Lung Repair and Granuloma Formation

Tubercle Bacilli Stimulated Neutrophils Release Chemotactic Factors for Monocytes

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The common underlying host response in tuberculosis and other granulomatous repair processes is formation of granulomata. These granulomata include mononuclear phagocytes which originate from peripheral blood monocytes (MN). Since neutrophils precede monocytes at areas of granulomatous inflammation, we hypothesized that neutrophils initiate these inflammatory responses by releasing chemotaxins which attract monocytes. Our results supported this premise (Fig 1). First, neutrophils stimulated by tubercle bacilli released chemotactic factors for monocytes, both in vivo and in vitro. Second, in nitrogen mustard treated rabbits given BCG intrapleurally, neutropenia was associated with decreased numbers of macrophages, increased BCG colonies recoverable from pleural fluid, and decreased granuloma formation on pleural surfaces.

METHODS

New Zealand white rabbits were immunized with intradermal bacillus Calmette-Guérin (BCG) and sensitization was confirmed by protein purified derivative (PPD) challenge. Rabbits then received intrapleural BCG via a percutaneous technique. For some experiments, rabbits were made neutropenic (<200 neutrophils/µl) with nitrogen mustard given in a single bolus dose (1.75 mg/kg) 60 hours prior to initiation of the experiment. In some experiments, immediately following BCG instillation, the pleural spaces of nitrogen mustard-treated rabbits were reconstituted with neutrophils isolated from peripheral blood. Exudative pleural effusions developed and were sampled sequentially via thoracocentesis over the next 120 hours. Pleural fluid was analyzed for cytology (Papanicolaou technique) and pleural fluid supernatants evaluated for their chemotactic activity for monocytes using modified Boyden chambers. Supernatants from serum-free mixtures of BCG and neutrophils incubated at 37°C for 4 hours also were evaluated for their chemotactic activity for monocytes in vitro.

RESULTS

Injection of BCG into the pleural spaces of normal rabbits caused large and reproducible influxes of cells (peak of 11,800 ± 3,000 [mean ± SEM] cells/µl by 4 hours). For the first 24 hours after BCG instillation, this cellular influx consisted primarily of neutrophils (>75%, Table I). However, 96 hours following injection of BCG, macrophages were the predominant cell recovered from pleural spaces (>60%). In contrast, in nitrogen mustard-treated rabbits there was an absence of neutrophils in pleural spaces (absolute count <200 cells) following injection of BCG. Furthermore, absence of neutrophils was followed by a lack of macrophages (<10%), increased numbers of BCG colonies recoverable from pleural fluids and decreased granulomata on the visceral and parietal pleural surfaces at autopsy. Importantly, in the reconstitution experiment, instillation of neutrophils into pleural spaces of nitrogen mustard-treated rabbits given BCG intrapleurally was followed by the usual influx of macrophages, decreased numbers of BCG colonies and granuloma formation. The aforementioned suggested that neutrophils might be producing chemotaxins for monocytes. This suggestion was supported when supernatants from pleural fluids obtained from normal rabbits treated with BCG showed a marked degree of chemotactic activity for monocytes. Furthermore, maximal chemotactic activity for monocytes was seen at 24 hours after BCG instillation and correlated with the number of neutrophils in pleural fluid. In contrast, pleural fluids from neutropenic rabbits did not show evidence of chemotactic activity.

HYPOTHESIS:

Neutrophils Initiate Macrophage Responses to T.B.

FIGURE 1. Hypothetical mechanism of neutrophil-initiated macrophage responses to tuberculosis.
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 granulomata 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 neutrophilic rabbits given intrapleural BCG. This phenomenon also was validated by finding similar monocyte chemotaxis 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**

2 Montgomery LG, Simon WS. The cellular reaction of the pleura

**Role of Fibronectin in Fibrotic Lung Disease**

**A Growth Factor for Human Lung Fibroblasts**

P. Bitterman, M.D.; S. Renard, M.D.; S. Adelberg, B.S.; and R. G. Crystal, M.D.

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.

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

<table>
<thead>
<tr>
<th></th>
<th>Total Cell Count</th>
<th>Neutrophils (% total)</th>
<th>Macrophages (% total)</th>
<th>Chemotactic Activity for monocytes</th>
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<tr>
<td>Normal rabbits given BCG</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 hr</td>
<td>1.7±0.4</td>
<td>2±0.3</td>
<td>8±1</td>
<td></td>
</tr>
<tr>
<td>12 hr</td>
<td>10.6±3.4†</td>
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<tr>
<td>48 hr</td>
<td>3.8±1</td>
<td>20±4†</td>
<td>32±2†</td>
<td>30±2†</td>
</tr>
<tr>
<td>96 hr</td>
<td>2.3±0</td>
<td>4±1</td>
<td>67±4†</td>
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<td>Neutropholic rabbits given BCG</td>
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<td></td>
<td></td>
</tr>
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<td>2±0.3</td>
<td>8±1</td>
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</table>

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