Experimental Hypersensitivity Lung Disease

Chronic Pulmonary Inflammation and Cell-Mediated Hypersensitivity by Exposure to Aerosolized Antigens in Rabbits

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In the group of diseases termed hypersensitivity pneumonitis (HP), lung lesions generally display chronic interstitial and alveolar granulomatous inflammation. Because of this pathology, it has been suggested that cell-mediated hypersensitivity (CMH) contributes to the pathogenesis of the group of diseases. In addition, other studies using blood and lung leukocytes have added impetus to this thesis. Chronic granulomatous pulmonary inflammation is best produced experimentally by the injection of particulate antigens such as BCG, *Mycoplasm psora faeni*, and antigens attached to large carriers such as Sepharose 4B. Attempts to produce chronic lung lesions in animals using soluble aerosolized antigens have been difficult. In the present study, we present evidence that chronic pulmonary inflammation can be produced in immunized animals after chronic exposure to aerosolized, soluble antigens. In addition, the inflammation was associated with CMH in bronchialveolar cells (BAC), was immunospecific, and was not transferrable to normal rabbits with large quantities of immune serum.

**MATERIALS AND METHODS**

New Zealand white rabbits of either sex were used in these studies. They were immunized subcutaneously in the inguinal area with 4 mg of antigen in complete Freund's adjuvant (CFA). Two weeks later, the animals were given booster injections as in the initial immunization procedure. Some control animals were immunized and given booster injections with only CFA.

Two weeks after the booster injection, the animals were insufflated five days per week for three weeks with approximately 50 ml of 1 mg of antigen/ml in an ultrasonic nebulizer DeVilbiss 65; this instrument delivers particles ranging from 2-10 μm in diameter.

Some animals were evaluated after immunization, but prior to insufflation for systemic CMH, CMH in the lung using BAC and migration inhibitory testing (MIT), for antibody activity in serum by passive hemagglutination (PHA) and tanned cell hemolysis (TCH), the latter a test for complement-fixing antibodies. Other animals were evaluated for these same parameters two days after their last aerosol exposure to aerosolized antigen.

Lungs from animals were evaluated histologically after immunization and booster injections, and two days after their last exposure to aerosolized antigens.

**RESULTS AND DISCUSSION**

Animals immunized and given booster injections (but not insufflated) developed high antibody activity in the blood as assessed by either PHA or TCH. These animals also developed activated lymphocytes in the spleen that were capable of incorporating increased quantities of 3HTdR in the presence of antigen. However, immunization did not result in the induction of CMH in BAC (only one animal of nine tested was positive). Thus,
immunization by the subcutaneous route resulted in the development of activated lymphocytes in the spleen, as well as high titers of circulating antibodies. However, immunization by this route did not result in the development of CMH in the lung by MIT or in detectable pulmonary inflammation.

Previous studies had demonstrated that long-term exposure of normal, non-immunized rabbits to PDE, one of the etiologic agents of HP, resulted in circulating anti-HGG immune serum per kg of body weight and minimal pulmonary inflammation. These studies indirectly suggest that activated lymphocytes (presumably in the blood, spleen, or lymph nodes), were responsible for the development of pulmonary inflammation and CMH in the lungs of actively-immunized animals.

These studies demonstrate that it is possible to produce chronic pulmonary inflammation in immunized animals by prolonged insufflation (three weeks) with soluble, aerosolized antigens. The development of pulmonary inflammation is similar to that reported by Richerson. Future studies are planned to examine the role of lymphocytes in the genesis of these lesions. In addition, dose-response and temporal experiments will be necessary to learn more about more extensive lesions such as fibrosis or of resolution of the inflammatory process.

**REFERENCES**


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**Table 1—Development of Chronic Pulmonary Inflammation and CMH* in Immunized Rabbits after Exposure to Aerosolized** **Antigen**

<table>
<thead>
<tr>
<th>Immunizing Antigen</th>
<th>Aerosol Challenge Antigen**</th>
<th>Pulmonary Inflammation</th>
<th>CMH in BAC***</th>
<th>Significant†</th>
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</thead>
<tbody>
<tr>
<td>PDE-CFA</td>
<td>PDE</td>
<td>Yes‡</td>
<td>Yes, 10/10*</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>HGG</td>
<td>No</td>
<td>No, 0/5</td>
<td>No</td>
</tr>
<tr>
<td>HGG-CFA</td>
<td>HGG</td>
<td>Yes</td>
<td>Yes, 7/8</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>PDE</td>
<td>No</td>
<td>No, 0/6</td>
<td>No</td>
</tr>
<tr>
<td>Saline-CFA</td>
<td>PDE</td>
<td>No</td>
<td>No, 2/8</td>
<td>No</td>
</tr>
</tbody>
</table>

Recipients of immune serum‡

- PDE: Minimal
- No, 1/9

*Assessed by migration inhibitory testing of BAC; 10/10 indicates number of animals with mean migration inhibition of 20 percent or greater.

**3 weeks of exposure to aerosolized antigen (5 time/week).

†Chi square analysis; data were considered significant when P<0.05.

‡Donors were immunized and boosted with PDE as described in MATERIALS AND METHODS. Recipients were given 40 ml of immune serum per kg of body weight and insufflated as described in MATERIALS AND METHODS. Ten days after insufflation was begun, each animal received another 25 ml of immune serum.

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**IMMUNOLOGY OF THE LUNG 275**
Influence of Immunity on the Absorption of Inhaled Antigen

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Previously we observed that following intratracheal administration of antigen, the concentration of antigen found in the blood was lower in immunized than in nonimmunized rabbits. To determine whether differences in the rate of absorption through the alveolo-capillary barrier could contribute to these differences in blood levels, we examined the absorption of inhaled soluble antigens through isolated blood perfused rabbit lungs. In this preparation, the only route by which antigen can enter the blood is via the alveolo-capillary barrier, and the rate of antigen removal from the blood by other organs of the body is not a factor influencing blood levels. We observed that when isolated lungs from normal non-immunized rabbits inhaled radiolabeled ovalbumin (OA) or human serum albumin (HSA), the label appeared in the blood in two fractions. One was precipitable with 5 percent trichloroacetic acid (TCA) or antiserum; the other fraction was TCA soluble and dialyzable. Thin layer chromatography of the TCA soluble fraction revealed radioactivity in several peaks, indicating that this fraction probably represented protein breakdown products. Immunization resulted in a decrease in the amounts of intact antigen and an increase in the TCA soluble fraction appearing in the blood after the aerosol exposure.

In the present study we have begun to examine the mechanisms whereby immunization alters the absorption of inhaled soluble antigen through the alveolo-capillary barrier.

Methods

New Zealand white rabbits were immunized by subcutaneous injection of human serum albumin (HSA) or ovalbumin (OA) in complete Freund's adjuvant, and the isolated lungs were prepared as previously described. The lungs were removed from normal and immunized rabbits, and the pulmonary artery, the left atrium and the trachea of each of the lungs were cannulated. They were then mounted in a chamber and aerated by negative pressure. The lungs were perfused at a constant flow rate (180 ml/min) with autacohromous blood at 37°C while breathing a mixture of respiratory gases which maintained normal blood gas composition. Arterial and venous blood pressures and the volume of blood in the perfusion circuit were monitored throughout the experiments.

The radiolabeled antigens (125I-HSA and/or 131I-OA in physiologic saline solution) were introduced into the isolated lung as an aerosol using an ultrasonic nebulizer (Devilbiss 65, Somerset, PA.) which was operated to produce vapor particles 2 to 10 μm in diameter. The lungs rebreathed the aerosol for 15 minutes. The nebulizer was then removed and the lungs ventilated and perfused for an additional four hours, during which time blood samples were taken for analysis of radioactivity in TCA-soluble and TCA-precipitable fractions as previously described.

Results and Discussion

Various experimental groups were studied. In order to determine whether the decrease in protein absorption caused by immunization was due to a specific mechanism directed at the antigen or to a nonspecific change initiated by antigen-antibody interaction, such as a decrease in permeability to protein in general, we immunized rabbits with either HSA or OA and instilled their isolated lungs with an aerosol containing both 125I-HSA and 131I-OA. Lungs from normal (non-immunized) rabbits were instilled with 125I-HSA and 131I-OA for comparison.

Table 1 shows the results. The percentage of intact radiolabeled protein appearing in the blood after four hours of perfusion was reduced for the antigen but not for the simultaneously inhaled nonspecific protein. This indicates that only absorption of the specific antigen was inhibited by immunization. There was also a tendency for a larger fraction of the inhaled proteins to appear in the blood as metabolites in immunized lung preparations than in non-immunized controls. This was true for both of the inhaled proteins, suggesting that an immunologic reaction to the antigen may alter protein metabolism and/or permeability to metabolites in a nonspecific manner.

To determine whether humoral immune mechanisms were involved in the inhibition of antigen absorption, normal rabbits were passively immunized by intravenous injection of rabbit anti-HSA serum 18 hours prior to isolation of the lung. Table 2 shows the results, which indicate that inhibition of intact HSA uptake could be passively transferred with serum.

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