Neutrophil Chemotactic Factor Release and Neutrophil Alveolitis in Asbestos-Exposed Individuals*

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Alveolar neutrophil accumulation occurs in asbestosis. To evaluate a possible role for release of neutrophil chemotactic factor (NCF) in the pathogenesis of asbestosis, spontaneous NCF release from alveolar macrophages obtained by bronchoalveolar lavage (BAL) in eight individuals with asbestosis, 13 asbestos-exposed individuals without asbestosis, and five control subjects has been studied. Alveolar macrophages were incubated in medium (four hours; 37°C), and neutrophil responses to the supernatants were assayed in a microchemotaxis chamber. Alveolar macrophages from subjects with asbestosis released more NCF (97±19 neutrophils per high-power field [N/HPF]) than controls (3±1 N/HPF; p<0.01). Alveolar macrophages from individuals with asbestos exposure and increased BAL neutrophil proportions (n=7) released more NCF (93±24 N/HPF) than individuals with asbestos exposure and normal BAL neutrophil proportions (n=6; 11±6 N/HPF; p<0.02). The results show that spontaneous NCF release occurs in asbestosis and that NCF release is associated with neutrophil alveolitis in asbestos-exposed individuals without asbestosis, suggesting a pathogenic role for NCF in mediating this neutrophil alveolitis. The results of the study also suggest that the presence of crackles is a better predictor of the presence of neutrophil alveolitis than is an abnormal chest x-ray film. (Chest 1988; 94:521-25)

Neutrophils are potent mediators of tissue damage, and their long-term presence in alveolar structures is often associated with marked tissue derangement.1,2 Studies using biopsy and bronchoalveolar lavage (BAL) show that neutrophils accumulate in the alveolar structures in asbestosis, and it is likely that neutrophils contribute to the tissue damage that occurs in this disease.3,4 In vitro studies have demonstrated that animal and human alveolar macrophages can be induced to release a neutrophil chemotactic factor (NCF) by asbestos fibers and that animals exposed to asbestos develop a neutrophil alveolitis in conjunction with spontaneous release of NCF by alveolar macrophages.5,9 This process has not been evaluated in humans with asbestos exposure. It is logical to expect that if alveolar macrophage-derived NCF is contributing to the alveolar neutrophil accumulation that occurs in human subjects with asbestosis, NCF would be released spontaneously in this disease and, in asbestos-exposed individuals without asbestosis by conventional diagnostic criteria, only those with alveolar neutrophil accumulation would demonstrate spontaneous NCF release. The aim of this study was to evaluate these two hypotheses.

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Subjects
A total of 21 asbestos-exposed male subjects were studied (Table 1). Details of asbestos exposure were obtained retrospectively from occupational history records, with 20 subjects being exposed to crocidolite and one to chrysotile. Crocidolite exposure occurred in subjects who had been employed in the mining of asbestos at Wittenoom in Western Australia. The one subject exposed to chrysotile had worked in the manufacture of asbestos-containing products.

The control group consisted of five subjects with no past history of asbestos exposure and no clinical, radiologic, or physiologic evidence of diffuse interstitial pulmonary disease, who were being investigated for localized pulmonary lesions (four) or were normal volunteers (one). Informed consent was obtained from all individuals, and the study was approved by the Human Research Ethics Committee of the University of Western Australia.

Clinical Assessment
One observer (A.W.M.) assessed all subjects by auscultation of the chest for the presence of inspiratory crackles which did not clear with coughing.

Chest Roentgenography
Routine posteroanterior chest roentgenograms were obtained with a focus-to-film distance of six feet, 150 kV for 6 to 10 ms using a falling load tube current with ionization chamber automatic exposure (Siemens Iontomat) and a chest Buckley. The roentgenograms were graded by two independent experienced observers and were defined as demonstrating asbestosis if, based on the International Labor Office classification of pneumoconiosis,9 diffuse irregular opacities of grade 1/0 or greater were considered present by both observers.

Pulmonary Function
Total lung capacity (TLC) and its subdivisions were measured in...
a pressure-compensated flow-plethysmograph (Collins 09103). The forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) were measured with a digital pneumotachograph (Hewlett-Packard 47303A). Gas transfer (Tlc) was assessed with a single-breath carbon monoxide technique, using a Resparameter (P.K. Morgan model TTB).

**Bronchoalveolar Lavage**

After local anesthesia to the upper and lower airways using 10 percent lidocaine (Xylocaine) spray and 2 percent lidocaine solution, the tip of a fiberoptic bronchoscope (Olympus BFIT10) was wedged in a lingular or middle lobe bronchus and 0.9 percent physiologic saline solution was instilled in six 50-ml aliquots which were immediately aspirated into a disposable sterile polycarbonate bottle (Polyvac). The fluid recovered from the first 50-ml aliquot was collected separately, and the fluid from the subsequent five aliquots was pooled. It was then transferred to 50-ml polypropylene test tubes (Nunc) for transport to the laboratory.

The BAL fluid was centrifuged (450 g; seven minutes; 22°C) and the cells washed in RPMI 1640 medium (Commonwealth Serum Laboratories, Melbourne). Cell viability was determined by trypan blue dye exclusion. Cytospin preparations were made with 106 cells per slide and were stained with May-Grünwald-Giemsa stain. Differential cell counts were performed on 200 cells, and the number of asbestos bodies per 106 cells was also recorded. Normal BAL neutrophil proportions were taken as less than 2 percent for nonsmokers, less than 3 percent for ex-smokers, and less than 4 percent for current smokers.3

**Production of NCF**

Alveolar macrophages at 106 cells per milliliter were cultured in 24-well plastic tissue culture plates (Nunc) for five hours (37°C), either in medium alone or in the presence of opsonized zymosan (0.1 mg/ml), a known stimulus for NCF release.11,12

The supernatants were then collected for measurement of released NCF. All supernatants were centrifuged (1,150 g; five minutes; 22°C) to remove cell debris and were stored at −20°C until assayed.

**Assay for NCF**

The NCF activity in the tested supernatants was assayed using a 48-well microchemotaxis chamber (Neuro Probe) with 3μ pore-size polyvinylpyrrolidone-free polycarbonate membrane filters (Nucleopore Corp.). The NCF test samples were placed in the lower compartments of the chemotaxis chamber. Neutrophils were prepared from heparinized blood by density gradient centrifugation in Monopoly Resolving Medium (Flow Laboratory, North Ryde, Australia) and were placed in the upper compartments of the

**Table 1—Characteristics of Subjects**

<table>
<thead>
<tr>
<th>Date</th>
<th>Asbestosis*</th>
<th>Raised BAL%N†</th>
<th>Normal BAL%N</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Clinical features</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>57±2</td>
<td>57±1</td>
<td>49±1</td>
<td>46±6</td>
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<tr>
<td>Crackles‡</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Asbestos exposure</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Duration, yr</td>
<td>9±4</td>
<td>3±1</td>
<td>2±1</td>
<td>0</td>
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<tr>
<td>Interval since first exposure, yr</td>
<td>29±1</td>
<td>27±2</td>
<td>24±1</td>
<td>0</td>
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<tr>
<td>Pulmonary function</td>
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<tr>
<td>TLC, percent of predicted</td>
<td>95±5</td>
<td>101±7</td>
<td>101±4</td>
<td>97±10</td>
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<td>VC, percent of predicted</td>
<td>82±3</td>
<td>90±5</td>
<td>98±6</td>
<td>96±9</td>
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<tr>
<td>FEV1, percent of predicted</td>
<td>75±4</td>
<td>76±6</td>
<td>105±7</td>
<td>96±9</td>
</tr>
<tr>
<td>FEV1/FVC, percent</td>
<td>70±3</td>
<td>68±7</td>
<td>94±1</td>
<td>81±4</td>
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<tr>
<td>T1,CO2, percent of predicted</td>
<td>82±5</td>
<td>82±7</td>
<td>105±6</td>
<td>94±9</td>
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<tr>
<td>Bronchoalveolar lavage</td>
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<tr>
<td>Percent recovery</td>
<td>44±5</td>
<td>51±5</td>
<td>65±6</td>
<td>58±6</td>
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<tr>
<td>Percent viability§</td>
<td>74±4</td>
<td>76±3</td>
<td>83±3</td>
<td>85±3</td>
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<tr>
<td>Differential cell count</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Macrophages, percent</td>
<td>83.0±2.0</td>
<td>89.0±1.0</td>
<td>90.0±2.0</td>
<td>93.0±2.0</td>
</tr>
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<td>Lymphocytes, percent</td>
<td>7.0±1.0</td>
<td>4.0±0.5</td>
<td>3.5±0.5</td>
<td>5.5±2.0</td>
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<tr>
<td>Neutrophils, percent</td>
<td>8.0±1.0</td>
<td>5.5±0.5</td>
<td>2.0±0.4</td>
<td>1.0±0.2</td>
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<tr>
<td>Eosinophils, percent</td>
<td>2.0±1.0</td>
<td>1.5±0.5</td>
<td>0.5±0.3</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>Asbestos bodies‡</td>
<td>112.0±67.0</td>
<td>4.0±1.0</td>
<td>4.0±2.0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Asbestosis defined according to presence of small irregular opacities of profusion 1/0 or greater on a chest roentgenogram according to ILO classification.
†No radiographic evidence of asbestosis but raised BAL neutrophil proportions (%N).
‡Presence of inspiratory crackles which did not clear on coughing.
§Trypan-blue dye exclusion.
[Cell counts performed on May-Grünwald-Giemsa-stained cytospin preparations (expressed as percent of total cells excluding bronchial epithelial cells).
†Number of asbestos bodies per 106 cells counted.
Chemotaxis chamber at a concentration of 2 × 10⁶/ml of medium. A complement fragment with NCF activity, C5a, produced by activation of human serum by endotoxin (Escherichia coli 055:B5, lipopolysaccharide; Sigma Chemical Co) was diluted 1:10 in medium and was used as a positive control for neutrophil chemotaxis. After incubation (45 minutes; 37°C) the filter was removed from the chamber, and nonmigrated cells were scraped from the upper surface of the filter to facilitate accurate counting of the migrated cells on the under surface of the filter. The filter was then fixed in methanol, stained in Diff Quik solution (Harleco, Philadelphia), and mounted on a glass slide. The NCF activity was quantified by counting the number of neutrophils in five adjacent high-power fields. In each experiment the number of neutrophils migrating in response to medium alone was subtracted from the number of cells migrating in response to the various NCF test samples. All experiments were carried out in duplicate.

Statistical Methods

All data are expressed as the mean ± standard error of the mean (SEM), and statistical comparisons are made with the Student's t-test.

Results

The subjects with asbestosis had been exposed to asbestos for longer than the other exposed groups and had a greater degree of impairment of pulmonary function (Table 1). There was a similar distribution of smoking histories in the four groups. Pulmonary function in the subjects without radiographic abnormality but with raised BAL neutrophil proportions was intermediate between that of subjects with asbestosis and subjects with asbestos exposure but normal BAL neutrophil proportions.

Of the asbestos-exposed subjects with normal chest roentgenograms, six of the seven with raised BAL neutrophil proportions had crackles on auscultation, but none of the subjects with normal BAL neutrophil proportions had crackles.

Whereas alveolar macrophages from the five control subjects released negligible amounts of NCF (3 ± 1 N/HPF) unless stimulated with opsonized zymosan (113 ± 25 N/HPF; p < 0.01; Fig 1), alveolar macrophages from the eight subjects with asbestosis released large amounts of NCF (97 ± 19 N/HPF) spontaneously. The amount of NCF released was similar to amounts produced by control alveolar macrophages when activated by opsonized zymosan. Further addition of opsonized zymosan to alveolar macrophages from individuals with asbestosis produced a further small increase in NCF production (total 125 ± 20 N/HPF; Fig 1).

Alveolar macrophages from the six asbestos-exposed subjects who had normal BAL neutrophil proportions did not spontaneously release significant amounts of NCF (11 ± 7 N/HPF), this amount being similar to the five control subjects. The addition of opsonized zymosan produced a similar further increase in NCF production which was similar to the control subjects (101 ± 17 N/HPF). In contrast, alveolar macrophages from the seven asbestos-exposed subjects who had increased BAL neutrophil proportions released increased amounts of NCF (93 ± 24 N/HPF) spontaneously, and the addition of opsonized zymosan produced a further increase in NCF production (148 ± 15 N/HPF).

Discussion

This study demonstrates that NCF is spontaneously released by alveolar macrophages in individuals exposed to asbestos who have clinical and radiologic evidence of asbestosis and also from individuals with-

![Figure 1. Release of neutrophil chemotactic factor (NCF) by alveolar macrophages (AM) in medium alone (+0) and following addition of opsonized zymosan (+O2) in subjects with radiographic asbestosis, asbestos-exposed individuals with normal chest radiographs (raised or normal BAL neutrophils) and control subjects. Normal BAL neutrophil proportions were taken as <2 percent for non-smokers, <3 percent for ex-smokers and <4 percent for current smokers.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21583/)
out radiographic evidence of asbestosis but who have crackles on auscultation. These findings suggest that locally released NCF mediates the neutrophil accumulation seen in asbestos-exposed individuals and that the presence of crackles may be a more reliable way of identifying alveolitis than the presence of radiographic changes.

The alveoli of the normal lung contain few neutrophils, but alveolar neutrophil accumulation is seen after asbestos inhalation in many animals\(^5^9\) and also in humans with asbestosis.\(^3^4\) Neutrophils are important cellular components of the inflammatory process and have the potential to cause pulmonary damage through the release of proteolytic enzymes and toxic oxygen metabolites.\(^15^17\) It is likely that neutrophils are responsible for some of the pathologic changes of asbestosis, and it is therefore important to understand the mechanisms underlying their accumulation in response to asbestos inhalation.

The alveolar macrophage is the major alveolar cell and has a central role in host defense.\(^18^19\) Normal human alveolar macrophages do not spontaneously release NCF, and this may partly explain the low numbers of neutrophils on the normal alveolar surface; however, alveolar macrophages do release NCF when stimulated by infectious, immune, or particulate agents.\(^11^18\) and it has been shown that exposure of alveolar macrophages to asbestos fibers (crocidolite or chrysotile) in vitro also induces NCF release.\(^20\) Nevertheless, it is unreasonable to extrapolate from these in vitro studies to the conclusion that this NCF release by alveolar macrophages mediates the alveolar neutrophil accumulation that is seen in vivo, particularly as these in vitro studies are performed over five hours, whereas in subjects included in this study, asbestos exposure lasted for several months and ceased at least 20 years ago. The levels of NCF released spontaneously by alveolar macrophages from the individuals with asbestosis were comparable to those released by alveolar macrophages in known activating agents such as opsonized zymosan.\(^11^13\) This suggests that alveolar macrophages in subjects with asbestos-induced alveolitis are continuously activated. This concept is supported by the knowledge that individuals with asbestosis exhibit enhanced pulmonary uptake of gallium,\(^21\) which is considered at least in part to reflect activation of alveolar macrophages.\(^22\)

Rather than being pathogenic, it would be postulated that NCF production by alveolar macrophages in asbestosis is a nonspecific feature either of asbestosis or asbestos exposure. In this study the individuals with asbestos exposure without radiographic abnormality showed BAL neutrophil proportions which were related to the levels of NCF. This implies that NCF release is not merely a manifestation of asbestos inhalation. The host factors regulating NCF release from alveolar macrophages in response to asbestos fibers are not known but may partly underlie the known heterogeneity of individual susceptibility to asbestos-induced pulmonary disease.\(^23\) In a sheep model of asbestosis, alveolitis has been found to be more closely related to alveolar retention of dust than the dose of exposure. Alveolar asbestos fiber clearance has not been directly studied in asbestos-exposed workers and may be an important factor in determining individual susceptibility to asbestosis.\(^24\)

Some asbestos-exposed individuals had no radiographic asbestosis but did demonstrate increased NCF production. All of these individuals had crackles. This suggests that the chest roentgenogram has a lower predictive value for the presence of alveolitis than the presence of crackles on auscultation of the lungs.

Asbestosis and idiopathic fibrosing alveolitis share similarities in clinical presentation, histologic findings, and progression of disease. Both diseases are characterized by a predominantly neutrophil alveolitis.\(^3\) In fibrosing alveolitis, alveolar macrophages release NCF spontaneously.\(^25\) This suggests that although the initiating stimulus is different from that observed in asbestosis, similar pathogenic mechanisms may underlie the neutrophil alveolitis of these two diseases.

Since NCF release by alveolar macrophages may be involved in the pathogenesis of both asbestosis and fibrosing alveolitis, the development of therapeutic agents to block NCF release may be of benefit in individuals with both of these diseases; for example, colchicine has been shown to suppress the release of NCF by alveolar macrophages in vitro,\(^20\) and it is possible that anti-inflammatory agents such as this may be effective in suppressing NCF release and its consequences.

References

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