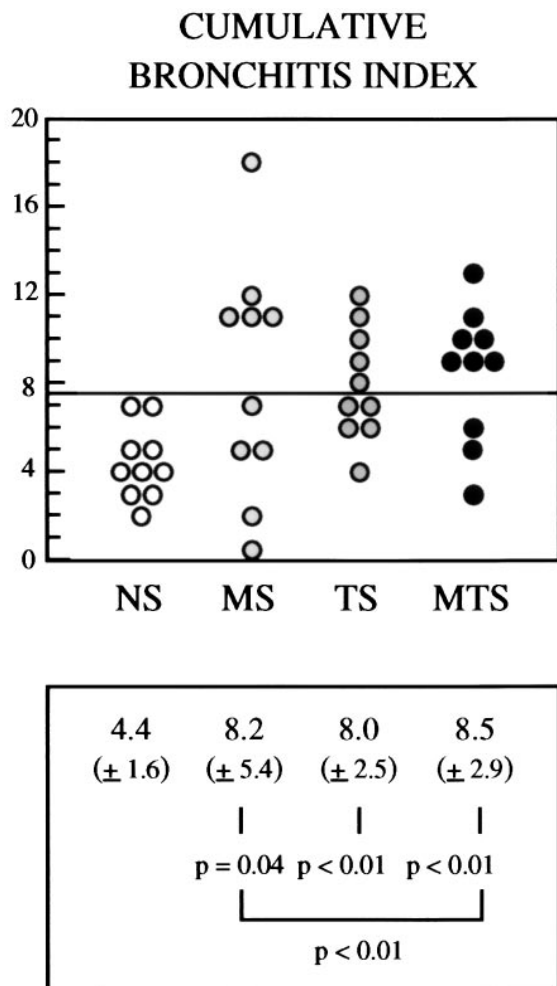


Figure 3. Bronchitis index scores for each subject according to parameter (erythema, edema, or secretions) and subject category (NS, MS, TS, MTS). The individual scores for each parameter were obtained by summing scores (0, 1+, 2+, or 3+) from each of the three tracheobronchial zones (maximum score of 9). All scores for NS subjects fell below the horizontal lines. The mean score (± SD) for each group is listed below and compared with the NS group (individually or all smoking groups pooled together) using a *t* test.



and 70% of MTS had BI values > 7. Using the criteria described in this study, both marijuana and tobacco smoking were associated with visual evidence of central airways inflammation in a majority of subjects. When analyzed according to airway location, the inflammatory changes in smokers were significantly more prominent in the upper central airways (BI = 3.47) than in the lower central airways (BI = 2.13, Figure 5). However, our results did not demonstrate any significant relationship between BI score and subject age, current or lifetime smoking histories, symptoms, or spirometry results.

Histopathology

Mucosal injury was also assessed by obtaining endobronchial biopsies from each subject and using light microscopy to determine the presence and severity of microvascular hyperplasia, submucosal edema and infiltrates, and goblet cell hyperplasia (Figure 6). In contrast to the visual finding of erythema in almost all of the subjects (Figure 2), the hyperplasia and ectasia of capillaries in the submucosa was an abnormality observed exclusively, and with high prevalence, in the smoking groups (Table 2). Histologic evidence of mucosal inflammation, including submucosal edema and the presence of inflammatory cells, occurred in 75 to 100% of smokers, as compared with 20 to 40% of NS. The degree of edema, when present, was greater in smokers than nonsmokers. Biopsies from two-thirds

Figure 4. Cumulative BI scores for all subjects by smoking category (NS, MS, TS, and MTS). The cumulative score for each subject was obtained by summing the total scores from each of the three parameters (erythema, edema, and secretions). All scores for NS subjects fell below the horizontal line. The mean score (± SD) for each group is listed below and compared with the NS group (individually or all smoking groups pooled together) using a *t* test.

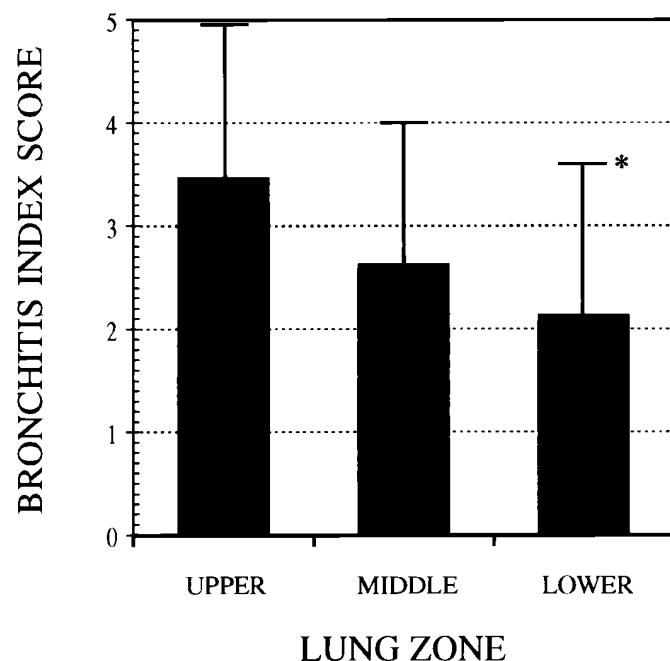


Figure 5. The BI was determined for all 30 smoking subjects (MS, TS, and MTS) according to the three different lung zones as defined in Figure 1. The BI for the upper zone was significantly greater than for the lower zone ($p \leq 0.05$).

of the smokers were graded as having 2+ edema, whereas biopsies from NS were graded as either normal or 1+. Goblet cell hyperplasia is only one of many mechanisms responsible for the increased mucoid secretions observed in inflamed airways. Despite this caveat, goblet cell hyperplasia was a frequent finding in smokers of marijuana and/or tobacco, although more so in TS and MTS than in MS (80 to 89% versus 60%).

In total, 100% of the biopsies from smoking subjects were graded as abnormal in at least one respect, 97% were abnormal by at least two criteria, 72% were abnormal by at least three of the criteria, and 48% were abnormal by all four criteria. By comparison, none of the biopsies from NS were positive for more than one histopathologic finding. For every parameter (erythema versus vascular hyperplasia; edema versus submucosal edema and/or infiltrates; or mucoid hypersecretion versus goblet cell hyperplasia), and for every smoking group, the number of subjects graded as abnormal by histopathologic criteria were greater than the number of subjects graded as abnormal by visual criteria. However, no significant correlations were observed between the presence or absence of an abnormality by histopathology and the cumulative score for the same parameter as measured by the BI.

Bronchial Lavage Findings

Bronchial lavage samples were evaluated for neutrophils as a measure of acute inflammation in the distal conducting airways (Figure 7). In every case, the percentage of neutrophils was $\leq 20\%$ in the lavage fluid from NS. In contrast, neutrophil counts in excess of 20% were observed in 1 of 9 MS, 2 of 9 TS, and 5 of 10 MTS. The finding of elevated percent neutrophils was only significant when the results for MTS were compared with those for NS or MS. As a correlate to neutrophilia, we also measured concentrations of the neutrophil chemotactic cytokine IL-8 in the lavage fluid. A similar pattern was observed to that for neutrophil counts, with significantly elevated levels of IL-8 found only in the samples from MTS (Figure 7). A linear regression analysis demonstrated a correlation between IL-8 levels and the percentage of neutrophils found in bronchial lavages (Figure 7). No significant correlations were observed between neutrophil counts and subject age, current or lifetime smoking histories, symptoms, spirometry results, or BI scores.

DISCUSSION

This study follows up on earlier findings and investigates two common misconceptions about marijuana and tobacco; first, that smoking marijuana does not injure the lung, and second, that smoking tobacco affects the lung infrequently and only in older, chronic smokers. To address these issues, we recruited young smokers of either marijuana, tobacco, or both. Using a combination of history, physical examination, and spirometry, we did not find any evidence of overt lung disease in these subjects—only a low incidence of cough, sputum, or wheeze. However, using videobronchoscopy, mucosal biopsy, and bronchial lavage, we found evidence of central airway inflammation in almost every smoker and signs of distal airway inflammation in one-half of MTS. The visual evidence of airway injury was at times striking. The cumulative BI scores were higher than normal (i.e., than that observed in any NS) in 57% of the smokers. Furthermore, histopathology identified at least two concurrent inflammatory changes (out of four) in 97% of the smokers as compared with none of the NS. While our small sample of recruited subjects may not represent the entire spectrum of smokers, these findings do suggest that habitual smoking of marijuana and/or tobacco produces airway inflammation, that this injury occurs in a high percentage of otherwise healthy smokers, and that routine physical examination and spirometry are insensitive measures of this injury.

The visual scoring used in our analysis was adapted from the bronchitis index described by Thompson and associates (7, 8). We made three modifications to their original technique. First, we videotaped the bronchoscopies in order to review them later in a blinded manner. Although not completely free

TABLE 2
HISTOPATHOLOGY OF BRONCHIAL BIOPSIES

	NS		MS		TS		MTS	
	% positive*	(average score) [†]	% positive	(average score) [†]	% positive	(average score) [†]	% positive	(average score) [†]
Vascular proliferation	0	(0)	70 [‡]	(0.9)	56 [‡]	(0.67)	89 [‡]	(1.1)
Edema	20	(0.2)	75 [‡]	(1.5)	88 [‡]	(1.2)	75 [‡]	(1.1)
Infiltrates	40		90		100 [‡]		100 [‡]	
Goblet cell hyperplasia	20		60		89 [‡]		80 [‡]	

* % of subjects with finding.

[†] (Average severity score on 0–2+ scale).

[‡] $p \leq 0.05$ compared with NS.

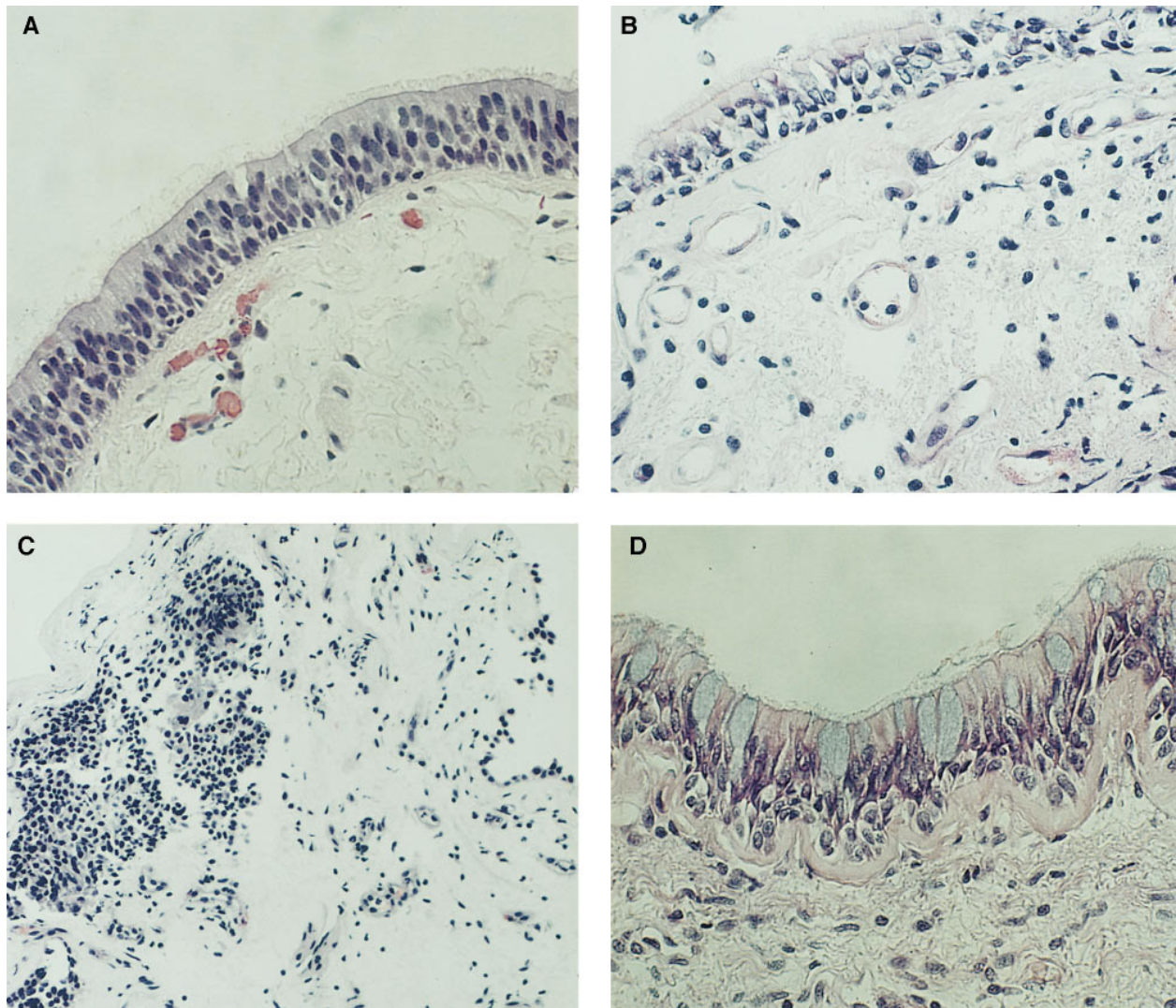


Figure 6. Representative photomicrographs of mucosal biopsies demonstrating normal mucosal histology (A); vascular hyperplasia and ectasia (B); submucosal edema and infiltrates (C); and goblet cell hyperplasia (D). Magnification $\times 320$ (C) or $\times 400$ (A, B, D). Hematoxylin-eosin stain.

from bias, this approach was more convenient and allowed for a single trained reviewer to score all of the bronchoscopies. Second, we eliminated friability as a scoring category because of its low incidence in our subject population. Finally, we di-

vided the central airway into regions and used the worst abnormality within a region as the recorded score. In contrast to the work by Thompson and colleagues (7, 8), which primarily evaluated patients with chronic bronchitis and asymptomatic

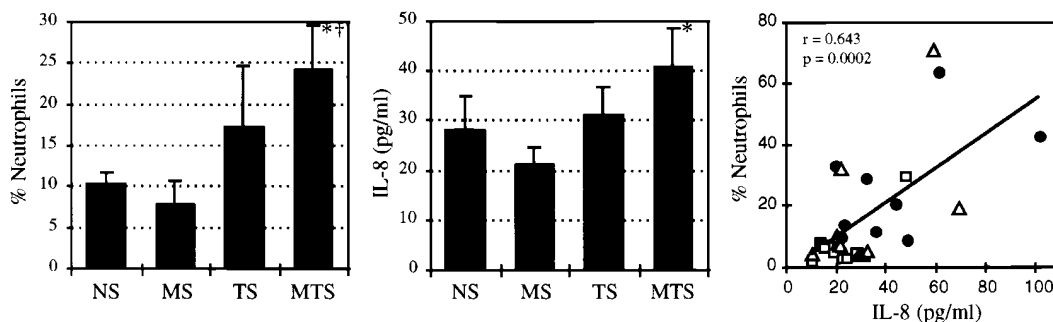


Figure 7. Bronchial lavage samples were analyzed for IL-8 concentration by cytokine-specific ELISA and cytocentrifuge preparations were stained with Wright-Giemsa and the percentage of neutrophils determined by counting 400 cells under the microscope. Bronchial lavage neutrophil counts were higher in MTS as compared with either NS ($^{\dagger}p < 0.05$) or MS ($^{*}p < 0.05$) and bronchial lavage IL-8 levels were higher in MTS as compared with MS ($^{*}p < 0.05$). A significant correlation existed between these two measurements for all of the smoking subjects (open squares, MS; open triangles, TS; closed circles, MTS).

tobacco smokers, we used this approach to evaluate nonsmokers as compared with otherwise healthy smokers of marijuana and tobacco. Despite these differences, our visual findings were similar and we noted a high incidence of erythema, edema, and airway secretions in both marijuana and tobacco users. Our results validate the use of a cumulative BI score as a mechanism for detecting and quantitating the airway inflammation that develops in smokers. With respect to lifetime smoking exposure, we did not observe the same correlation between smoking history and BI score as previously noted (7, 8). This difference likely reflects the weak nature of this relationship (reported by Thompson and associates as $r = 0.285$), the relatively moderate smoking history of our subjects (averaging only 26 tobacco cigarettes/day and 22 pack-years as compared with the 50 cigarettes/day and 36 pack-years in the subjects studied by Thompson and associates), the fact that we did not include older individuals with symptomatic chronic bronchitis, and the relatively small size of our study which limited the statistical power of our analysis.

The broad-ranging histopathologic effects of marijuana and/or tobacco on the bronchial mucosa have been described in detail before and include mucosal inflammation, cellular atypia, tissue metaplasia, and even frank dysplasia (4–6). In the present study we extend these prior observations by concentrating on four indicators of acute and chronic inflammation (vascular hyperplasia, submucosal edema, infiltration by inflammatory cells, and goblet hyperplasia) as potential correlates for the different parameters of the BI. Despite this hypothesis, we failed to find any correlation between the different histopathology scores and their corresponding visual BI scores. Mucosal biopsy was far more sensitive and specific than visual scoring at detecting the inflammatory effects of smoking. There are many reasons for this. First, the BI was designed as a survey technique and uses a single “cumulative” number to describe the prevalence, extent, and severity of each finding. Distinguishing between normal and abnormal therefore depends upon the extent of visual findings—not merely their presence or absence. In contrast, the pathologic criteria were designed *a priori* to identify abnormalities in smokers and therefore are mostly a measure of prevalence. It is possible that a different approach, such as scoring each of the five biopsies separately and summing the results, might have provided a better correlation to the BI. In addition, a single pathologic feature such as vascular hyperplasia may not adequately explain a complex visual finding such as mucosal erythema. Despite these limitations, however, the comparison of visual and pathologic findings suggests several important points. For example, while some degree of airway erythema was observed in almost all subjects, including both smokers and NS, pathologic evidence of capillary hyperplasia was found only in smokers. This finding implies that mild to moderate erythema is a normal consequence of the bronchoscopy procedure itself, likely resulting from procedure-related coughing. By contrast, 56 to 89% of the smokers demonstrated smoking-related vascular changes that were statistically related to smoking. This smoking-related capillary hyperplasia has not previously been reported and deserves further evaluation. Cigarette smoking is known to activate vascular endothelium in humans (15), and animal studies have identified increased endothelial proliferation in the bronchial wall of tobacco-exposed rats (16).

The mucosa was judged to be significantly edematous (score of > 4) in only 10 to 30% of smokers by visual criteria, but submucosal edema and inflammatory cell infiltrates were present in 75 to 100% of smoking subjects according to their biopsies. Most of this discrepancy is accounted for by the cut-

off value of 4 that was used to discriminate between normal and abnormal. While this score set the specificity at 100%, it allowed many smokers with disease-related edema to be characterized as normal. However, the statistical limitations of the BI should not detract from the importance of the visual findings. The degree of airway edema was at times visually striking, including near-occlusion of a segmental orifice and dramatic narrowing of lobar and mainstem bronchi. Interestingly, none of the subjects with 3+ edema (which occurred only in smokers) had symptomatic complaints or abnormal measurements for FEV₁ or FEV₁/FVC. However, more sensitive measures of large airway narrowing, such as specific airway conductance and airway resistance, have been noted to be abnormal in marijuana and tobacco smokers in prior investigations (3). The central airway edema described in this report supports these earlier physiologic findings.

The histopathologic finding of airway edema and cellular infiltrates in a small percentage of normal nonsmoking subjects is also interesting. While these subjects were carefully screened to exclude active smoking or high-risk occupational exposures, we cannot exclude the possibility that exposure to second-hand smoke, ambient air pollution or other inhaled irritants might account for these findings (17–19). Environmental pollutants, such as ozone, are clearly capable of inducing bronchial inflammation (17). The preparation for bronchoscopy, and the coughing that sometimes develops during the procedure, may be another factor producing background levels of airway inflammation. While our study identified airway edema and cellular infiltration in some nonsmokers, the percentage of subjects with these findings and the severity of these findings were clearly less than that observed in active smokers of marijuana and tobacco.

Goblet cell hyperplasia is only one of many factors responsible for the increased secretions observed in smokers. Other factors include hypertrophy of submucosal glands, disruption of mucociliary transport by a variety of mechanical factors such as squamous metaplasia and bronchiectatic changes, and ciliotoxic effects of gas-phase smoke components which impair ciliary function (20). Whereas goblet cell hyperplasia was a frequent finding in biopsies from MS, TS, and MTS, it was somewhat less prevalent in MS than in the other smoking categories. One explanation for this heterogeneity is the frequent finding of squamous metaplasia in biopsies from MS (6). In cases of severe squamous metaplasia, both ciliated columnar cells and goblet cells were replaced. While these subjects were therefore graded as negative, they had other reasons for abnormal mucous clearance.

Perhaps the most striking result of our study is the fact that MS, who smoked an average of only a few joints per day, had the same degree of visually evident and histopathologically confirmed airway injury as that detected in tobacco smokers, who smoked 20 to 30 cigarettes per day. This finding confirms our prior observations (4). While the major constituents of marijuana and tobacco smoke are quite similar (21, 22), it is the method of smoking which likely accounts for this finding (23, 24). Marijuana is smoked as a loosely packed, unfiltered cigarette, whereas tobacco is tightly rolled and nearly always filtered. This results in a hotter smoke and a higher delivery of particulates from marijuana joints. In addition, marijuana smoke is inhaled with a larger puff volume and a 4-fold greater retention time (breathholding time) than tobacco smoke (23). Because of these differences, smoking a marijuana joint deposits over four times as much particulates in the lung than smoking an average tobacco cigarette. The same is also true for the delivery and retention of noxious gases such as carbon monoxide. The pattern of injury that we observed, which di-

minished as a function of distance down the airway, is consistent with injury from relatively large respirable particulates, water-soluble constituents of smoke, and/or heat.

In addition to directly examining the central airways, we used bronchial lavage to sample lining fluid and cells from the small conducting airways of our subjects (25, 26). Many groups have reported an increased percentage of neutrophils in bronchial samples from smokers with chronic bronchitis (27–29). Even in asymptomatic smokers, Thompson and associates (7) reported that about half of their subjects had elevated percentages of neutrophils in their bronchial lavage. The results were less striking in our subjects with only 1 of 9 MS, 2 of 9 TS, and 5 of 10 MTS exhibiting neutrophil percentages greater than that observed in any NS (> 20% neutrophils). Again, this might be explained by the relatively low intensity of tobacco smoking in our subjects. As an alternate measure of distal airway inflammation we also determined the concentration of IL-8 in bronchial lavage fluid. IL-8 is elevated in many inflammatory lung diseases and appears to be the primary cytokine responsible for neutrophil chemotactic activity in the bronchi of smokers with chronic bronchitis (30–32). IL-8 concentrations correlated with bronchial lavage neutrophil counts and were significantly increased in MTS. With respect to distal airway inflammation, it is possible that the combined smoking of marijuana and tobacco may be more injurious than smoking either substance alone. However, due to the small number of subjects and the relatively low frequency of abnormal findings, further study is warranted before drawing any firm conclusions.

In summary, our observations suggest that regular smoking of marijuana and/or tobacco by young adults is associated with a high frequency of central airway inflammation. This injury is visually evident by bronchoscopy and is sometimes quite striking. At the microscopic level, there is evidence of airway inflammation in almost all smokers. These changes occur even in the absence of any symptoms or physiologic evidence of injury. The evidence for small airways inflammation was less striking in smokers of marijuana or tobacco alone, but quite prevalent in combined MTS. Collectively, our findings strongly suggest that smoking marijuana and/or tobacco has significant injurious effects on the central and peripheral airways, even in young and otherwise asymptomatic adults.

Acknowledgment: Bronchoscopy equipment and support were provided by the UCLA Medical Plaza Outpatient Procedure Suite under the direction of Sheridan Wiggett, R.N. Excellent nursing and technical assistance were provided by Jane Glade, R.N., and Jerrine Sauntry, R.N. Subject recruitment and screening were performed by John Dermand, Laura Jeremiah, and Wesley Pfenning.

References

1. Johnston, L. D., P. M. O'Malley, and J. G. Bachman. 1994. National Survey on Drug Use from The Monitoring the Future Study, 1975–1993, Vol. I. Secondary School Students. National Institute on Drug Abuse, Government Printing Office, Washington, DC. NIH Publication No. 94-3809.
2. Johnston, L. D., P. M. O'Malley, and J. G. Bachman. 1994. National Survey on Drug Use from The Monitoring the Future Study, 1975–1993, Vol. II. College Students and Young Adults. National Institute on Drug Abuse, Government Printing Office, Washington, DC. NIH Publication No. 94-3809.
3. Tashkin, D. P., A. H. Coulson, V. A. Clark, M. Simmons, L. B. Bourque, S. Duann, G. H. Spivey, and H. Gong. 1987. Respiratory symptoms and lung function in habitual heavy smokers of marijuana alone, smokers of marijuana and tobacco, smokers of tobacco alone, and nonsmokers. *Am. Rev. Respir. Dis.* 136:209–216.
4. Gong, H., Jr., S. Fligiel, D. P. Tashkin, and R. G. Barbers. 1987. Tracheobronchial changes in habitual, heavy smokers of marijuana with and without tobacco. *Am. Rev. Respir. Dis.* 136:142–149.
5. Fligiel, S. E. G., H. Venkat, H. Gong, Jr., and D. P. Tashkin. 1988. Bronchial pathology in chronic marijuana smokers: a light and electron microscopy study. *J. Psychoactive Drugs* 20:33–42.
6. Fligiel, S. E. G., M. D. Roth, E. C. Kleerup, S. H. Barsky, and D. P. Tashkin. 1997. Tracheobronchial histopathology in habitual smokers of cocaine, marijuana and/or tobacco. *Chest* 112:319–326.
7. Thompson, A. B., D. Daughton, R. A. Robbins, J. H. Sisson, J. R. Spurzem, S. Von Essen, K. A. Rickard, D. J. Romberger, I. Rubinstein, M. Ghafouri, D. M. Daughton, and S. I. Rennard. 1993. The bronchitis index: a semiquantitative visual scale for the assessment of airways inflammation. *Chest* 103:1482–1488.
8. Sherman, M. P., L. A. Campbell, H. Gong, M. D. Roth, and D. P. Tashkin. 1991. Antimicrobial and respiratory burst characteristics of pulmonary alveolar macrophages recovered from smokers of marijuana and tobacco, and nonsmokers. *Am. Rev. Respir. Dis.* 144:1351–1356.
9. American Thoracic Society. 1995. Standardization of spirometry, 1994 update. *Am. J. Respir. Crit. Care Med.* 152:1107–1136.
10. Morris, J. F., A. Koski, and L. C. Johnson. 1971. Spirometric standards for healthy nonsmoking adults. *Am. Rev. Respir. Dis.* 103:57–67.
11. Lam, S., C. MacAulay, J. Hung, J. LeRiche, A. E. Profio, and B. Palcic. 1993. Detection of dysplasia and carcinoma in situ with a lung imaging fluorescence endoscope device. *J. Thorac. Cardiovasc. Surg.* 105:1035–1040.
12. MacAulay, C., S. Lam, P. W. Payne, J. C. LeRiche, and B. Palcic. 1995. Malignancy-associated changes in bronchial epithelial cells in biopsy specimens. *Anal. Quant. Cytol. Histol.* 17:55–61.
13. Barbers, R. G., H. Gong, Jr., D. P. Tashkin, J. Oishi, and J. M. Wallace. 1987. Differential examination of bronchoalveolar lavage cells in tobacco cigarette and marijuana smokers. *Am. Rev. Respir. Dis.* 135:1271–1275.
14. Blann, A. D., and C. N. McCollum. 1993. Adverse influence of cigarette smoking on the endothelium. *Thromb. Haemost.* 70:707–711.
15. Sekhon, H. S., J. L. Wright, and A. Churg. 1994. Cigarette smoke causes rapid cell proliferation in small airways and associated pulmonary arteries. *Am. J. Physiol.* 267(5, Pt. 1):L557–L563.
16. Scannell, C., L. Chen, R. M. Aris, I. Tager, D. Christan, R. Ferrando, B. Welch, T. Kelly, and J. R. Balmes. 1996. Greater ozone-induced inflammatory responses in subjects with asthma. *Am. J. Respir. Crit. Care Med.* 154:24–29.
17. Karlsson-Stiber, C., J. Hojer, A. Sjöholm, G. Bluhm, and H. Salmonson. 1996. Nitrogen dioxide pneumonitis in ice hockey players. *J. Internat. Med.* 239:451–456.
18. Xu, X., and B. Li. 1995. Exposure–response relationship between passive smoking and adult pulmonary function. *Am. J. Respir. Crit. Care Med.* 151:41–46.
19. U.S. Public Health Services. 1979. Smoking and Health: A Report of the Surgeon General. U.S. Government Printing Office, Washington, DC. DHEW Publication No. (PHS) 79-5000. 66:32–33.
20. Hoffman, D., D. K. Brunnerman, G. B. Gori, and E. L. Wynder. 1975. On the carcinogenicity of marijuana smoke. *Recent Adv. Phytochem.* 9:63–81.
21. Novotny, M., F. Merli, D. Weisler, M. Fencl, and T. Saeed. 1982. Fractionation and capillary gas chromatographic-mass spectrometric characterization of the neutral components in marijuana and tobacco smoke condensates. *J. Chromatogr.* 238:141–150.
22. Wu, T.-C., D. P. Tashkin, B. Djahed, and J. E. Rose. 1988. Pulmonary hazards of smoking marijuana as compared with tobacco. *N. Engl. J. Med.* 318:347–351.
23. Tashkin, D. P. 1990. Pulmonary complications of smoked substance abuse. *West. J. Med.* 152:525–530.
24. Thompson, A. B., and S. I. Rennard. 1988. Assessment of airways inflammation utilizing bronchoalveolar lavage. *Clin. Chest Med.* 9:635–642.
25. Kelly, C. A., C. J. Kotre, C. Ward, D. J. Hendrick, and E. H. Walters. 1987. Anatomical distribution of bronchoalveolar lavage fluid as assessed by digital subtraction radiography. *Thorax* 42:624–628.
26. Kuschner, W. G., A. D'Alessandro, H. Wong, and P. D. Blanc. 1996. Dose-dependent cigarette smoking-related inflammatory responses in healthy adults. *Eur. Respir. J.* 9:1989–1994.
27. Wesselius, L. J., M. E. Nelson, K. Bailey, and A. R. O'Brien-Ladner. 1997. Rapid lung cytokine accumulation and neutrophil recruitment after lipopolysaccharide inhalation by cigarette smokers and non-smokers. *J. Lab. Clin. Med.* 129:106–114.

29. Riise, G. C., S. Ahlstedt, S. Larsson, I. Enander, I. Jones, P. Larsson, and B. Andersson. 1995. Bronchial inflammation in chronic bronchitis assessed by measurement of cell products in bronchial lavage fluid. *Thorax* 50:360–365.
30. Keatings, V. M., P. D. Collins, D. M. Scott, and P. J. Barnes. 1996. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am. J. Respir. Crit. Care Med.* 153:530–534.
31. Chanez, P., I. Enander, I. Jones, P. Godard, and J. Bousque. 1996. Interleukin 8 in bronchoalveolar lavage of asthmatic and chronic bronchitis patients. *Int. Arch. Allergy Immunol.* 111:83–88.
32. Richman-Eisenstat, J. B., P. G. Jorens, C. A. Hebert, I. Ueki, and J. A. Nadel. 1993. Interleukin-8: an important chemoattractant in sputum of patients with chronic inflammatory airway diseases. *Am. J. Physiol.* 264:L413–L418.