Dr. Becklake: Did the fibers get further out to the pleura?

Dr. Brody: In animals which were dusted for five hours a day for several weeks, the dust extends to the alveolar duct surface, but still not to the individual alveolar spaces. Such small fibrils, when inhaled to the most peripheral alveolar ducts, would have direct access to the pleura.

Dr. Brain: If particles penetrate through type I cells by some sort of pinocytic process, what happens to the material when it gains access to the basement membrane?

Dr. Brody: I believe the fiber size determines whether it's going to be picked up in a phagosomal type situation in which the particles are membrane bound, or whether the particles will be free in cytoplasm. We fortuitously were able to see along the axis of the fibers in some sections and they appear to line up along the plane of the basement membranes, suggesting that these small fibrils could then move to the peribronchiolar lymphatics.

Inorganic Particulates Associated with Desquamative Interstitial Pneumonia*

Jerrold L. Abraham, M.D.; and Michael A. Hertzberg, B.A.†

Environmental exposure to inorganic particulates is one recognized cause of desquamative interstitial pneumonia (DIP).1-3 This study is part of a series aimed at discovering possible etiologic agents associated with lung diseases which are otherwise considered idiopathic.4-7 The lung retains an inventory of inorganic particulate agents which are amenable to analysis using microanalytic techniques.7,8 We feel that the detection of previously unrecognized etiologic agents is important for several reasons: 1) these data may serve to direct future studies of epidemiology and mechanisms, 2) the results may facilitate more accurate diagnoses, and 3) most importantly, these data may be of use for primary prevention.

Whether one views DIP as a specific entity or merely as part of the spectrum of changes seen in interstitial pneumonia,8 it has been well documented that patients with the histology typical of DIP have a better prognosis than those with usual interstitial pneumonia (UIP).10

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Table 1—Certainty of Diagnosis of DIP in Microanalyzed Cases and Others

<table>
<thead>
<tr>
<th>Category</th>
<th>1(%)</th>
<th>2(%)</th>
<th>3(%)</th>
<th>4(%)</th>
<th>5(%)</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microanalyzed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26(100)</td>
</tr>
<tr>
<td>Non-Analyzed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>67(100)</td>
</tr>
<tr>
<td>Cases</td>
<td>15(23)</td>
<td>23(35)</td>
<td>9(13)</td>
<td>12(18)</td>
<td>8(12)</td>
<td>67(100)</td>
</tr>
<tr>
<td>All Cases</td>
<td>22(24)</td>
<td>28(30)</td>
<td>12(13)</td>
<td>17(18)</td>
<td>14(15)</td>
<td>93(100)</td>
</tr>
</tbody>
</table>

Materials and Methods

Cases for scanning electron microscopy (SEM) and energy dispersive x-ray analysis (EDXA) were selected from the Averill A. Liebow Pulmonary Pathology Collection based only on an indexed diagnosis of DIP plus the availability of paraffin blocks. Five μm thick sections were mounted on carbon discs, deparaffinized and dried. Sections were searched at a standard magnification (6000X) and all inorganic particulates revealed in the back-scattered electron image7 were individually analyzed using EDXA. This SEM approach allows much more efficient searching of

Figure 1. Light micrographs. A, upper, (original magnification, 104X) showing classic features of DIP.1,10 Note that lymphoid aggregate partially narrows small airways. B, lower, (original magnification, 416X), partially crossed polarizers, showing presence of strongly birefringent plate-like particles in macrophages (H & E stained 5μm section).
Table 2—DIP: Data on All 93 Cases

<table>
<thead>
<tr>
<th>Diagnostic Certainty</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Totals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satisfactory Env Hx avail</td>
<td>11 (50%)</td>
<td>12 (43%)</td>
<td>7 (58%)</td>
<td>3 (18%)</td>
<td>3 (21%)</td>
<td>36 (39%)</td>
</tr>
<tr>
<td># c biref particles‡</td>
<td>11 (50%)</td>
<td>10 (36%)</td>
<td>2 (17%)</td>
<td>1 (6%)</td>
<td>2 (14%)</td>
<td>26 (28%)</td>
</tr>
<tr>
<td># c Fe stain§</td>
<td>11 (50%)</td>
<td>12 (43%)</td>
<td>4 (33%)</td>
<td>8 (47%)</td>
<td>7 (50%)</td>
<td>42 (45%)</td>
</tr>
<tr>
<td># c asb bod¶</td>
<td>3 (14%)</td>
<td>1 (4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Totals¶</td>
<td>22</td>
<td>28</td>
<td>12</td>
<td>17</td>
<td>14</td>
<td>93 (100%)</td>
</tr>
</tbody>
</table>

*? = unknown  
†Age groups: 1: <20; 2:20-40; 3: >40 years  
‡Number of cases showing increased numbers of birefringent particles  
§Number of cases having iron-stained section available  
¶Number of cases in which typical asbestos bodies were found  
*Unfortunately, only 13 (14%) had available smoking histories. 3 had never smoked.

tissue (10,000 μm²/field) than ultra thin (100nm) section analysis (31 μm²/field) (volume ratio is 300:1). This may account for the failure of previous studies to find inorganic particulates in DIP cases. As an assessment of selection, a total of 93 indexed DIP cases (including the analyzed cases) were reviewed independently by light microscopy. We graded the certainty of diagnosis using Carrington's scheme (1 to 5 in order of decreasing certainty), age, sex, availability of environmental history, abundance of birefringent particles, and presence of asbestos bodies.

RESULTS

Table 1 shows some of the data on the cases analyzed compared to those not analyzed and the total 93 cases reviewed. Other diagnoses were preferred over DIP in 31 cases (33%). These included honeycombing (9), lesions too unevenly distributed. UIP (4), granulomatous disease (4), bronchiolitis (3), eosinophilic granuloma (2), hemosiderosis (2), and insufficient biopsy (3). Ages ranged from infancy to the ninth decade. As shown in Table 2, the sex ratio was male-predominant in the more certain cases. Of special note was the finding of asbestos bodies only in cases in the most certain diagnostic categories (1 and 2). In total we searched over 2,000 fields and analyzed over 1,100 particles. A sample of the detailed information and correlation in those analyzed cases with available exposure history is given in Table 3. Figures 1 and 2 illustrate some of the light microscopic and SEM appearances seen in this study.

Table 3—Inorganic Particulates Associated with DIP: Cases with Environmental History

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, Sex</th>
<th>Particles/cm²**</th>
<th>% &lt;1μm†</th>
<th>M T‡</th>
<th>A Y Siliates</th>
<th>J P cates</th>
<th>O E</th>
<th>R S Other</th>
<th>History</th>
<th>Comment</th>
<th>Certainty of Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>63,M</td>
<td>6.3x10⁷</td>
<td>41</td>
<td>SiO₂</td>
<td>13%</td>
<td>Si,Al,K</td>
<td></td>
<td>28%</td>
<td>Truck Driver</td>
<td>Silicates</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>61,F</td>
<td>&gt;10⁴</td>
<td>&gt;90</td>
<td>SiO₂</td>
<td>13%</td>
<td>Si,Al,K</td>
<td></td>
<td>&gt;90%</td>
<td>Tool Grinder</td>
<td>Hard Metal</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>41,M</td>
<td>&gt;10⁴</td>
<td>≈60</td>
<td>SiO₂</td>
<td>13%</td>
<td>Si,Al,K</td>
<td></td>
<td>&gt;90%</td>
<td>Arc Polisher</td>
<td>Al &amp; Fumes</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>50,M</td>
<td>5.5x10⁷</td>
<td>87</td>
<td>SiO₂</td>
<td>13%</td>
<td>Si,Mg,Fe</td>
<td></td>
<td>72%</td>
<td>Manager Tire Shop</td>
<td>Tale Metals</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>65,M</td>
<td>&gt;10⁴</td>
<td>≈50</td>
<td>SiO₂</td>
<td>13%</td>
<td>Si,Mg,Fe</td>
<td></td>
<td>≈10%</td>
<td>Plastics Machinist</td>
<td>Diatoms</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>37,F</td>
<td>&gt;10⁴</td>
<td>&gt;90</td>
<td>SiO₂</td>
<td>13%</td>
<td>Si,Mg,Fe</td>
<td></td>
<td>&gt;90%</td>
<td>Tool Grinder</td>
<td>Al &amp; Fumes</td>
<td>3</td>
</tr>
<tr>
<td>23</td>
<td>34,M</td>
<td>&gt;10⁴</td>
<td>&gt;90</td>
<td>SiO₂</td>
<td>13%</td>
<td>Si,Mg,Fe</td>
<td></td>
<td>&gt;90%</td>
<td>Al Are Welder</td>
<td>Al &amp; Fumes</td>
<td>1</td>
</tr>
</tbody>
</table>

**Particles/cm² calculated from particles/field searched and known volume/field  
†% of particles with diameter <1μm  
‡ Analyzed particles grouped into three major types and % in each type, with major identified particles listed.
Fig 2. Scanning electron micrographs. A. Low magnification 5μm section on carbon. Marker = 100μm. B. High magnification backscattered electron (BSE) image of same case as in Fig 1B showing dark (higher atomic number) particles analyzed as talc. Marker = 1μm. C. High magnification BSE image of another DIP case with many tiny particles in giant cells. Many of the particles are too small to resolve by light microscopy. Analysis revealed W, Ta, Ti & Co. Marker = 10μm.

Discussion

Of the 17 DIP cases quantitatively analyzed, there were $5.8 \pm 1.1 \times 10^7$ particles/cm$^3$ and $0.8 \pm 0.63 \times 10^4$ in controls (N=5) (P<0.001). Cases used as controls were other air space filling processes with known nonparticulate etiology (eg Pneumocystis carinii). We found that 23 of 25 (92%) of DIP cases analyzed had particle concentrations well above that in control tissues. It is noteworthy that 14 of these 23 cases had not shown increased numbers of birefringent particles, confirming that much of the particulate burden goes undetected by light microscopy.

In our 93 cases, of the 39 with available environmental history, 28 (72%) had a history of dust exposure of one kind or another. The major environmental exposures documented included 15 with metal or welding fume exposure (5 AL, 2 tool grinding, and 13 various other metals) and 11 with silicate exposure (6 talc and 5 asbestos). None of these cases showed SiO$_2$ as a major particulate. In fact, it is interesting that the only overall statistically significant difference between the findings in the DIP cases and the pulmonary alveolar proteinosis (PAP) cases$^+$ was between the % SiO$_2$ (5.7 ± 1.2, N=17, DIP; 20.9 ± 4.6, N=22, PAP) (P<0.005). Probably those cases with abundant giant cells and presence of tungsten, etc should be regarded as giant cell interstitial pneumonia (GIP)$^{+}$ or hard metal pneumoconiosis.$^{12}$ Other interesting exposures documented include aluminum metal and diatomaceous earth.

Conclusion

We have shown that in a group of 93 previously idiopathic cases of DIP, approximately 75% (of those with environmental history available) have a history of dust or fume exposure, and that specific types of particulate exposures are documented in 92% of cases subjected to tissue microanalysis. These findings show associations between DIP and certain particulate exposures and only hint at possible etiologic relationships in need of future investigation.

References

7 Abraham JL. Recent advances in pneumoconiosis-The pathologist's role in etiologic diagnosis. In: The lung (Thurlbeck WT, Abell M, eds) Baltimore: Williams and Wilkins 1978; 96-137
9 Liebow AA. Definition and classification of interstitial pneumonias in human pathology. Prog Resp Res 1975; 8:1-33

The environment and the lung 68S
Inflammation and Asbestosis: Characterization and Maintenance of Alveolitis following Acute Asbestos Exposure*

C. Schoenberger, M.D.; G. Hunninghake, M.D.; J. Cadek, M.D.; and R. Crystal, M.D.

Asbestos is a chronic interstitial lung disease associated with the inhalation of asbestos fibers. Although the parenchymal injury which is characteristic of this disorder is clearly mediated by the presence of asbestos fibers within the lung, the mechanisms by which these fibers injure the lung is poorly understood. To determine whether asbestos fibers trigger an acute inflammatory response in the lung, guinea pigs were exposed via a single intratracheal (IT) injection to 25 mg of chrysotile asbestos fibers in 1 ml saline solution or 1 ml saline alone.

At four hours and ten days following injection, the inflammatory and immune effector cells that were present within the lung were evaluated by light microscopy and by analysis of the cell populations that were present in bronchoalveolar lavage fluid (BAL). Microscopically, at both four hours and ten days following injection, the lungs of control animals were characterized by the absence of neutrophils and by the presence of small numbers of alveolar macrophages. Compared to controls at both time points, the lungs of asbestos-treated animals demonstrated peribronchial and alveolar collections of both neutrophils and alveolar macrophages. The presence of neutrophils in the lungs of asbestos treated animals was confirmed by analysis of the proportions of BAL cells that were neutrophils (4 hr, asbestos 28 ± 8% vs saline 4 ± 1%; 10 days, asbestos 38 ± 9% vs saline 10 ± 3%).

To determine whether alveolar macrophages play a role in attracting neutrophils to the lung in this disorder, alveolar macrophages were exposed for three hours in vitro to chrysotile asbestos and supernatants of these alveolar filling processes that are known to be non-environmental, such as pneumocystis or bacterial pneumonia. They demonstrated only background levels of silicate from the soil exposure in that region.

Dr. Brody: Isn’t it possible that the people had an alveolar filling disease, DIP, and then inhaled particles which were more efficiently trapped by the presence of macrophage?

Dr. Abraham: That’s possible; however, most of the agents can produce the same lesions experimentally. The controls also had alveolar filling diseases.

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**DISCUSSION**

Dr. Thurlbeck: Have you studied the effect of feeding macrophage particles that don’t cause fibrosis in the lung?

Dr. Schoenberger: A group in Amersham, England has injected asbestos-like fibers intratracheally in a similar rat model. They have shown that fibers which produce long-term toxicity such as silica or asbestos, are associated with an influx of neutrophils which persists for a long period of time, while non-toxic fibers result in mild neutrophil accumulation in the first day or so and that then disappears.

Dr. Brody: Will an inhalation model produce neutrophil accumulation in the lungs?

Dr. Schoenberger: Other investigators using inhalation models to produce fibrosis have demonstrated neutrophils on pathology, but this has not received much emphasis.

Dr. Goodman: Will these animals develop chronic pulmonary fibrosis similar to the human model, and have you repeated these experiments with silica or other things?