The Relationship between Large Airway Inflammation and Airway Metaplasia*

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To assess the role of acute inftammatory cells in large airways in the pathogenesis of metaplasia, we performed HAL (divided into aliquots) and mucosal biopsies on asbestos workers. They had evidence of asbestos-related lung injury. We found that acute in8ammatory cells were significantly increased in the 6rst aliquot. Ex-smokers had a greater percentage of PMN compared with nonsmokers and current smokers. The subjects were subgrouped with respect to biopsy-detected metaplasia. There was no difference between these groups for percentage or total number of PMN in the first aliquot. However, subjects with metaplasia had significant reduction in FEV_1/FVC compared with those without. We conclude that there are significant differences in cells between the Orst and subsequent aliquots. Although in8ammatory stimuli may be important in the pathogenesis of metaplasia, PMN present in the first aliquot could not be related to the severity of the metaplastic changes in these workers. *(Chest* 1991; 100:131-35)

Dco = diffusing capacity of carbon monoxide; PMN = polymorphonuclear leukocytes; TLC = total lung capacity; TNC = totaI nucleated cells; VO = volume recovered

Individuals who smoke and were exposed to asbestos have an increased risk of pulmonary carcinoma.[1] Metaplasia is an important coexisting lesion in the airways of cigarette smokers who develop lung cancer.[2,3] Moreover, it is a common histologic feature of animal models which results ultimately in the development of lung tumors.V It also has been suggested to be an important lesion when seen in asbestos-related disease." The role of inflammatory stimuli in the pathogenesis of metaplasia and cancer has been suggested by several observations. First, inflammatory lesions in mucosal surfaces often are accompanied by metaplastic change.[2,7,8] Second, subjects with chronic inflammatory lung disease may be at an increased risk of lung cancer.[9,10] In fact, this has been suggested as an important predisposing factor in asbestos-related lung cancer.[11] Finally, inHammatory cells are capable of releasing factors in *vitro* which cause mutations in simple bacterial assay systems.[12,13] These observations suggest that inHammatory stimuli may be important in the development of both metaplasia and subsequent respiratory tract cancers.

Other investigators have noted that one may separate the sequential BAL into an early fraction composed of the first aliquot and a later fraction composed of a pool of all the subsequent aliquots.w'? Analysis of lavage Huids separated in this fashion suggests that the first aliquot derives from the large airways and that subsequent aliquots are sampling more distal lung units," Most studies of this type have shown an increase in the number of neutrophils recovered in the first aliquot.[14-16] Because of the prior suggestions that inHammatory cells might be important stimuli for metaplasia, we assessed the relationship between acute inflammatory cells in large airways and histologic metaplasia demonstrated in airway biopsies obtained from a population of subjects with significant past exposure to asbestos. This is a particularly relevant target population because they are known to have an increased risk of cancer and metaplasia in large airways." As noted below these studies confirm prior observations of others that the first aliquot is different from the pooled fluid recovered from subsequent aliquots. Moreover, these two cell populations appeared to be unrelated to one another. However, we were unable to demonstrate in these studies any relationship between inHammatory cells in the first aliquot and histologic detection of metaplasia in airway biopsy specimens.

METHODS

Study Population

Subjects with a history of occupational exposure to asbestos were recruited from an occupational medicine clinic. Clinic records were screened and workers were recruited from among a subgroup of individuals with abnormal chest radiographs indicative of significant prior asbestos exposure. A careful occupational history was obtained from each subject by a trained occupational therapist. A history of prior cigarette smoking, including age at the time the first cigarette was smoked, average packs per day, duration of smoking and years since the last cigarette was smoked, was also obtained. Full pulmonary function tests were performed after subject selection (Collins DS II, Braintree, MA). All studies were approved by the Yale Committee on Human Investigation.

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Bronchoalveolar Lavage

Bronchoscopy was performed as described previously.[19,20] Briefly, the nose and upper airways were anesthetized with a topical anesthetic and a fiberoptic bronchoscope was passed via the mouth. The airways were initially inspected carefully for abnormal lesions. If none was found, attempts were made to remove three to six mucosal biopsy specimens at random from subsegmental branch points in the right lower lobe. Attempts were terminated when the operator determined that three visible pieces had been recovered.

The bronchoscope was then maneuvered into a wedged position in the lingula, and BAL was performed by alternately instilling and aspirating 50-ml aliquots of room temperature isotonic sodium chloride solution. A total of 5 aliquots were employed.

Processing of Recovered Fluid

The recovered fluid was filtered through a single layer of sterile surgical gauze to remove gross mucous particles, and the individual aliquots were centrifuged at 1,000 g for 10 min at 4°C to pellet cellular elements. Cells from the first aliquot were processed alone, whereas the cells recovered from subsequent aliquots were pooled for further analysis. Fifty to 100,000 cells from each cell population were subsequently pelleted onto glass slides by cytocentrifugation and stained with modified Wright-Giemsa (Dif-quick, Harleco) for subsequent analysis.

Processing of Biopsies

Biopsies were fixed for 6 h in glutaraldehyde-paraformaldehyde fixative, embedded in paraffin and 4-μ sections were cut and stained with hematoxylin and eosin. All biopsies were scored in blinded fashion by one of us (D.C.) who was unaware of the identity or lavage findings of study subjects. The biopsy material was scored with respect to the worst metaplastic lesion present similar to the studies of Auerbach et al.[2-4] Biopsies were judged to be adequate if mucosa was seen on cut tissue sections.

Statistical Analysis

The data were screened for normality and normally distributed continuous variables were assessed by paired and unpaired t tests where appropriate. Abnormally distributed data were assessed by the Mann-Whitney U test. The biopsy results and smoking history comprised a set of discontinuous data, and for this analysis the study population was separated into groups and assessed by the chi square test, analysis of variance and the Kruskal-Wallis test as appropriate.

RESULTS

Demographic Variables

Fifty men averaging 60 ± 6 years of age (mean ± SD) were studied. The study subjects included eight nonsmokers, 13 current smokers and 29 ex-smokers. Smokers had smoked for an average of 41 pack-years. Ex-smokers had discontinued tobacco use an average of 13 years prior to study. The men had been exposed to asbestos from work in the construction trades and asbestos products manufacturing. Age at first asbestos exposure averaged 25 years. Asbestos exposure averaged 14.8 (range, 2.5 to 36) insulator-year equivalents as estimated by the method of Nicholson et al.[21] Mean latency between beginning exposure and study was 35 years. Of these subjects, 36 had both adequate biopsy material and sufficient cellular material in the first aliquot for analysis. Inadequate material was usually

Table I—BAL *Fluid Recovery and PMN in Aliquot 1*

BAL Sample	VO*	TNC (10^6)	% PMN
Aliquot 1	15±10	0.5±0.7	15.5±17
Pooled cells†	112±20	52±6	6.6±11

*For aliquot 1, 50 ml instilled. Total lavage, 250 ml.
†Pooled cells represent cells from aliquots 2 to 5 of the lavage.

due to poor recovery of aliquot 1. Inadequate biopsy material was recovered from two subjects and two subjects could not be studied due to coughing or presyncope. Subjects with inadequate cells in aliquot 1 were from most path score categories (score 1, 5; 2, 0; 3, 3). Thus, the lost subjects came from both extremes of the path scores and were not overrepresented in either.

Lavage Analysis

The recovery of lavage fluid in the first and subsequent aliquots and the numbers and percentage of PMN are shown in Table 1. A relatively small number of cells was recovered in the first aliquot. There was, however, a disproportionate increase in the percentage of PMN in this aliquot compared with that in the pooled specimen (p<0.0001). Despite this percentage increase, the total number of neutrophils contained in this aliquot was relatively small because of the relatively small number of cells. Relationships between cell percentages in the first and the pooled aliquots were sought by linear regression and rank correlation. We could detect no obvious association between these variables (r=0.12, Fig 1). As shown in the figure, ex-smokers tended to have higher numbers of PMN in aliquot 1 (9.6 ± 8 percent) compared with those who had never smoked (2.4 ± 2 percent) and current smokers (5±7 percent, p<0.03, Kruskal-Wallis test). Current smokers and those who had never smoked were

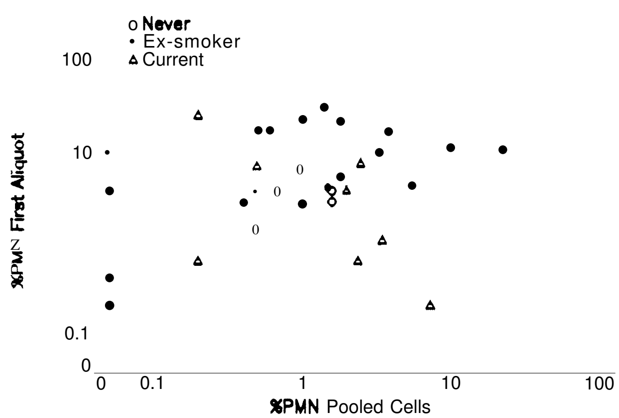

FIGURE 1. Relationship between neutrophils in the first and pooled aliquots. The percentage of PMN in the first aliquot (ordinate) is plotted against the percentage of PMN in the pooled cell population. The percentage of PMN in aliquot 1 exceeded that in the pooled cells (p<0.0001). There was no relationship between the two variables (r=0.12, p>0.1). Ex-smokers had a higher percentage of PMN in aliquot 1 compared with other groups (p<0.03).

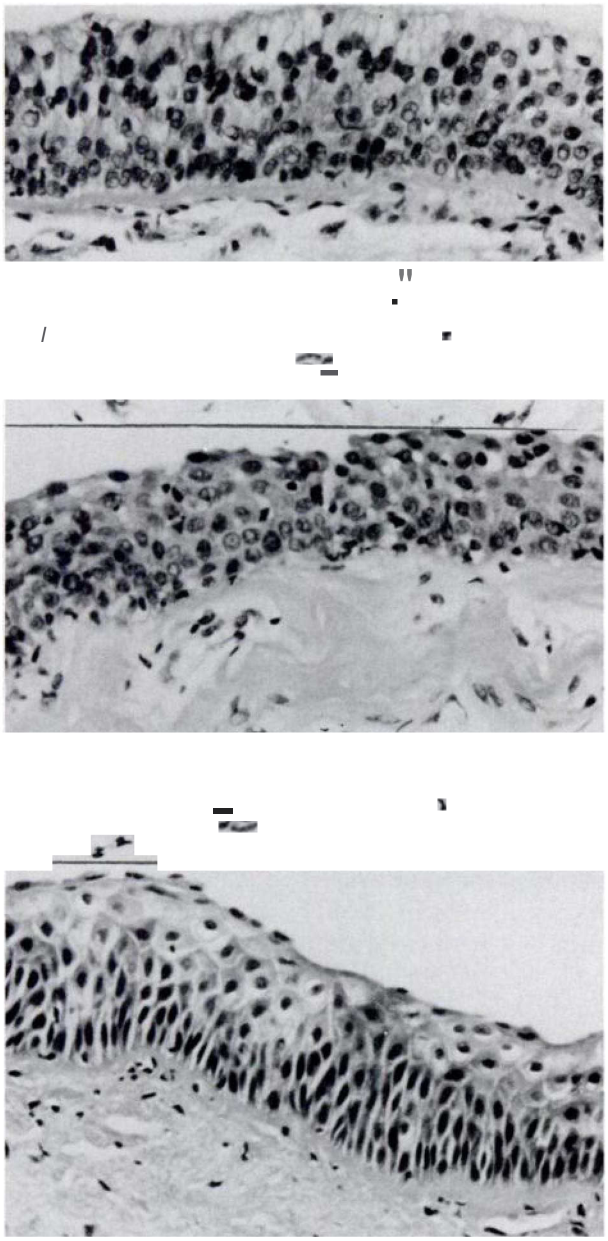

FIGURE 2. Histologic scoring sytem. Histologic specimens of pooled biopsy material from each study subject were scored for the worst mucosal lesion seen in multiple cuts. ***Top***, Basal cell hyperplasia. The basal cells (normally a single layer) are increased to three or more cell layers. The basement membrane is thickened and variable amounts of goblet cell hyperplasia were noted. This lesion was seen in all subjects and was the worst lesion seen in subject 27. ***Center***, Full-thickness hyperplasia. seen as the worst lesion in four subjects. Basalcells form the full thickness of the membrane but keratinization is absent. *Bottom*. An example of keratinization. a lesion seen in 15 study subjects. Changes are similar to those seen in full-thickness hyperplasia but include keratinization indicated by numerous intercellular bridges.

not different from one another. We could detect no relationship between smoking history or pulmonary function tests and the percentage ofPMN in aliquot 1.

Bronchial Mucosal Biopsies

Adequate biopsy material was recovered from 46 of 50 study subjects. As might be expected among these study subjects, significant abnormalities of bronchial mucosa were seen frequently (Fig 2). As shown in Table 2, metaplasia was seen with equal frequency in each ILO radiographic category. Pulmonary function parameters were similar for most tests in subjects with and without squamous metaplasia. However, those with metaplasia evidenced on biopsy specimens had significant reduction in FEV.IFVC compared with those with less severe changes ($p<0.04$).

Table 2—***Metaplasia** and IW **Radiographic Category***

Clinical Marker	Metaplasia Present	Metaplasia Absent
ILO Score"		
010	1	0
011	6	14
1/0	0	9
VI	5	5
1/2	1	1
2/1	1	1
2/2	1	0
312	0	1
Pulmonary Functiont		
TLC	76 ± 17	73 ± 14
Dco	71 ± 25	81 ± 20
FEV,IFVC	71 ± 8	77 ± 8‡

·ILO Score is radiographic score of a certified B reader who was unaware of the lavage and biopsy findings. Data shown are numbers of subjects with each score separated by the presence or absence of squamous metaplasia.

tPulmonary function data are expressed as **mean** ± SD percentage of predicted value.

‡**FEV_1/FVC** of subjects with metaplasia significantly less than the ratio of subjects without metaplasia. $p<0.04$.

Metaplasia was more common among current and ex-smokers ($X^2=7.1$, $p<0.05$); however, no combination of smoking or asbestos exposure history either alone or together was predictive of the pathologic score. The values for a single and multiple variable regression analysis revealed small coefficients with $p>0.15$ for each individual association. Moreover, no combination of these variables was able to predict accurately the percentage or number of acute inflammatory cells present in the first aliquot.

Given the inability of history to predict either metaplasia or the presence of large airway inflammatory cells, we assessed the role of these cells as independent predictors of metaplasia. The results of this analysis disclosed no tendency for subjects with metaplasia to have higher numbers of inflammatory cells in the first aliquot. In fact, a weak tendency for the reverse was found. Subjects with evidence of metaplasia (n = 15) had slightly lower mean percentage of PMN in aliquot 1 (mean, 4.5 percent) compared with that in subjects with less severe lesions (7.1 percent, $t=1.13$ $p>0.1$).

DISCUSSION

Prior authors have shown clearly that there are important differences in cellular and protein content of the first aliquot of sequential BAL when that sample is compared with material pooled from subsequent aliquots. Cenerally neutrophils have been significantly increased in the first aliquot.[14-16] One published exception is the result reported by a group who use a much different lavage technique.P However, this group also found differences with respect to protein concentration. Based on the results noted by prior investigators and the data from our own study, we agree that the first BAL aliquot is sampling a different pool of cells (and probably proteins) from those sampled by subsequent aliquots. Although a variety of differences are noted, the most important seems to be an increase in the number of acute inflammatory cells present in the first aliquot.

We attempted to utilize this phenomenon to explore the role of inflammation in large airways as a pathogenic factor in the development of bronchial metaplasia. The role of inHammation in the pathogenesis of metaplasia is somewhat uncertain. However, several papers published in the recent past suggest that there may be an important connection between inHammation and metaplastic change in mucosal epithelium and perhaps in the evolution of epithelial cancers. Bacterial inflammatory stimuli can be associated with metaplastic change of both the respiratory tract[2] and gastric mucosa.[23,24] Moreover, irritant types of inHammatory stimuli can cause similar ehanges.P The most Horid example of this link is the possible association between inflammatory and fibrotic lung diseases and cancers of the respiratory tract.[9,10] Mechanisms by which inHammatory cells might be related to subsequent metaplastic and neoplastic events must be complex. However, one possible mechanism is suggested by observations from two laboratories that inHammatory cells, when cultured with bacteria in the Ames test, can cause mutations in bacterial cells. These studies have suggested a role for neutrophil oxidant generating systems in the production of these changes.[12,13]

In our studies, because metaplasia could not be predicted by demographic characteristics of the study population, we sought to assess the role of large airway inflammatory cells as possible markers of events leading to this histologic change. The feasibility of this was suggested by our data and those of others that these cells probably derive from the large airways and are independent of the presence of inHammatory cells present in subsequent aliquots. Moreover, the presence of these cells in the large airways did not appear to be related to other demographic features of the population. Therefore, it seems that these cells denote some independent feature of the large airways that might be related to subsequent metaplastic change.

In fact, we were unable to detect any association between the number and types of inHammatory cells present in the first BAL aliquot (presumably derived from large airways) and histologic evidence of metaplasia. "Airway" cells were not related to FEV.IFVC. This observation is in contrast to that of Martin et al[14] who found a relationship between airflow limitation and first aliquot PMN. However, these investigators chose a population with obstructive airway disease for testing and we assessed a group with a tendency toward restriction. We did note, however, that those with metaplasia evidenced on a biopsy specimen had significantly lower FEV.IFVC than those without this change.

It is possible that our inability to find an association was based on our method oflavaging one segment and biopsying a second (contralateral) segment. However, we believe this to be unlikely. Other investigators'" have shown that a random biopsy technique could detect histopathologic evidence of metaplasia in subjects at risk. We believe that the implication of this finding (which has been replicated by others) is that the airways are diffusely affected by the pathogenic stimulus (in this case asbestos and cigarette smoking) and that random sampling would have an excellent chance of finding the disease. That fact formed a logical foundation to sample two different sites in the hope of finding associations because of the diffuse nature of the airway injury. It is likely that the small biopsy samples are useful because the biopsies are, of necessity, performed at branch points in the airways, and these points are characteristically most heavily affected by the metaplastic process. Lavage, by its nature, is a diffuse procedure, sampling all the segments distal to the wedged scope. Therefore, this procedure also should have great power to assess a diffuse process.

Unfortunately, the precise meaning of our findings is unclear. Conceivably, metaplasia and PMN in aliquot 1 are unrelated because the PMN is not causing mucosal injury in the airway Alternatively the PMN detected in aliquot 1 may have migrated into the lung at more distal sites and have been carried to larger airways by the mucociliary escalator. The observation that cell percentages in first and subsequent aliquots were unrelated seems to be against this assessment. Sampling error of a random biopsy might also inject error. However, others have shown usefulness of this technique to study metaplasia and its response to therapy.[26] Moreover, we could show a relationship between this change and lung physiology Finally, there may be other, less obvious factors which regulate this process. Perhaps submucosal inHammatory substances" or even molecules made by epithelial cellsthemselves" may be important.

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