activity for monocytes while nitrogen mustard-treated rabbits reconstituted with neutrophils showed chemotactic activity for monocytes (Table 1). Supernates from mixtures of BCG and neutrophils also contained chemotactic activity for monocytes (80 ± 6 MN/10 HPF) which was greater than that seen in supernates from mixtures of BCG (15 ± 2) or neutrophils (10 ± 4) alone.

**DISCUSSION**

Considerable evidence was obtained to support the premise that neutrophils play an important role in granulomatous inflammation. First, neutrophils preceded influxes of macrophages into pleural spaces of rabbits given BCG. Second, in nitrogen mustard-treated rabbits, neutropenia was associated with an absence of macrophage influx, increases in the number of BCG colonies recoverable from pleural fluids and a lack of well-defined granulomas on pleural surfaces. The absence of the usual macrophage influx could not be explained on the basis of the direct effect of nitrogen mustard on monocytes, since following reconstitution with neutrophils, the usual macrophage influx occurred. Since neutrophils were the only variable injected into pleural spaces of nitrogen mustard-treated rabbits and since neutrophil injection was followed by the appearance of macrophages, it appeared that neutrophils were involved in the recruitment of monocytes. This impression was substantiated when chemotactic activity for monocytes was found in pleural fluids of normal and neutrophil-reconstituted rabbits, but not in neutropenic rabbits given intrapleural BCG. This phenomenon also was validated by finding similar monocyte chemotaxins in in vitro mixtures of neutrophils and BCG. The present observations support the possibility that neutrophils participate in monocyte recruitment and granuloma formation and suggest that this mechanism may be involved in granulomatous inflammation and repair.

**REFERENCES**


**Table 1—Pleural Fluid Characteristics in Normal and Nitrogen Mustard-Treated Neutropenic Rabbits Given BCG Intrathoracically**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Normal Rabbits</th>
<th>Neutrophils</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.7 ± 0.4</td>
<td>2 ± 0.3</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>12</td>
<td>10.6 ± 3</td>
<td>76 ± 31</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>48</td>
<td>3.6 ± 1</td>
<td>20 ± 41</td>
<td>32 ± 21</td>
</tr>
<tr>
<td>96</td>
<td>2.5 ± 0</td>
<td>4 ± 1</td>
<td>67 ± 41</td>
</tr>
</tbody>
</table>

Activity for monocytes (MN/10 HPF)

*Mean ± SEM (of 18 or more determinations)
†Value significantly (p<0.05) increased compared to value for neutrophils injected with BCG.

**Role of Fibronectin in Fibrotic Lung Disease**

A Growth Factor for Human Lung Fibroblasts

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In addition to an accumulation of collagen, the alveolar structures of patients with fibrotic lung disease are characterized by increased numbers of fibroblasts and increased amount of fibronectin, a glycoprotein that mediates the attachment of fibroblasts to the extracellular matrix. To evaluate the concept that extracellular matrix elements are important modulators of cell number, the ability of fibronectin to augment human lung fibroblast replication was evaluated. To accomplish this, human lung fibroblasts (HFL-1) were cultured in defined medium (Dulbecco's modified Eagle's medium + 1% bovine serum albumin + 5 μg/ml transferrin) to maintain the fibroblasts in a viable, but nonreplicating state. Fibronectin, purified from human plasma by gelatin sepharose affinity chromatography followed by gel filtration, was added to the fibroblast cultures (0 to 2 μg/ml) alone or together with alveolar macrophage derived growth factor (0 to 100nM), a growth factor for lung fibroblasts that is released by the activated macrophages present in the alveolar structures of most patients with fibrotic lung disease. Following growth factor addition, fibroblasts were cultured 72 hr, then counted. Cells cultured with fibronectin or alveolar macrophage-derived growth factor alone showed little increase in cell number above that seen in defined medium (control), p >0.2. In marked contrast, fibroblasts cultured with both fibronectin and alveolar macrophage-derived growth factor showed a dramatic dose-dependent increase in cell number ranging from 88% above control (fibronectin 0.2 μg/ml and alveolar macrophage-derived growth factor 1nM) up to 185% above control (fibronectin 2μg/ml + alveolar macrophage derived growth factor 100nM, p <0.001, both comparisons with control). Thus, the presence of increased amounts of the matrix component fibronectin together with alveolar macrophage-derived growth factor within the alveolar structures of patients with fibrotic lung disease may play a significant role in the fibrosis characteristic of these disorders, by amplifying the numbers of fibroblasts in the alveolar structures of these patients.

*From the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda.