Association between Acute Inflammatory Cells in Lavage Fluid and Bronchial Metaplasia*

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In epidemiologic studies, airway disease and parenchymal injury are known morbid outcomes of occupational exposure to asbestos. However, the relationship of inflammatory events considered to be responsible for parenchymal injury to the subsequent development of airway injury is unknown. To assess this we performed bronchoalveolar lavage (BAL) and airway biopsies on a population of subjects with exposure to asbestos in the workplace. As an index of airway injury, we employed histologic metaplasia seen in mucosal biopsy specimens. Lung BAL fluid was analyzed for two potentially relevant protein markers and for inflammatory cells recovered from the lower respiratory tract. We related metaplasia to demographic features of this study population (*eg*, smoking history and asbestos exposure data) and also to the protein and cellular markers recovered by BAL. We studied 50 workers and detected keratinizing metaplasia in 15 and varying lesser abnormalities in the other 28. Cigarette smoking was not associated with the presence of metaplasia ($p<0.2$). Smoking status was associated with an increase in BAL cells ($p<0.02$); however, neither the percent nor concentration of acute inflammatory cells was significantly increased. Acute inflammatory cells (percent and cells per milliliter of BAL fluid) were significantly increased among the subjects with severe metaplasia compared with other study subjects. This increase was true of both neutrophils and eosinophils and the sum of these two ($p<0.02$). Stratification of subjects by smoking status demonstrated a persistent association of inflammatory cells with metaplasia. By logistic regression analysis, polymorphonuclear leukocytes per milliliter and eosinophils per milliliter were significantly related to the presence of metaplasia in two independent models (odds ratios, 9.9 and 7.6, respectively). Cigarette smoking and other demographic or BAL variables were not significantly associated with metaplasia in these models. *(Chest 1992; 102:688-93)*

ELISA = enzyme-linked immunosorbent assay; TNC = total nucleated cells

Workers with occupational exposure to asbestos are afflicted by a variety of diseases that can be related epidemiologically to their occupation.[1,2] Asbestos can affect the alveolar region of the lung or the airways. Clinically, the two most important diseases are progressive fibrosis, which occurs in up to 10 percent of workers with significant past exposure, and bronchogenic carcinoma, the most important airway lesion. Recently, parenchymal injury has been linked to inflammatory pathways in the lower respiratory tract by the research use of bronchoalveolar lavage (BAL) to sample the cells and proteins of the alveolar space.[3,4] These studies have shown the tendency for workers with asbestosis to have an increase in the number of acute inflammatory cells recoverable by BAL. Evidence implicating macrophages as important regulatory elements in this cascade of inflammation has also been reported.[5-7]

Some factors that regulate the subsequent development of cancer in this patient population are also known. Although epidemiologic studies indicate that asbestos exposure by itself can also increase the risk of cancer,[8] cigarette smoking is an important copredictor, increasing the risk of cancer 10- to 15-fold. The role of inflammatory and fibrotic pathways in the pathogenesis of cancer has also been explored epidemiologically with somewhat variable results. For example, one group of investigators found that virtually all asbestos workers with lung cancer had evidence of fibrosis at autopsy or in surgically-resected specimens,[9] while another group was unable to document this association.[10]

To assess factors that might be associated with airway injury in this population, we performed fiberoptic bronchoscopies on a population of workers with known prior exposure to asbestos. We used metaplasia, detectable in random bronchial mucosal biopsy specimens, as our index of mucosal injury.[11,12] We related this to demographic variables in the subject population and also to a variety of cellular and protein constituents of fluid recovered from the lower respiratory tract. Specifically we assessed free secretory component and the keratins because in prior studies we noted an alteration in these proteins (reduction in free secretory component and an increase in the keratins) in cigarette-smoking normal volunteers.[13] More severe ab-

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normalities were seen in patients with lung cancer.[14] We also assessed acute inflammatory cells because others have shown these cells to be associated with the evolution of parenchymal[3] and airway injury.[15]

Materials and Methods

Study Population and Protocol

Volunteer subjects were recruited from among over 1,000 patients in the Yale Occupational Medicine Program.[16] Patients underwent bronchoscopy and BAL as described previously.[13] In brief, the upper airway was anesthetized with a topical agent and a fiberoptic scope was passed through the mouth. Airways were inspected carefully for abnormalities. If none were found, three to six mucosal biopsy specimens were obtained from random subsegmental branch points in the right lower lobe. Biopsies were terminated when two to three generous pieces of mucosal tissue had been obtained. The bronchoscope was then wedged into the lingula. BAL was performed by five alternate instillations/aspirations of 50-ml aliquots of isotonic saline solution at room temperature. The entire protocol had been reviewed and approved by the Yale Human Investigation Committee prior to initiation of the study.

Exposure Analysis

Since virtually none of the subjects had worked in an environment from which adequate environmental sampling for asbestos had taken place during the periods of exposure, average and cumulative doses were calculated using the relative scale as described by Nicholson et al.[16,17]

Analysis of BAL Fluid

Recovered fluid was filtered through a layer of gauze to remove mucus and centrifuged to pellet cellular elements. Cells and fluid from aliquot 1 were analyzed separately from the remaining fluid, which was pooled for subsequent analysis.[18-20] Total nucleated cells (TNC) were quantified by hemocytometer; 10^5 TNC were pelleted onto glass slides by cytocentrifugation and stained by modified Wright's Giemsa (Dif quick, Harleco). Differential cell counts were performed by counting the percentage from 500 stained cells. Total protein was assessed by Coomassie blue using serum albumin as standard and free secretory component and the keratins were quantified by enzyme-linked immunosorbent assay (ELISA) as described previously in detail.[14,21]

Several systems to express the final BAL data have been proposed;[22-24] the lack of uniformity reflects the absence of a consensus on the most appropriate system. A recent cooperative study has published values for normal volunteers and ranges for several disease states.[25] Our method for BAL is similar to theirs and we have adopted their method for data expression of all variables. Therefore, we counted TNC recovered and differential percentage of each cell type. These values and BAL effluent volume were used to calculate cell concentrations per milliliter of recovered fluid. Proteins were expressed as their concentration in BAL fluid as discussed.[25,p175]

Processing of Biopsy Specimens

Biopsy specimens were fixed for 6 h in glutaraldehyde-paraformaldehyde fixative and embedded in paraffin, and two slides containing 12 4-μm sections were cut and stained with hematoxylin-eosin. All biopsy specimens were scored in blinded fashion by one of us (D.C.) who was unaware of the identity or BAL findings of study subjects. The biopsy material was scored with respect to the worst metaplastic lesion present on a three-point scale similar to the description of Gouveia et al.[12] Briefly, histologic specimens of pooled biopsy material from each study subject were scored for the worst mucosal lesion seen in multiple cuts as described above. When basal cells (normally a single layer) were increased to three or more cell layers, basal cell hyperplasia was diagnosed. Basement membrane thickening and goblet cell hyperplasia were often noted. This lesion was seen in all subjects and was the worst lesion seen in 27. Full-thickness hyperplasia, seen in four subjects, was diagnosed when basal cells formed the full thickness of the membrane but keratinization was absent. Keratinization, present in 15 subjects, was determined by the presence of numerous intercellular bridges.

Data Analysis and Exclusions

A total of 50 subjects had bronchoscopy. Two subjects tolerated the procedure poorly (cough, one; presyncope, one) and no results were obtained. Two subjects had no mucosa visible in biopsy tissue and were excluded. Finally, a processing error occurred during preparation of cell differential counts of three subjects and their cytocentrifuge preparations could not be interpreted. The remaining 43 cases are reported in detail below. Results of all evaluations and analyses were coded on a microcomputer (Macintosh II) and analyzed (using Data Desk Professional, Odesta, Northbrook, Ill). For nonparametric analyses, a computer (IBM PC AT) and statistical software (CRUNCH 4, Crunch Software, Oakland, Calif) were employed. The distribution of all relevant variables was first evaluated for normality and appropriate transformations were made where necessary. Correlations among paired variables were explored using Pearson correlation for continuous variables and Spearman ranks for categoric data such as metaplasia grades. Subsequent statistical comparisons were made using Student's t test and analysis of variance for continuous variables, χ^2 analysis for proportions, and Kruskall-Wallis and Mann-Whitney U test for nonparametric analyses. Multiple logistic regression analyses were used to evaluate relationships between acute inflammatory cells and metaplasia, controlling for demographic variables.

Results

Detection of Metaplasia and Demographic Variables

Assessable biopsy specimens were obtained successfully from 46 of the study subjects. Two subjects did not complete the protocol because of technical factors and in two subjects epithelium was not detected. The most severe pathologic change (metaplasia with keratinization) was detected in 15 subjects (one third of the biopsy specimens). Four subjects had full-thickness hyperplasia and 27 subjects had basal cell hyperplasia. The presence of cigarette smoking—whether scored as ever smoked vs never smoked; as current smokers, ex-smokers, and never smokers; or as pack-years of smoking—was not significantly related to the presence of metaplasia on biopsy specimens ($p>0.2$ for all comparisons). As shown in Table 1, no other demographic variables were specifically related to the presence of metaplasia ($p>0.1$ for all comparisons).

Smoking and BAL Parameters

We assessed the effect of smoking on recovery of acute inflammatory cells. Smoking increased significantly the recovery of TNC from the lower respiratory tract (Table 2). Mean numbers of acute inflammatory cells were lowest in never smokers and highest in current smokers. Ex-smokers were intermediate between these groups. However, none of these differences was statistically significant ($p>0.2$ for all com-

Table 1—*Demographic Variables and Metaplasia*

Demographic Variable	Metaplasia* (−)	(+)
Age, yr	60.9	60.8
Smoking		
Pack-years	35±28	43±40
Ex-years	10.5±9	13±10
Asbestos exposure		
Age first, yr	25±7	24±6
Latency, yr	34.8±10	36±10
Total dose	13±8	16±10

*Metaplasia (+) refers to the presence of keratinizing squamous metaplasia in random biopsy specimen. Lesser degrees of bronchial mucosal injury are scored metaplasia (−). Ex-years = years since last cigarette; age first = age at first exposure to asbestos; total dose = total dose of asbestos in insulator-years. The method of assessment is described in reference 14. Values represent mean ± SD.

parisons by Kruskal-Wallis test).

Metaplasia and BAL Parameters

We assessed the relationship of concentrations of free secretory component and the keratins in BAL samples to the presence of metaplasia in the biopsy specimens. There was no specific relationship of either of these protein parameters to the presence or the degree of metaplastic abnormality in bronchial biopsy specimens. In fact, the presence of keratins in bronchial wash fluid was more common among subjects who did not have severe metaplastic lesions on biopsy specimen, although this difference was not significant.

By contrast, both neutrophilic granulocytes and eosinophils were more commonly seen in the pooled cell populations of subjects with keratinizing squamous metaplasia compared with those without this change (Table 3). Their sum (neutrophils + eosinophils) was also significantly increased. In an attempt to control for the effect of smoking on lung inflammatory cell populations, we analyzed the populations subgrouped by cigarette smoking status and by the presence or absence of keratinizing metaplasia. Subgroup analysis indicated that inflammation was still related to metaplasia in all groups; however, this was significant only for the largest group. For example, although most comparisons demonstrated higher numbers of acute inflammatory cells in subjects with metaplasia (Table 3), p values achieved significance only for ex-smokers ($p<0.05$, Mann-Whitney *U* test). Among never smokers, the trend for polymorphonuclear leukocytes per milliliter was in the same direction ($p<0.09$, *t* test). For each smoking subgroup, smoking intensity and duration (pack-years) were not different among those with and without keratinization.

Table 3—*BAL Inflammatory Cells in Metaplasia: Subjects Separated by Smoking Class**

Class	n	M	Neut/ml	EOS/ml	Gran/ml
All	29	(−)	3.7±6.0	7.2±25	5.8±12
All	14	(+)	13±18†	8.7±14	22±29†
Never	6	(−)	1.1±1.3	0.48±0.56	1.62±1.6
Never	2	(+)	4.2±3.2	1.96±2.7	6.2±6.0
Ex	17	(−)	3.2±5.2	1.6±2.8	4.8±6.7
Ex	8	(+)	15.5±20†	10.9±18†	26.5±37†
Smoke	6	(−)	4.9±8.1	23±54	28.5±61
Smoke	4	(+)	14±18	7.5±5.7	21.9±20

*All indicates all study subjects; M +/− refers to the presence or absence of keratinizing squamous metaplasia. Other abbreviations are similar to Table 2.

†$p<0.05$ for ex-smokers with metaplasia compared with ex-smokers without metaplasia.

Finally, we then assessed the relationship between acute inflammatory cells and metaplasia with logistic regression analysis. In these models, squamous metaplasia was the outcome variable and the concentrations of acute inflammatory cells (neutrophils in one model and eosinophils in the second) were the primary predictor variables. Prior analyses demonstrated that values for neutrophils and eosinophils per milliliter of BAL fluid were not normally distributed. Therefore, we divided the population into groups with high and low (greater or less than 5×10^3 cells per milliliter, respectively) neutrophil concentration. Subjects were similarly subdivided by eosinophil concentration for the second analysis; however, a third group was used (eosinophils = 0/ml; >0, $<5\times10^3$; $>5\times10^3$) because of the relatively large number of subjects with no eosinophils. Results of these analyses revealed a significant association between neutrophil concentration and squamous metaplasia after controlling for other variables (odds ratio, 9.91; range, 1.62 to 61). Eosinophils showed a weaker association (odds ratio, 7.6; 95 percent CI, 1.05 to 55.2) for subjects with the highest number of eosinophils in a separate analysis.

Table 2—*Effect of Smoking on Cell Populations**

Class	TNC	%Neut	Neut/ml	%EOS	EO/ml	%Gran	Gran/ml
Never	27.4±20	0.7±0.8	1.9±2.2	0.34±0.38	0.85±1.3	1.0±1.0	2.7±3.3
Ex	70.2±138	4.0±9.2	9.4±15	2.3±6.8	6.5±13	6.3±15	16±26
Smoke	117.6±90†	1.0±1.1	8.7±13	2.3±2.3	17±41	2.3±2.3	11±16

*Class = smoking classification; never = lifelong nonsmokers; ex = subjects who had discontinued smoking; smoke = current smokers; TNC = total nucleated cells recovered by BAL; %Neut, %EOS, %Gran = percentages of neutrophils, eosinophils, and their sum, respectively; Neut/ml, EOS/ml, and Gran/ml = numbers of neutrophils, eosinophils, and their sum per mililiter of recovered BAL fluid.

†$p<0.02$ for smokers vs other groups by Kruskal-Wallis test.

Discussion

In these experiments, we have assessed the role of a variety of cellular and protein parameters quantified in BAL fluid as markers of a tendency toward severe bronchial mucosal injury. We chose this end-point because in many animal models[26,27] and in human pathologic specimens,[28,29] metaplastic change in the bronchial mucosa is an important marker of illness. For example, while this biologic end-point does not obligate the future development of a tumor, metaplasia is invariably seen in the presence of pulmonary carcinoma in both humans and animals.[28] Moreover, others have shown a significant ability for random biopsy specimens to detect this change and also to demonstrate its resolution with appropriate intervention.[11,12] Thus, this tissue change seems to be a reasonable surrogate for severe bronchial injury.

Subjects with severe metaplastic injury detected by biopsy specimen had significant increases in the number of recoverable acute inflammatory cells. Smoking did have an effect on cell recovery. The total number of cells recoverable by BAL of the lower respiratory tract was increased by smoking; however, numbers of acute inflammatory cells were not (Table 2). When subjects were grouped by smoking class, those with metaplasia still tended to have higher numbers of acute inflammatory cells (Table 3). However, these results were significant only for the largest subgroup. Significantly, the effect of inflammatory cells on keratinizing metaplasia held true for cells from the pooled alveolar sample. We have reported previously that studies of cells in the first aliquot of BAL, presumably derived from airways,[18-20] bore no specific relationship to the presence of metaplasia in large airway biopsy specimens.[30] Moreover, these cells bore no specific relationship to the acute inflammatory cells recovered in subsequent aliquots. Thus, it may be reasonable to assume that inflammatory cells in the "alveolar" sample are markers of reactions in the more distal airways or alveolar space and that it is alveolar space inflammation that is associated with metaplasia.

Although cigarette smoking is an important epidemiologic factor in the evolution of mucosal injury, it was not associated with the presence of metaplasia in airway biopsy specimens in our study whether analyzed by category or by pack-year. For example, two of eight subjects who had never smoked had detectable metaplasia while 21 of 35 smokers and ex-smokers appeared to escape this problem. There was a tendency for subjects who currently smoked or those with a smoking history to have a higher frequency of metaplasia. This tendency was not significant, perhaps because of the presence of two factors (smoking and asbestos exposure) capable of causing airway injury. Perhaps our data are a correlate of the epidemiologic observations concerning cancer development in smokers and asbestos workers.[31] Conceivably the synergistic effects of two stimuli are necessary for maximum effect. Smoking has been associated with the development of small irregular opacities on chest roentgenograms[32,33] that are thought to be markers of asbestos-induced parenchymal injury. Moreover, in one study, pulmonary carcinomas were always found in the setting of lung fibrosis.[9] Our data, although not definitive, are suggestive that the inflammatory effects of the two stimuli may well be an important common pathway for bronchial injury and the development of cancer.

Presumably, cigarette smoking predisposes to lung cancer because of the presence of carcinogens contained within smoke, carcinogens synergistic with asbestos.[8] However, smoking also increases the number and types of inflammatory cells recoverable from the lower respiratory tract.[34] Conceivably, this increase of inflammatory cells is also important in the predisposition to metaplasia. Infectious and irritant stimuli are both associated with the presence of metaplastic change in both the respiratory[27] and the gastrointestinal tracts.[35] Inflammation has also been linked to metaplastic lesions among asthmatics[15] and coke oven workers.[36] Moreover, chronic inflammatory and fibrotic diseases of unknown cause are also occasionally complicated by the development of pulmonary carcinoma.[37,38] Therefore, it is certainly conceivable that the tendency for both smoking and asbestos exposure to increase the local recruitment of inflammatory cells to the lung is an important synergistic mechanism both for the development of fibrosis and for the production of bronchial metaplasia.

The mechanisms that might cause this association are not well known; however, some have been proposed. For example, neutrophils when activated *in vitro* can cause alterations in bacterial DNA, and this is blocked by the presence of antioxidants in the media.[39] Activated neutrophils can also "activate" smoke carcinogens by an oxidative mechanism.[40] Alternatively, the presence of neutrophils and eosinophils may be a marker for activation of other cells. Activated macrophages can release a variety of stimuli that can alter the growth and differentiation of other cells.[6,41,42] These factors can also affect bronchial epithelial cells, changing their differentiation and gene expression.[43,44] Finally, bronchial epithelial cells, stimulated with inflammatory agonists, can themselves express growth factors.[45] This observation suggests that in certain situations, epithelial cells may participate in this process in an autocrine fashion.

Although the precise pathways are uncertain, our data suggest an important link between inflammation, induced by the stimuli of smoking and asbestos, and the development of metaplasia in large airways. This suggests a need for evaluation of the role of inflammatory cells and their products in the processes of cell transformation. Moreover, if these studies are confirmed, they may indicate a role for the bronchoscopic assessment of inflammation in the clinical assessment of exposed patients.

References

1 Seidman H, Selikoff IJ, Gelb SK. Mortality experience of amosite asbestos factory workers: dose-response relationships 5 to 40 years after onset of short-term work exposure. Am J Ind Med 1986; 10:479-514

2 Johansson LG, Albin MP, Jakobsson KM, Welinder HE, Ranstam PJ, Attewell RG. Ferruginous bodies and pulmonary fibrosis in dead low to moderately exposed asbestos cement workers: histological examination. Br J Ind Med 1987; 44:550-58

3 Robinson BW, Rose AH, James A, Whitaker D, Musk AW. Alveolitis of pulmonary asbestosis: bronchoalveolar lavage studies in crocidolite- and chrysotile-exposed individuals. Chest 1986; 90:396-402

4 Rom WN, Bitterman PB, Rennard SI, Cantin A, Crystal RG. Characterization of the lower respiratory tract inflammation of nonsmoking individuals with interstitial lung disease associated with chronic inhalation of inorganic dusts. Am Rev Respir Dis 1987; 136:1429-34

5 Schoenberger CI, Hunninghake GW, Kawanami O, Ferrans VJ, Crystal RG. Role of alveolar macrophages in asbestosis: modulation of neutrophil migration to the lung after acute asbestos exposure. Thorax 1982; 37:803-09

6 Rom WN, Basset P, Fells GA, Nukiwa T, Trapnell BC, Crystal RG. Alveolar macrophages release an insulin-like growth factor I-type molecule. J Clin Invest 1988; 82:1685-93

7 Hayes AA, Rose AH, Musk AW, Robinson BW. Neutrophil chemotactic factor release and neutrophil alveolitis in asbestos-exposed individuals. Chest 1988; 94:521-25

8 Mossman BT, Bignon J, Corn M, Seaton A, Gee JBL. Asbestos: scientific developments and implications for public policy. Science 1990; 294:247-53

9 Kipen HM, Lilis R, Suzuki Y, Valcuikas JA, Selikoff IJ. Pulmonary fibrosis in asbestos workers with lung cancer: a radiologic and histopathologic evaluation. Br J Ind Med 1987; 44:96-100

10 Hnizdo E, Sluis-Cremer GK. Effect of tobacco smoking on the presence of asbestosis at postmortem and on the reading of irregular opacities on roentgenograms in asbestos-exposed workers. Am Rev Respir Dis 1988; 138:1207-12

11 Bertram JF, Rogers AW. Recovery of the bronchial epithelium on stopping smoking. Br J Med 1981; 283:1567-69

12 Gouveia J, Hercend T, Lemaigre G, et al. Degree of bronchial metaplasia in heavy smokers and its regression after treatment with a retinoid. Lancet 1982; 1:710-12

13 Merrill WW, Goodenberger D, Strober W, Matthay RA, Naegel GP, Reynolds HY. Free secretory component and other proteins in human lung lavage. Am Rev Respir Dis 1980; 122:156-61

14 Merrill WW, Barwick KW, Madri J, Strober W, Matthay RA, Olchowski J, et al. Bronchial lavage proteins as correlates of histopathologic airway changes in healthy smokers and patients with pulmonary carcinoma. Am Rev Respir Dis 1984; 130:905-09

15 Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma: an ultrastructural study and correlation with hyperreactivity. Am Rev Respir Dis 1989; 140: 1745-53

16 Cullen MR, Merrill WW. The relationship between bronchoalveolar lavage cells and proteins and asbestosis in asbestos-exposed subjects after control of exposure.

17 Nicholson WJ, Perkel G, Selikoff IJ. Occupational exposure to asbestos: population at risk and projected mortality 1980-2030. Am J Ind Med 1982; 2:259-311

18 Martin TR, Raghu G, Maunder RJ, Springmeyer SC. The effects of chronic bronchitis and chronic air-flow obstruction on lung cell populations recovered by bronchoalveolar lavage. Am Rev Respir Dis 1985; 132:254-60

19 Robinson BW, James A, Rose AH, Sterrett GF, Musk AW. Bronchoalveolar lavage sampling of airway and alveolar cells. Br J Dis Chest 1988; 82:45-55

20 Rennard SI, Ghafouri M, Thompson AB, et al. Fractional processing of sequential bronchoalveolar lavage to separate bronchial and alveolar samples. Am Rev Respir Dis 1990; 141: 208-17

21 Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY. Immunoglobulin G subclass proteins in serum and lavage fluid of normal subjects: quantitation and comparison with immunoglobulins A and E. Am Rev Respir Dis 1985; 131:584-87

22 Merrill WW, Naegel GP, Reynolds HY. Reaginic antibody in the lung lining fluid: analysis of normal human bronchoalveolar lavage fluid IgE and comparison to immunoglobulins G and A. J Lab Clin Med 1980; 96:494-500

23 Rennard SI, Basset G, Lacossier D, O'Donnell KM, Pinkston P, Martin PG, et al. Estimation of volume of extravascular lung water recovered by lavage using urea as a marker of dilution. J Appl Physiol 1986; 60:532-41

24 Merrill WW, Marcy TA, Reynolds HY. Bronchoalveolar lavage: clinical role and quantitative assessment. Respiration 1988; 54(S1):3-8

25 The BAL Cooperative Group Steering Committee. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected diseases. Am Rev Respir Dis 1990; 141(5 pt 2)

26 Akagi G, Stanton M, Miller E, May M, Poore C. Ultrastructural observations on pre-neoplastic epidermoid metaplasia in the lungs of rats. J Environ Pathol Toxicol 1980; 4:71-85

27 Keenan KP, Saffiotti U, Stinson SF, Riggs CW, McDowell EM. Multifactorial hamster respiratory carcinogenesis with interdependent effects of cannula-induced mucosal wounding, saline, ferric oxide, benzo[a]pyrene and N-methyl-N-nitrosourea. Cancer Res 1989; 49:1528-40

28 Auerbach O, Hammond EC, Garfinkle L. Changes in bronchial epithelium in relation to smoking: 1955-60 vs 1970-77. N Engl J Med 1979; 300:381-86

29 Woodworth CD, Mossman BT, Craighead JE. Squamous metaplasia of the respiratory tract: possible pathogenic role in asbestos-associated bronchogenic carcinoma. Lab Invest 1983; 48:587-94

30 Merrill WW, Carter D, Cullen MR. Relationship between leukocytes in large airways and large airway metaplasia. Chest (in press)

31 Blanc PD, Golden JA, Gamsu G, Aberle DR, Gold WM. Asbestos esposure-cigarette smoking interactions among shipyard workers. JAMA 1988; 259:370-73

32 Weiss W. Cigarette smoke, asbestos, and small irregular opacities. Am Rev Respir Dis 1984; 130:293-301

33 Kilburn KH, Warshaw R, Thornton JC. Asbestos diseases and pulmonary symptoms and signs in shipyard workers and their families in Los Angeles. Arch Intern Med 1986; 146:2213-20

34 Reynolds HY, Merrill WW. Airway changes in young smokers that may antedate chronic obstructive lung disease. Med Clin North Am 1981; 65:667-89

35 Carrick J, Lee A, Hazell S, Ralston M, Daskalopoulos G. *Campylobacter pylori*, duodenal ulcer, and gastric metaplasia: possible role of functional heterotopic tissue in ulcerogenesis. Gut 1989; 30:790-97

36 Madison R, Afifi AA, Mittman C. Respiratory impairment in coke oven workers: relationship to work exposure and bronchial

inflammation detected by sputum cytology. J Chronic Dis 1984; 37:167-76
37 Kuhara H, Wakabayashi T, Kishimoto H, Morishita M, Suzuki T, Yamamoto M. Synchronous bilateral double primary lung cancer associated with diffuse interstitial fibrosing pneumonitis (DIFP). Acta Pathol Jpn 1984; 34:617-29
38 Kuhara H, Wakabayashi T, Kishimoto H, Ichimura K, Torii Y, Yamamoto M. Lung cancer and chronic interstitial pneumonia associated with systemic sarcoidosis. Acta Pathol Jpn 1985; 35: 199-211
39 Weitzman SA, Stossel TP. Effects of oxygen radical scavengers and antioxidants on phagocyte-induced mutagenesis. J Immunol 1982; 128:2770-72
40 Trush MA, Seed JL, Kensler TW. Oxidant-dependent metabolic activation of polycyclic aromatic hydrocarbons by phorbol ester-stimulated human polymorphonuclear leukocytes: possible link between inflammation and cancer. Proc Natl Acad Sci USA. 1985; 82:5194-98
41 Assoian RK, Fleurdelys BE, Stevenson HC, et al. Expression and secretion of type B transforming growth factor by activated human macrophages. Proc Natl Acad Sci USA 1987; 84:6020-24
42 Leslie CC, McCormick-Shannon K, Mason RJ. Bronchoalveolar lavage fluid from normal rats stimulates DNA synthesis in rat alveolar type II cells. Am Rev Respir Dis 1989; 139:360-66
43 Siegfried JM, Owens SE. Response of primary lung carcinomas to autocrine growth factors produced by a lung carcinoma cell line. Cancer Res 1988; 48:4976-81
44 Jetten AM, Vollberg TM, Nervi C, George MD. Positive and negative regulation of proliferation and differentiation in tracheobronchial epithelial cells. Am Rev Respir Dis 1990; 142(6 pt 2):S36-9
45 Smith SM, Lee DKP, Lacy J, Coleman DL. Rat tracheal epithelial cells produce granulocyte/macrophage colony-stimulating factor. Am Rev Respir Cell Mol Biol 1990; 2:59-68