the lysozyme activity for the entire cell population was not significantly different from control animals suggesting that the total number of activated macrophages was small. Intratracheal inoculation with \textit{M. faeni} antigen resulted in the production of anti-\textit{M. faeni} precipitating antibodies in serum of all inoculated animals. Anti-\textit{M. faeni} precipitins demonstrated by counterimmunoelectrophoresis were also found in 14 of 16 concentrated lung wash specimens at 14 days and in 15 of 16 animals at 21 days. Determination of immunoglobulins by single radial diffusion in bronchial lavage specimens of all control and \textit{M. faeni} inoculated animals revealed IgG and IgA to be present at 14 and 21 days. IgM was noted in only 6 of the 40 total specimens analyzed and when detected was present in low concentrations at both 14 and 21 days. Levels of IgG and IgA were 384 ± 31 and 101 ± 12 mg % respectively in normal controls. Immune rabbits had significantly increased IgG and IgA levels (614 ± 34 and 219 ± mg % respectively).

The conclusions that one may draw from this study are as follows: 1) intratracheal inoculation of rabbits with \textit{M. faeni} produces a reversible mononuclear interstitial pneumonitis similar to that seen in man; 2) this pneumonitis is associated with intraalveolar accumulation of macrophages with increased percentages of lymphocytes and granulocytes early in the course of the lesion; 3) associated with this proliferative cellular response is evidence of macrophage stimulation and MIF production by bronchoalveolar lymphocytes. These data, together with other studies demonstrating ability to produce the same pathologic lesions by passive transfer of sensitized lymphoid cells followed by respiratory tract challenge,\textsuperscript{a} suggest that delayed (cell-mediated) hypersensitivity plays a role in the pathogenesis of these experimental lesions in rabbits; 4) in addition, the presence of precipitins and increased immunoglobulin levels in bronchial secretions suggest a role for humoral immune mechanisms in either disease pathogenesis or host defense against inhaled actinomycete antigen.

The heterogeneous immune response noted in this study is consistent with current findings in hypersensitivity pneumonitis in man\textsuperscript{7} and it is reasonable to postulate that both humoral and cellular hypersensitivity may play a role in the pathogenesis of this pulmonary disorder.

\textbf{References}


\textbf{Experimental Granulomatous Pneumonitis: Immunologic, Histologic, and Ultrastructural Correlations*}

\textit{Ray E. Standford, M.D.; and John E. Salvaggio, M.D.}

Allergic diseases of the bronchi and lungs are being recognized with increasing frequency, due in part to an increasing number of known potential allergens present in the atmosphere. One group of such diseases, called variously extrinsic allergic alveolitis or hypersensitivity pneumonitis, may be caused by exposure to any of an ever-expanding list of noninfectious bacterial, fungal, or higher plant/animal particles. Repeated exposure may result in chronic granulomatous inflammation of airways and lung parenchyma, leading to pulmonary fibrosis and respiratory failure. Among the more common organisms causing this type of allergic lung disease are various thermophilic actinomycetes, especially \textit{Microcytopora faeni} (in farmer’s lung) and species of thermoactinomycyes (in bagassosis and humidifier lung). Both humoral and cell-mediated immune mechanisms have been implicated in pathogenesis. Nonimmunologic mechanisms may be operative in addition, but their relative importance, as well as host susceptibility factors, remain unknown. Dr. Allen has just told us of possible genetic predisposing factors in his patients, and in agreement with recent published work from other centers has suggested that cell-mediated immunity (CMI) may be of paramount importance in the pathogenesis of chronic hypersensitivity pneumonitis.\textsuperscript{1,2}

Several animal models for these diseases have been developed, but they have either utilized antigen in complete Freund’s adjuvant (which can produce pulmonary granulomas by itself) or have shown lesions different from those of the chronic human disease.\textsuperscript{3,4} The purpose of this report is to present detailed histologic and ultrastructural features of pulmonary lesions in various stages of development in rabbits exposed to particulate \textit{M. faeni} antigen, and to correlate these findings with evidence of cell-mediated hypersensitivity.

\textbf{Methods}

Rabbits were given up to three intratracheal inoculations, at three-day intervals, with 50 mg in 1 ml saline solution of a homogenized, lyophilized, reconstituted preparation derived from cultured \textit{M. faeni}. Controls received similar inoculations of saline solution alone. Skin tests with 10 and 100 \textmu g

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Table 1—Summary of Histologic Findings

<table>
<thead>
<tr>
<th>Day</th>
<th>Summary of Findings</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td>acute necrotizing bronchiolitis</td>
</tr>
<tr>
<td>9</td>
<td>organizing and interstitial bronchiolitis</td>
</tr>
<tr>
<td>14</td>
<td>early granulomatous peribronchiolitis and</td>
</tr>
<tr>
<td></td>
<td>obstructive (&quot;lipoid&quot;) pneumonitis</td>
</tr>
<tr>
<td>21</td>
<td>well-formed granulomas; reduced pneumonitis</td>
</tr>
<tr>
<td>28</td>
<td>small granulomas; fibrosis of bronchioles;</td>
</tr>
<tr>
<td></td>
<td>centriacinar emphysema</td>
</tr>
</tbody>
</table>

dialyzed antigen were done at various intervals, read at 20 minutes, 6, 24, and 48 hours, and biopsied at 48 hours. Animals were sacrificed by intravenous pentobarbital at 4, 9, 14, 21, and 28 days after first inoculation. Lungs were washed immediately to obtain alveolar macrophages for migration inhibition studies, then inflation-fixed with 1.7 percent glutaraldehyde for histologic and ultrastructural examination. A small portion of lung was quick-frozen for immunofluorescent microscopy prior to washing and fixation. Precipitins to \( M. faeni \) were determined by counterimmunoelectrophoresis of serum obtained and frozen at the time of sacrifice. Details of methods may be found elsewhere.

RESULTS

The histologic findings are summarized in Table 1. Necrotizing inflammation of terminal and respiratory bronchioles was observed on day 4 (Fig 1A) following inoculation with \( M. faeni \) antigen. Granulomas were first seen in lungs of animals sacrificed on day 14, and were well-developed by day 21 (Fig 1B). Obstructive pneumonia developed distal to the bronchiolar lesions in many animals, and a few showed destructive lesions similar to centriacinar emphysema by day 28. Control animals developed no lesions, except that one died with fulminant bronchopneumonia.

Electron microscopy was done on ultrathin sections of osmium postfixed, Epon-embedded, uranyl acetate/lead citrate stained lung tissue. Hyperplasia of type II pneumocytes was evident by day 14, maximum on day 21, and had subsided greatly by day 28 (Fig 2). Histiocytes within granulomas and alveolar macrophages contained various sized cytoplasmic inclusions thought to be phagocytized antigen particles (Fig 3). In no instance were subendothelial, subepithelial or membranous deposits observed.

Fluorescence microscopy revealed \( C_4 \) within a few alveolar macrophages, and sparse intracellular (plasma cell) IgG and IgA in developing interstitial lesions, but no vascular or membranous immunoglobulin or \( C_4 \) deposits to suggest the presence of immune complexes.

Concurrent with granuloma formation, \( M. faeni \) induced alveolar macrophage migration in vitro became efficient.
and Roger Wheelis, M.D.

*From the Virginia Mason Research Center, Seattle. This work was supported by USPHS-NIH Grant No. PRO5588-08.

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Immunologic Mechanisms in Experimental Interstitial Pneumonitis*

William F. Willoughby, M.D.,** Judith E. Barbaras, M.D.,† and Roger Wheelis, M.D.

Figure 4. Alveolar macrophage migration inhibition by \textit{M. faeni} (direct assay). Values between +20 and -20 are plotted as zero.

progressively inhibited (Fig 4) and the animals developed both precipitating serum antibodies and delayed skin reactivity. Skin biopsies at 48 hours revealed perivascular infiltration by lymphocytes and histiocytes characteristic of cell-mediated hypersensitivity, superimposed upon a nonspecific acute inflammatory reaction.

**DISCUSSION**

The granulomatous pneumonitis which we observed is similar to that seen in many cases of chronic farmer's lung and other human hypersensitivity pneumonitides. The early bronchiolar lesions, occurring as early as four days after inoculation of previously unsensitized animals, are considered to represent direct irritation of the tissue by the relatively large (about 1 micron) and antigenically complex \textit{M. faeni} spores and hyphae. This interpretation is consistent with the fact that skin tests with \textit{M. faeni} antigen are notoriously unreliable clinically because of irritative properties. The bronchiolar localization of lesions is probably also related to the particle size, and it would be of interest to perform similar experiments with smaller particles—perhaps purified antigenic components adsorbed onto inert particles of known size and shape. The later granulomatous lesions strongly suggest cell-mediated hypersensitivity to the antigen, which is supported by the MIF and skin test data. That the skin tests read at 48 hours reflect at least in part CMI is illustrated in Figure 5 which shows a rough linear correlation between the skin test size and percent inhibition of alveolar macrophages.

between the skin test size and percent inhibition of alveolar macrophages obtained at subsequent sacrifice. An additional hypothesis, that \textit{M. faeni} particles may act as an immunologic adjuvant, is discussed in detail elsewhere, but remains speculative at present. We found no evidence for a type III (immune complex) reaction in our animals, but recognize that this and perhaps other mechanisms in addition to CMI may be important in the pathogenesis of hypersensitivity pneumonitis in some instances.

REFERENCES


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The lung, by virtue of its unique exposure to both the circulation and the external environment, is particularly vulnerable to immunologic insult. Modes of well-documented immune pulmonary injury include alveolar capillary injury following either sequestration of circulating immune complexes, or fixation of antivascular basement membrane antibody.12 In addition, inhaled antigens are known to provoke asthmatic-type reactions in atopic individuals, but their role in inducing interstitial disease is less clear.

We have employed an animal model to assess pulmo-