THE ROLE OF PRECIPITINS IN ALLERGIC LUNG DISEASE

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Summary

Using an experimental model of hypersensitivity pneumonitis in rabbits wherein depressions in arterial oxygen tension are monitored following aerosol challenge with antigen, an attempt was made to determine the effect of humoral antibody on the pulmonary physiologic impairment. Rabbits that had been passively immunized with large quantities of anti-human serum albumin (HSA) serum failed to respond with depressions in PaO2 following aerosol challenge with antigen as opposed to actively immunized animals. Rabbits that were actively immunized with HSA by the aerosol route did respond with various patterns of pulmonary physiologic impairment with time following subsequent challenge, but these defects could not be correlated with the presence of precipitins.

Many experimental models of hypersensitivity pneumonitis have been devised and most are designed to produce pathology representing end stage, chronic disease, but little information is available on how the disease is initiated in either man or experimental animals. Earlier workers had postulated that precipitating antibodies were the cause of the tissue injury through the development of a pulmonary Arthus reaction (1). Other than for the demonstration that such antibodies are often present in patients with this disease, there is very little experimental evidence for this concept. Recently a model was designed in our laboratories to look specifically at the initial events that might ultimately develop into hypersensitivity pneumonitis (2,3). This model is based on physiologic and immunologic changes as well as changes in platelet function and number provoked in unimmunized animals following single aerosol challenge with Aspergillus spores.

Following such challenge, unimmunized rabbits respond with depressed arterial oxygen tensions (PaO2), hemolytic complement activities (CH50), and platelet counts. In vitro, Aspergillus extracts caused the release of platelet histamine. It was concluded from these studies that precipitins were not involved in bringing about these changes, but that non-specific complement activation, possibly via the alternative pathway, was responsible for platelet mediator release, as exemplified by histamine, resulting in damage to the alveolar membranes and altered arterial oxygen tensions. Although the bulk of our data supports the non-specific complement activation mechanism of the initiation of hypersensitivity pneumonitis, we have also obtained data showing that for a different antigen (human serum albumin), prior immunization was first necessary to bring about similar blood gas changes following aerosol challenge, thus suggesting that more than one antigen dependent mechanism might be involved.
The purpose of this paper is to study further the role of pre-formed antibody on the induction of the physiologic impairment seen in this model.

Materials and Methods

Antigens. Human serum albumin (HSA) 4 x crystalline was prepared at a concentration of 1% (w/v) in 0.02M phosphate buffered saline, pH 7.2 (PBS).

Aspergillus fumigatus antigens were prepared in three different forms:

a) Spore aerosols: Dextrose-casein hydrolysate agar plates were inoculated from a single stock culture in a manner to produce confluent growth in three weeks at room temperature.

b) Particle-free extract, aerosols: Two week old, shake broth cultures were grown at 30°C, homogenized in a blender, and subjected to intense disruption with a homogenizer for 20 minutes. The homogenates were lyophilized, reconstituted to one-fifth of original volume in distilled water, centrifuged, and filter sterilized before storage at -20°C.

c) Serologic analysis antigens: A culture extract of A. fumigatus was also prepared and used for skin testing and serological analyses from organisms harvested from agar cultures. The resulting combination of spores and mycelia was passed a single time through a modified Hughes freeze press (4) at 15,000 to 18,000 psi. The disrupted material was clarified, concentrated, and frozen.

Laboratory Animals. Outbred female New Zealand White rabbits weighing approximately 2-2.5 kg were obtained from local suppliers for use in this study.

Active Immunization.

Intradermal groups: Intradermal injections of 2 mg HSA were made every other day for up to 3 months. Arthus skin reactivity was determined by the appearance of a large (2-5 cm) erythematous reaction in 6-8 hrs. This reaction usually led to necrosis within 24 hr.

Likewise, sensitization to A. fumigatus extract (serologic form) was attempted by intradermal injections of 1 mg protein N of the extract every other day for 4 months. Arthus skin reactivity was not achieved by this protocol.

Inhalation groups: A third group of rabbits received 37-48 aerosol challenges with 1% HSA over a 12 month period by a procedure described previously (2). Each aerosol dose consisted of aerosolizing 3.0 ml of 1% HSA into a 1.5 x 10^4 cm^3 chamber over a 30 min period. The chamber was constructed to accommodate the heads of four rabbits simultaneously. It was fitted with exhaust filters and air was passed through it at the rate of 13 l/min.

Passive Immunization. High titered rabbit antisera were pooled and heat inactivated at 56°C for 30 min. They were then filter sterilized using 0.45μ membrane filters and divided into aliquots of 25 ml each. Each 25 ml aliquot was injected intraperitoneally into a normal unsensitized rabbit. This was repeated every 24 hours for 3 days. Before the first injection (day 0) and after the last injection (day 3) a serum sample was taken and subjected to counterimmunoelectrophoresis and precipitin quantitation.
Blood Gas Analyses and Inhalation Challenge Protocol. These procedures and serological analyses were previously described (2).

Complement Assay. All animals used for passive transfer experiments were screened semi-quantitatively for normal levels of peripheral C3 prior to experimentation by immunoelectrophoresis of the serum developed with goat antirabbit C3 (β1C/β1A) (Cappell Laboratories, Downington PA, USA). Hemolytic complement activities, expressed as CH50 units/ml, were assayed according to the method described by Mayer (5).

Results

Inhalation immunization with HSA. Since it was known that rabbits repeatedly immunized intradermally to the point of strong Arthus reactivity produced various patterns of depressed PaO2 following aerosol challenge with soluble antigen, the first experiment was performed to see if this effect was also produced following respiratory immunization. Eight rabbits received 36-47 30 min aerosols of 1% HSA over a period of 8-10 months. When arterial blood samples were examined for PaO2, PaCO2, and pH, only one of the rabbits (receiving 37 aerosols) failed to respond significantly to the final aerosol challenge. With one exception, response patterns were similar to those seen previously (2) with intradermally immunized rabbits. As presented in Figure 1, four rabbits showed a significant drop in PaO2 at one hour followed by a further drop at eight hours post-challenge (Pattern A). Three others responded with the major drop at one hour only (Pattern B) and one rabbit failed to respond (Pattern D). No eight hour only responses (Pattern C) were obtained. Control data represent the means and standard deviations of responses of 16 unimmunized rabbits which received a single aerosol of HSA. The PaCO2 and pH values of all animals remained stable.

Although rabbits which responded to aerosol challenge following intradermal immunization with HSA produced circulating precipitins on the order of 1.5-7.0 mg Ab N/ml (2), only two of the eight rabbits receiving aerosol immunization in this current study produced measurable precipitins (1.215 and 2.080 mg AbN/ml respectively), and the latter was the one which exhibited no pulmonary response following challenge.

Passive transfer of precipitating antibody. Since there appeared to be no correlation of precipitins with the response to provocation, the following experiments were then undertaken. Preliminary experiments indicated that maximum antibody transfer to the circulation was afforded by three daily I.P. injections of 25 ml each of antiserum. One unimmunized group of four rabbits served as controls while another group received the 3 x 25 ml regimen of passive immunization with precipitin rich anti-HSA. The efficacy of this passive immunization was seen by the demonstration of from 290-405 μg AbN/ml in the recipient's serum as a result of injecting donor serum containing 1247 μg AbN/ml. Following aerosol challenge with antigen, none of the animals showed statistically significant depressions in PaO2 or CH50 at one, four, six, or twenty-four hour intervals nor could any differences be discerned between the responses of the two groups, unlike when responses from actively immunized animals are observed.

Similarly, two further groups of four rabbits each were aerosol challenged with the particle-free extract of Aspergillus fumigatus (particulate aerosols of this fungus were shown previously to cause non-specific depressions in arterial oxygen tensions and hemolytic complement activity).
Comparison of relative changes in arterial oxygen tensions in three types of response patterns from immunized animals with unimmunized controls following aerosol challenge with antigen. The shaded area represents depressions of chance variation beyond which any changes are statistically significant (P<0.05) by the Dunnett's Test (6).

Again, one group was immunized passively with 3 x 25 ml regimens of anti-A. fumigatus. The donor serum, containing 882 µg AbN/ml of precipitating antibody, produced recipient levels of 144-278 µg AbN/ml. Following a 30 min aerosol of 3.0 ml of the A. fumigatus extract, no depressions in PaO2 or CH50 were obtained in any animal from either group.

Histologic examinations performed on all experimental animals failed to show any treatment-related changes different from those normal changes seen in untreated controls. Although this examination was done 24-48 hours after the experiment, well beyond the time of any physiologic abnormality, it must be pointed out that the design of these experiments was to permit continuous physiologic monitoring, uninterrupted by sacrificing experimental animals.
In an experimental model of extrinsic allergic alveolitis constructed to be assessed in the same manner used to diagnose spontaneous human disease (monitoring pulmonary function following aerosol challenge), rabbits previously immunized via the respiratory route with HSA responded with various patterns of decreased arterial oxygen tension in a manner similar to rabbits intradermally immunized. These patterns of pulmonary impairment were similar to those described for spontaneous human disease (1). Unlike the intradermally immunized animals, the aerosol immunized animals responded poorly in producing precipitins. This failure might be because the antigen was unable to cross the pulmonary barrier and enter the systemic circulation (7). The relative inability to induce the production of circulating antibody by repeated inhalation challenge with 3 ml of 1% HSA is not surprising since these rabbits received only a total exposure of 19-24 hours. Fink, Hensley, and Barboriak, (8) exposed rats to a total of 20 hours of aerosol of 12-15 ml 10% bovine serum albumin (BSA) or BSA with 0.01% endotoxin, and no animals produced precipitins.

Claims of producing pathology by aerosol challenge of monkeys passively immunized with precipitin rich antiserum have been made by Golbert and Patterson (9) in one animal and Hensley, Fink, and Barboriak (10) using a huge aerosol dose (35-40 ml). On the other hand, Richerson (11) passively immunized rabbits with anti-ovalbumin 48 hrs prior to homologous antigen challenge via aerosol and failed to find histopathology consistent with hypersensitivity pneumonitis and concluded that precipitins per se were not enough for disease production. None of these three studies used physiologic studies for evidence of pulmonary injury as is the case with the diagnosis of human disease.

Our results confirm the studies of Richerson (11) from two different aspects. First, what little histopathology that he was able to obtain (minimal and scattered alveolitis) was seen in our animals, also. But more importantly, no physiologic responses were obtained following challenge with HSA in the passively immunized animals which stands in marked contrast to the responses evoked by actively immunized animals following challenge. The amount of antibody detected in our passively immunized animals was lower than that seen in the intradermally immunized donors, but much greater than in six of the eight aerosol immunized. Taken together with the data that one of the non-responding animals did produce abundant antibody, it was concluded that although active immunization was necessary to produce a HSA-responsive rabbit, precipitating antibody had nothing to do with that response.

A different problem is encountered when using aerosols of Aspergillus spores in this model because such exposure results in striking depressions in arterial oxygen tension and hemolytic complement in unimmunized animals (2). For this reason, particle free extracts of the fungus had to be used since it could be shown that aerosols of it did not evoke similar responses. It was antigenic, however, as seen by the production of precipitins when these extracts were used as immunogens. Using such antisera for passive immunization, no pulmonary or complement aberrations could be elicited following aerosol challenge.

These results strongly suggest that precipitins per se are inconsequential in bringing about the disease or at least the physiologic impairment that has been interpreted as the hallmark of it. It also suggests that other humoral antibodies e.g., homocytotropic, are not involved either since the immunizing regimen of a total of 75 ml of homologous antiserum
would have afforded ample opportunity for such antibody to have become fixed and exposed to antigen. Although the presence of such non-precipitating antibodies was not sought in this study, results from additional experiments to be reported elsewhere failed to show the presence of these antibodies by passive cutaneous anaphylaxis in unheated sera from unimmunised rabbits responding to aerosol challenge. Even though the 30 min heat treatment of the antiserum in these experiments may have modified the cytotropic activity of IgE antibody (usually 2 hours at 56 C is required to remove this biologic activity) we do not feel that IgE can explain the results of previous experiments, i.e., it can not fix complement by the classical pathway and it would require an enormous amount of aggregated, antigen-specific IgE working via the alternative pathway to account for the tremendous drop in circulating hemolytic complement activity in rabbits exposed to Aspergillus spores.

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