Genetic Control of Experimental Murine Hypersensitivity Pneumonitis*

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Hypersensitivity pneumonitis (HP) is an environmental lung disease involving the distal portion of the respiratory tract. The inflammatory response is variable, but usually consists of chronic granulomatous inflammation. Physiologic manifestations of these diseases include a peripheral neutrophilia, fever, and tachypnea occurring 4 to 8 hours after exposure to the antigen. The mechanisms of pathogenesis are not yet clarified, but probably involve both immune complex disease and cellular immunity. Why only certain individuals in exposed populations develop disease has not been determined to date. Studies in individuals with disease have not revealed associations with either HLA haplotypes or immunoglobulin allotypes. However, it is possible that appropriate genetic markers within these complexes were not examined. To study the possible role of genetic factors in the development of HP, we initiated studies of an animal model of HP in inbred strains of mice.

**MATERIALS AND METHODS**

**Animals**

The following strains of mice were purchased from the Jackson Laboratory, Bar Harbor, Maine: C3H/He, C57Bl/10, DBA/2, B10.BR, C3H.SW, B10.A, B10.A(2R), and B10.A(4R). F1 and F2 mice from a cross between C3H/He and C57Bl/10 parents were bred in the Animal Research Facility of the Wood VA Medical Center which is fully approved by the American Association for Accreditation of Laboratory Animal Care. F1 progeny from between B10.A(2R) and B10.A(4R) mice were also bred in this facility. Female mice, 7 to 12 weeks of age, were used for all experiments.

**Production of Pulmonary Inflammation**

Mice were immunized subcutaneously in the two inguinal areas with 1 mg of pigeon droppings extract (PDE) prepared, as previously described, in complete Freund’s adjuvant (CFA; Difco Laboratories, Detroit, Michigan). The animals were boosted in the same manner 2 weeks later. Aerosolization with soluble PDE followed in 2 weeks using the same techniques as previously described in rabbits. Controls were only immunized or aerosolized. Aerosol challenge was carried out 5 times per week for 2 weeks.

Lung index was used to quantify the pulmonary inflammation response. In chronic pulmonary inflammation, the increase in lung weight has been shown to be due to the influx of inflammatory cells. Lung index was calculated as described in the figure.

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**RESULTS AND DISCUSSION**

C3H/He mice that were immunized and aerosol challenged with PDE developed extensive interstitial infiltrations of macrophages and multinucleated giant cells that were most prominent around respiratory bronchioles but also extended into the alveolar septa. There were also associated perivascular lymphocytic and plasma cell infiltrates. Neither immunization nor aerosol challenge per se resulted in pulmonary inflammation. In contrast, C57Bl/10 mice that were immunized and aerosol challenged developed only a mild perivascular lymphocytic infiltrate. Pulmonary inflammation was quantified by calculating lung indices (Fig 1). High responder C3H/He mice developed greater than twofold increases in lung weight as a result of immunization and aerosol challenge (Fig 1, Table 1). Other strains of mice (C57Bl/10, DBA/2) developed only a minimal increase in lung weight. These data indicate that only certain strains of inbred mice develop a substantial inflammatory response to PDE when immunized and aerosol challenged, suggesting that the production of PDE-induced pulmonary inflammation in mice is under genetic control.

Since there were high and low responders with respect to the development of PDE-induced pulmonary inflammation, we performed breeding studies in high responder C3H/He and low responder C57Bl/10 mice and their F1 and F2 progeny (Fig 1). (C3H/He × C57Bl/10)F1 mice developed intermediate responses between high and low responsiveness, suggesting that pulmonary inflammation was not under the control of single dominant or recessive genes. The data in [(C3H/He × C57Bl/10)F1 × (C3H/He × C57Bl/10)F1,F] mice suggest that multiple genes influence the development of inflammation since F1 animals did not segregate into proportions expected for single gene control (single dominant gene: 75% high responders, 25% low responders; single recessive gene: 75% low responders, 25% high responders) (Fig 1).

To further analyze the role of H-2-linked genes in the development of the inflammatory response, we used H-2 congenic and recombinant mice with C57Bl/10 backgrounds in order to minimize the influence of non-H-2 genes (Table 1). The data indicate that high responsiveness is associated with H-2b, but not with H-2a or H-2d. Data in B10.A mice, which

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lung Index</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H/He</td>
<td>2.42 ± 0.11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C57Bl/10</td>
<td>1.31 ± 0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(C3H/He × C57Bl/10)F1</td>
<td>1.71 ± 0.06</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values expressed as Mean ± S.E.

**FIGURE 1.** Quantification of pulmonary inflammation in C3H/He, C57Bl/10 and F1 and F2 progeny. The animals were immunized, boosted and aerosol challenged with soluble PDE 5 days per week for 2 weeks. The animals were killed, weighed, their lungs removed and weighed and lung indices calculated according to the formula shown.
Table 1—Development of Pulmonary Inflammation Associated with H-2\(^*\) and Within the 1-B Subregion

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>No.</th>
<th>K</th>
<th>A</th>
<th>B</th>
<th>J</th>
<th>E</th>
<th>C</th>
<th>S</th>
<th>G</th>
<th>D</th>
<th>Lung Index*</th>
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<tr>
<td>C3H/He</td>
<td>10</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>2.18±0.12</td>
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<tr>
<td>DBA/2</td>
<td>5</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>1.10±0.06</td>
</tr>
<tr>
<td>C57BL/10</td>
<td>10</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>1.20±0.10</td>
</tr>
<tr>
<td>B10.BR</td>
<td>22</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>2.20±0.05</td>
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<td>B10.A</td>
<td>15</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>k</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>2.00±0.05</td>
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<tr>
<td>B10.A(4R)</td>
<td>13</td>
<td>k</td>
<td>k</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>1.12±0.06</td>
</tr>
<tr>
<td>B10.A(5R)</td>
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<td>b</td>
<td>b</td>
<td>b</td>
<td>k</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>1.30±0.03</td>
</tr>
<tr>
<td>[B10.A(4R)\times B10.A(5R)] F(_1)</td>
<td>23</td>
<td>k</td>
<td>k</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>1.12±0.03</td>
</tr>
</tbody>
</table>

*bMean ± standard error of the mean calculated according to the formula in Figure 1.

are recombinants within the H-2 complex, indicate that genes somewhere within H-2 K through H-2 1-E regions influence responsiveness. Data in the other recombinant mice showed that responsiveness is not controlled by either H-2 K and H-2 1-A [B10.A(4R)] or by H-2 1-J and H-2 1-E [B10.A(5R)]. Data in [B10.A(5R)\times B10.A(4R)] F\(_1\) mice indicate that control is not by complementation between H-2 1-A and H-2 1-E. Therefore, the most simple explanation, through a process of elimination, is that H-2-linked genes within the 1-B subregion influence PDE-induced pulmonary inflammation. The functional significance of this finding is not known at this time, since the 1-B region has not been shown to code for H antigens. However, Nadler et al showed that Ia-bearing macrophages were necessary to generate an immune response to staphylococcal nuclease. It is therefore possible that H-2-linked genetic restriction in PDE-induced inflammation is at the level of Ia-bearing macrophages. However, this needs to be tested.

In data not shown, C3H/He high responder mice developed delayed hypersensitivity to PDE in the footpad after immunization; low responder C57BL/10 mice failed to develop this reactivity. Thus, the phenotypic expression of some aspect of the genetic control of PDE-induced pulmonary inflammation appears to be at the level of the development of immunologic reactivity to this antigen.

Overall, these studies show that the development of pulmonary inflammation by an etiologic agent of human lung disease is under genetic control; the response is multigenic; and that gene products encoded by the major histocompatibility complex are important determinants of the development of inflammation.

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REFERENCES


A Murine Model of Asbestosis*


In recent years, animal models of asbestosis which employ aerosolized exposure to the fibers have been limited primarily to the rat. More importantly, documentation of disease progression into a pathologic form similar to that found in human cases of asbestosis has not been demonstrated. In this communication, we will describe a murine model of asbestos-induced pulmonary inflammation which offers both a host with a well-defined immune system closely linked to that of man's, and a disease morphologically similar to human asbestosis.

In order to investigate the pulmonary reaction to inhaled asbestos in this species, we exposed a group of 150 2-month old Balb/c mice to chrysotile fibers. The animals were subjected to an environment containing an average of 10.9 mg of respirable chrysotile/m\(^3\) for 3 hours a day, 5 days per week, for up to 3 months. After 40 days of exposure, this procedure resulted in significant accumulation of asbestos (40 \(\mu\)g) within the lungs of exposed mice as compared to confined non-exposed controls.

The first histologic alteration noted within the pulmonary compartment of experimental mice occurred after 4 to 5 consecutive days of exposure and consisted of an influx of a homogeneous population of macrophages into the alveolar septum and alveolar spaces adjacent to respiratory bronchi. *From the Clinical Immunology Section, Tulane University School of Medicine, New Orleans. Reprint requests: Dr. Bozelka, Tulane University Medical School, 1700 Perdido Street, New Orleans 70112