The role of precipitins and complement activation in the etiology of allergic lung disease

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An experimental model of allergic lung disease has been described that is monitored by analysis of arterial oxygen tension following aerosol challenge with antigen. Rabbits immunized to a classical soluble antigen, human serum albumin (HSA), to the point where severe Arthus skin reactivity was demonstrable, were aerosol-challenged with antigen. Arterial oxygen tension measurements made on pre- and post-challenge samples yielded early, late, and continuous response patterns, reminiscent of those obtained in humans following provocation testing. Aerosol challenge of unimmunized animals with HSA resulted in no change from baseline conditions. Unimmunized rabbits exposed to small and massive (10X) aerosols of Aspergillus *spores also demonstrated various postchallenge depressions in arterial oxygen tension as well as decreased levels in hemolytic complement activity, depending on the species of fungus and dose of spores used. Unimmunized animals pretreated with cobra venom factor in a manner known to achieve complement depletion failed to respond with altered arterial oxygen tensions following similar aerosol challenge. It is postulated that although precipitins may play a role in artificial disease initiated by soluble antigens, nonspecific complement activation may be more important in understanding the etiology of spontaneous disease in humans brought about by inhalation of moldy particulate matter.*

Although it is generally agreed that there is an immunologic etiology of hypersensitivity pneumonitis, opinion is divided as to the exact type of immune injury involved. One of the leading theories proposes that hypersensitivity pneumonitis is an Arthus-like, immune complex or Type III disease of the lung.[1] The main argument in favor of this hypothesis is the high incidence of precipitins that is found in patients with the disease, although precipitins may be absent in clinically ill patients and present in normal individuals.[2-4] Obtaining Arthus-like skin test reactions has also been reported in certain forms of this disease,[1] although its specificity has been questioned.[5]

On the negative side, the elicitation of Arthus skin test reactions is not universal[6, 7] and, more importantly, the histology of lung biopsies from clinical material does not have the characteristics of an Arthus reaction.[8, 9]

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There has been some suggestion that a Type II etiology is involved, i.e., that humoral antibodies reacting with environmental antigens adsorbed to lung tissue bring about cytotoxic reactions,[9, 10] but the data available for support of this are scarce and indirect.

Ample evidence exists that Type IV or cell-mediated, delayed hypersensitivity reactions accompany hypersensitivity pneumonitis. Evidence for this has been obtained from stimulating patients' lymphocytes with offending antigen,[5, 11, 12] from stimulating lymphocytes from immunized, experimental animals with antigens,[13, 14] and by passive transfer of disease by lymphoid cells.[15, 16]

All of these components plus Type I, reagin-dependent atopy have been shown to accompany various forms of hypersensitivity pneumonitis, but recently two additional suggestions have also been made. First is the possibility that some of the inhaled incitants of this disease can activate the complement cascade. Patients' sera have been demonstrated to have had the alternate complement pathway activated following incubation with antigen,[17, 18] while other studies have shown depressed CH_{50} levels in asymptomatic pigeon breeders following inhalation challenge.[5] Evidence has been obtained indicating that complement activation may take place following in vitro incubation with many known fungal incitants even in the absence of detectable antibody.[19]

Finally, the possibility exists that following sufficient inhalation to certain fungi, a primary intoxication due to innate properties of the fungal incitants may occur.[20, 21]

The purpose of this report is to present a series of experiments originally designed to test the suitability of the Type III Arthus theory in explaining the cause of hypersensitivity pneumonitis. Our model is based on the demonstration of altered pulmonary function as reflected in changes in arterial blood gases, precipitin production, Arthus reactivity, and complement levels in immunized rabbits following aerosol challenge with homologous antigen.

MATERIALS AND METHODS

Animals

Outbred New Zealand female rabbits weighing approximately 2-2.5 kg were obtained from Hilltop Laboratory Animals, Inc. (Scottdale, Pa.). A second control group of 8 rabbits of similar weight were obtained from Ancare Corporation (Manhasset, N. Y.) one year after the first Hilltop control group.

Blood gas analyses

Prior to examination of blood for the partial pressure of arterial oxygen (Pa_{O_2}), carbon dioxide (Pa_{CO_2}), and pH, each animal received a single intramuscular injection of 4-6 mg/kg chlorpromazine (Thorazine, Smith, Kline, & French Laboratories, Philadelphia, Pa.) 1 hr prior to the baseline and once again prior to the 24-hr bleedings. After approximately 1 hr, the animals demonstrated a tranquil, even respiratory rate. Each animal was placed in a restrainer for 10 min prior to bleeding, a time period found necessary to prevent hyperventilation due to excitability from handling. A 3-ml sample was then withdrawn from the medial ear artery into a heparinized plastic syringe. The needle was removed and replaced with a B-D ("dead-ender") adaptor (Becton, Dickinson, & Co., Rutherford, N. J.) that contained 3 drops of mercury. The mercury mechanically aided mixture of the blood and heparin, and, with the adaptor, prevented contamination of the specimen by air. The closed syringe was then placed in an ice water bath. Determinations for Pa_{O_2}, Pa_{CO_2}, and pH were made within 15 min on a Corning

TABLE I. Blood gas data for normal resting rabbits

Blood gas		Range (mm Hg)	Mean	SD
Group I				
Pa_{O_2}	(N = 58)	59.4 -88.0	73.6	6.1 (8.3%)
Pa_{CO_2}	(N = 58)	25.2 -40.5	34.1	3.2 (9.4%)
pH	(N = 50)	7.41- 7.53	7.46	0.03 (0.4%)
Group II				
Pa_{O_2}	(N = 16)	69.6 -78.9	73.1	3.5 (4.8%)
Pa_{CO_2}	(N = 16)	24.5 -36.8	32.2	3.6 (11.2%)
pH	(N = 16)	7.45- 7.53	7.49	0.02 (0.3%)

Model 16 pH blood gas analyzing system with a Model R blood pH electrode (Corning Scientific Instruments, Corning, N. Y.). Calibration of the blood gas analyzing system was performed between each reading using humidified compressed gas mixtures containing 5.14% CO_2/N_2 balance and 10.8% CO_2/O_2 balance (Matheson Gas Products, East Rutherford, N. J.) and room air. Calculations of gas tensions were corrected daily for barometric pressure and the vapor pressure of water. The blood pH electrode was calibrated with pH buffer standards of 7.382 ± 0.005[22] and 6.838 ± 0.005 (Corning Scientific Instruments). Both instruments maintained an operating temperature of 37° C and received power from a regulated voltage source.

Sensitization

Rabbits of the first experimental group received intradermal injections of 1 mg human serum albumin (HSA, 4× crystalline, Nutritional Biochemicals Corporation, Cleveland, Ohio) in 0.02 M phosphate-buffered saline, pH 7.2 (PBS) every other day for 3 mo. Arthus reactivity was determined by the appearance of a large (2-5 cm) erythematous reaction in 6-8 hr, which usually led to necrosis by 24 hr.

Serological analyses

All sera from HSA-immunized or challenged animals were assayed for precipitins against 0.05% antigen by standard quantitative techniques.[23] Sera were assayed for *Aspergillus* precipitins by counterimmunoelectrophoresis[24] using freeze-pressed extracts of homogenized 2-week-old broth cultures of the respective fungi as antigens. The fungi were grown on a glucose and Polypeptone (Baltimore Biological Laboratories, Baltimore, Md.) broth (10 gm/L each).

Inhalation challenge protocol

Aerosols were administered to a 1.5×10^4 cm^3 Lucite chamber capable of accommodating the heads of 4 rabbits simultaneously. Collars made of rubber dams were fitted over the ports of the chamber through which the animals' heads were placed, thus ensuring a closed system. Antigens used for aerosolization included a filter-sterilized 1% solution of HSA or mature agar cultures of either *Aspergillus fumigatus* (WVU clinical isolate) or *Aspergillus terreus* (WVU stock culture). The fungi were grown on polypeptone-glucose agar at R° for 3 wk, at which time the surfaces of the plates were covered with confluent growth consisting of well-developed conidiophores.

Exactly 3.0 ml of the HSA were aerosolized over a 30-min period by means of a No. 40 DeVilbiss nebulizer attached to a pressure pump at 2 psi and delivering a flow rate of 13 L/min through the chamber. When fungi were to be aerosolized, an open plate was placed on the floor of the chamber. A jet of air at 2 psi delivered through a Pasteur pipette was then played over the surface of this plate for 30 min to disseminate the spores. Such an exposure was referred to as 1X and resulted in the retention of $4\text{-}5 \times 10^4$ viable spores per gram of lung parenchyma following the 30-min exposure. Massive, 10X doses were delivered by aerosolizing the spores from three plates of such mature cultures, and this resulted in a retention of 3×10^5 viable spores per gram of lung parenchyma. The spore counts were obtained by standard

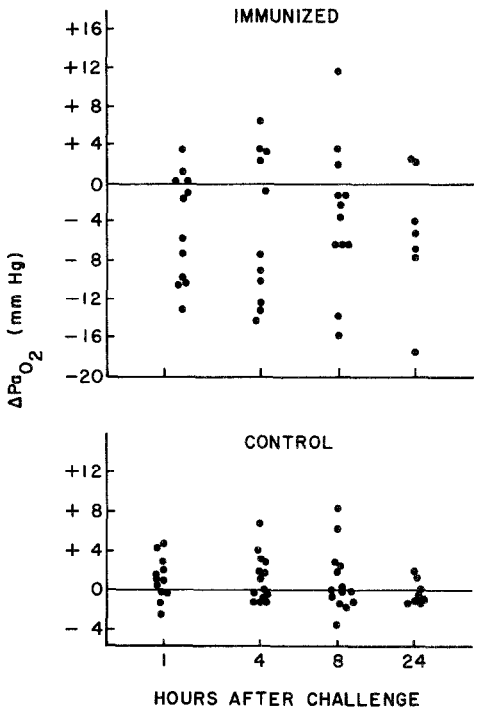

FIG. 1. Scattergram comparison of changes in arterial oxygen tensions in two distinct groups of animals following aerosol challenge with antigen.

surface colony counts of 10-fold dilutions of lung homogenate. Extreme care was taken to ensure a closed system by using filters installed on the outlet ports in order to prevent an escape of spores into the laboratory.

Complement assays

Hemolytic assays of complement activity in CH_{50} U/ml were performed according to the method of Mayer.[25] Fresh rabbit plasma was diluted either 1/10 or 1/15 in veronal-buffered saline prior to evaluation, while that obtained from cobra venom–treated animals was diluted 1/5 for assessment. Prechallenge samples and all subsequent samples were mixed with approximately 15 U of heparin per milliliter, an amount which is far less than that known to induce nonspecific consumption of complement.[26]

Complement depletion

To further study the effect of complement on the physiologic reactions of rabbits to aerosolized fungal spores, 4 fresh, unimmunized rabbits, weighing approximately 1.8 kg each, received injections of a purified anticomplementary factor (CoF) prepared from cobra venom (Cordis Laboratories, Miami Fla.). A total of 250 U/kg was injected intraperitoneally into each animal in 4 equal doses at 0, 4, 10, and 24 hr, according to the method of Cochrane, Müller-Eberhard, and Aikin.[27] One hour after the final CoF injection, the animals were exposed to a 10X dose of aerosolized *A. terreus* spores. The fate of C3 was demonstrated by immunoelectrophoresis of plasma before and after treatment with CoF. The slides were developed with goat antirabbit C3 (β_{1C}/β_{1A}) obtained from Cappell Laboratories, Downington, Pa. If C3 is cleaved, a complete shift of the β_{1C} component arc to the β_{1A} arc with extension into the alpha-2 globulin region is observed.

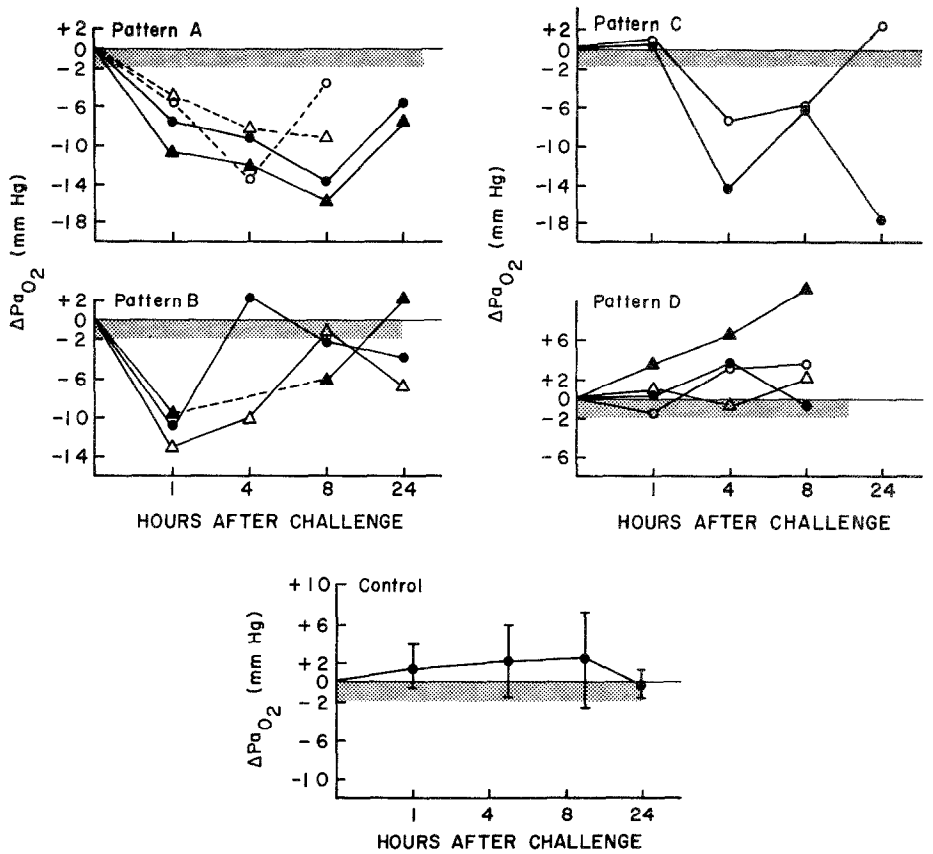

FIG. 2. Comparison of relative changes in arterial oxygen tensions in four types of response patterns from immunized animals with unimmunized controls following aerosol challenge with antigen. Data are shown so that sequential values of each animal may be seen. The shaded area represents depressions of chance variation beyond which any changes are significant at the 0.05 level (critical difference). Control data represent the means and standard deviations of responses from 16 unimmunized animals. A single response from only one animal fell below the critical difference at the 8-hour postchallenge point.

RESULTS

Reproducibility of method used in assessment of the model

In order to demonstrate the usefulness and reproducibility of measuring blood gases in the experimental animal of choice, arterial oxygen and CO_2 tensions and pH values were obtained on random days and at random times on two different control groups of rabbits. The summarized data from over two years of observations are presented in Table I. The range of Group I was extended artificially high by a single reading of 88 mm Hg due to a hyperventilating rabbit; otherwise the range of data from the two groups was markedly consistent and produced rather narrow standard deviations for the three parameters measured.

Correction of the rabbit hemoglobin dissociation curve[28] for in vivo pH resulted in the determination of oxygen saturation values of 97.0 and 96.5% for each group, respectively.

Effect of HSA aerosol challenge on unimmunized and immunized rabbits

When the 7 animals sensitized to HSA were challenged with aerosolized antigen and their blood gas data compared with that obtained from unimmunized animals similarly treated, obvious differences in Pa_{O_2} were seen. When the entire group of immunized animals is compared with all of the controls, a marked tendency to respond to aerosol challenge by reduction in arterial oxygen tensions can be seen in the immunized group (Fig. 1). Unimmunized control animals cluster around the baseline or prechallenge Pa_{O_2} while the immunized animals, when examined as a group, show marked depressions. This figure does not represent each animal's individual response pattern, but clearly defines a trend in challenge-induced pulmonary response.

Examination of Pa_{CO_2} changes from baseline values with time after challenge demonstrated no remarkable differences between the immunized and unimmunized groups of animals, i.e., there was no significant difference between the standard deviations or means obtained at each sampling period for each group. For this reason, only the Pa_{O_2} data will be expanded further in this report.

In order to study each animal's individual response against time, the data obtained from four types of response compared with the controls are replotted in Fig. 2. When compared with the means of the 16 controls, 13 response measurements from 7 immunized animals fell into different patterns. Reaction pattern A, here represented by the 4 animals exhibiting an almost continuous response, consisted of significant decreases at 1 hr and also at 4 to 8 hr postchallenge. Reaction pattern B, here represented by the 3 animals exhibiting this response, consisted of a marked 1-hr decrease that subsequently returned to near normal values later. Two others exhibited significant Pa_{O_2} depressions at 4 hr after challenge (C) and 4 more exhibited no significant responses at any interval (D).

Decreases from the baseline, prechallenge, level of Pa_{O_2} were statistically analyzed for each rabbit by Dunnett's test.[29] The critical difference from the baseline which indicates statistical significance at the 0.05 level varied with each response analyzed. However, the shaded area in Fig. 2 represents the largest critical difference calculated for any response, indicating that most decreases in Pa_{O_2} of the immunized animals were significant.

Not only did the individual animals vary in the quality of their responses, but also the responses from individual animals were shown to change with time following immunization. For instance, in one animal an immediate, 1-hr response was all that was seen at 12 wk postimmunization, but this developed into a biphasic pattern 3 wk later. Finally, the animal's immune response had matured such that by 22 wk after immunization, no evidence of blood gas change was seen following challenge. In order to prevent similar inductive changes in the controls, no control unimmunized animal ever received an aerosol challenge more than

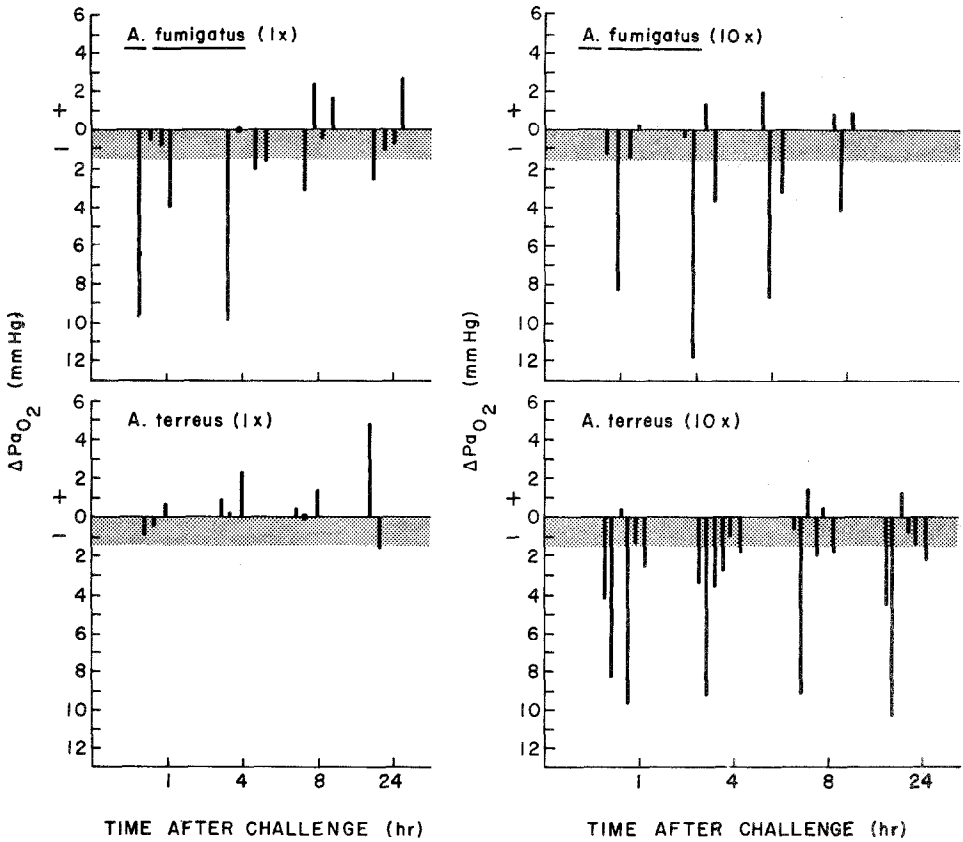

FIG. 3. Changes in arterial oxygen tensions in separate groups of unimmunized rabbits with time after aerosol challenge with 1X or massive, 10X doses of two species of fungal spores. The responses from each animal are plotted at the same relative position at each observation interval, i.e., the first line at each point is from the same animal, the second line is for the second animal, etc.

once. Quantitative precipitin values for the same three observation points for the above animals were as follows: 12 wk = 3.425 mg Ab N/ml; 15 wk = 1.550 mg Ab N/ml; and 22 wk = 1.360 mg Ab N/ml.

Effect of fungal spore inhalation on Pa_{O_2} values of unimmunized animals

In order to determine what relevance the above findings had to spontaneous hypersensitivity pneumonitis, it was planned to repeat the preceding experiments using fungal spore aerosols, but first it was necessary to determine the effect of such aerosols on unimmunized animals. Fresh rabbits were given an aerosol and their blood gas values were determined. This initial exposure would then constitute the first immunization dose of a planned series to follow. Fig. 3 presents the results of arterial Pa_{O_2} responses following initial challenge with two species of *Aspergillus*. The responses from each animal were used to calculate a

TABLE II. Percent CH_{50} decreases in unimmunized rabbits following inhalation of fungal spore aerosols

1X spores					*10X spores*				
Rabbit	Hours after challenge				Rabbit	Hours after challenge			
No.	1	4	8	24	No.	1	4	8	24
A. fumigatus									
316	—	—	6.2	*	320	—	—	—	—
319	8.2	*	*	—	328	—	—	—	—
322	—	—	*	—	329	28.9	—	—	—
323	—	—	*	—	331	15.0	27.2	—	—
A. terreus									
317	—	—	—	—	574	—	19.6	7.4	—
318	*	6.4	—	—	585	—	—	7.2	—
321	—	—	—	—	324	10.5	5.2	6.4	—
					327	32.2	24.1	20.7	—
					330	—	—	*	6.4
					332	—	—	*	—

—, Little change from normal, prechallenge level.
*CH_{50} measurable but less than 5% decrease.

critical difference from its own baseline value, below which any responses were statistically significant at the 0.05 level. The largest critical difference from any animal is represented by the shaded area in Fig. 3. It may be seen that even though these animals had received no known prior immunization, several significant reductions in arterial oxygen tension were noticed in the group of animals challenged with *A. fumigatus*.

The serum from each of these animals was screened for precipitins and only one animal from each group demonstrated such antibody, but neither produced significant Pa_{O_2} depression. Alone, these data were not exceptionally striking, but when fresh groups of unimmunized rabbits were given aerosols with spore loads 10X greater than before, the contrast was quite evident. As before, a similar number of significant depressions were seen in those animals receiving *A. fumigatus* spores, but now many more depressions were obtained from those animals receiving *A. terreus* spores. In fact, every single rabbit had a significant depression 4 hr after challenge. (Although the fifth rabbit's response is within the critical difference area of Fig. 3, it should be recalled that this area was drawn from the largest such difference of any animal. When the fifth animal's response is compared with its own critical difference, a statistically significant value was obtained.) Thus the dose of spores as well as the species of fungus influenced the frequency of arterial oxygen tension depressions. One precipitin line each was found in only one of the *A. fumigatus* and in two of the *A. terreus* rabbits.

Effect of fungal spore inhalation on complement levels of unimmunized rabbits

In an effort to determine whether nonspecific complement activation was involved following fungal spore inhalation, total hemolytic activity was assayed in a separate aliquot of each plasma sample obtained for the arterial oxygen tension measurements exhibited in Fig. 3. These data, expressed as percent reductions in CH_{50} U/ml, are presented in Table II. A few animals showed a 5% to 10% decrease in CH_{50} units following aerosol challenge with 1X spore

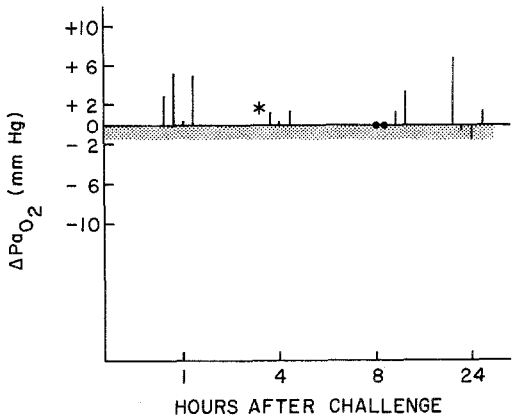

Fig. 4. Changes in arterial oxygen tensions with time in unimmunized animals treated with cobra venom factor and exposed to 10X aerosol challenge of **A. terreus** spores. Asterisk indicates artificial hyperventilation occurred. The responses from each animal are plotted at the same relative position at each observation interval so that sequential responses from individual animals may be followed.

doses, but when the exposure was increased 10X, several marked CH_{50} depletions were noted. As before, the *A. terreus* seemed to be more effective in bringing about this reaction.

Effect of fungal spore inhalation on complement-depleted rabbits

It remained to be seen whether the activation of complement was tied in with the pulmonary damage as reflected by decreases in Pa_{O_2}. Accordingly, the same type of experiment was repeated in four unimmunized rabbits that were pretreated with cobra venom factor in a manner known to severely deplete C3.[27] That such treatment did in fact greatly decrease the complement was shown by the disappearance of the β_{1C} line on immunoelectrophoresis when developed with anti-C3 serum[30] and by greatly decreased ($> 50\%$) CH_{50} values in each of the sera from the treated animals when compared with pretreatment baseline bleedings. After receiving a 10X challenge of *A. terreus* spores, arterial blood gas values were obtained from each cobra venom factor–treated rabbit as before. None of the bleedings taken from any of these treated animals following aerosol challenge exhibited a decrease in Pa_{O_2} of more than 1.3 mm Hg from that animal's baseline value, indicating that the arterial oxygen tensions were well within normal limits despite massive inhalation with the active *A. terreus* spores (see Fig. 4).

DISCUSSION

Several experimental models of hypersensitivity pneumonitis or similar forms of allergic lung disease have been described, but many are deficient in design because either very artificial means were employed to induce lesions, enormous doses of antigen were employed, or the antigens used, e.g., HSA, were not similar to those that bring about spontaneous disease in man. Human hypersensitivity pneumonitis is diagnosed by history, roentgenographs, pulmonary function, and serologic evidence, but seldom on histologic findings.[31] In particular, much re-

liance has been placed on precipitin production and reduction in ventilatory capacity and oxygen transfer. With this in mind, it was decided to establish an animal model in which these diagnostic parameters would be used to assess responses following aerosol exposure to fungal antigens.

Since it is obviously impossible to utilize such effort-dependent pulmonary function measurements as the FEV_1 repeatedly in animals, an alternate, convenient method had to be established for demonstrating changes in pulmonary function. Previous work from these laboratories used the CO diffusing capacity,[32] but it was found exceedingly cumbersome and impossible to use on groups of animals required for statistical analysis.

When compared to effort-dependent parameters, Lopez Merino and associates[33] found significant correlation between changes in FEV_1 and changes in Pa_{O_2}. Likewise, Palmer and Kelman[34] found that the Pa_{O_2} correlated with dynamic lung volumes and the residual volume to total lung capacity ratio (RV/TLC) in humans. While small interlaboratory differences in the absolute values of Pa_{O_2}, Pa_{CO_2}, and pH may occur,[35] relative changes from baseline levels can represent significant decreases of pulmonary function.

Nearly identical results obtained from two groups of animals measured at widely disparate times indicate that measurement of blood gas tensions and percent saturation can be applied to the rabbit with a great deal of confidence.

The mean Pa_{O_2} values found were slightly lower than other published measurements,[36] but those for Pa_{CO_2} and pH fell within previously reported ranges.[36, 37] This demonstrates that our animals for the most part were not hyperventilating and were tranquil because of the chlorpromazine medication.

Since it has been postulated that this disease is the equivalent of an Arthus reaction in the lung, experiments were begun by immunizing rabbits with a classic protein antigen, HSA, in a manner well known to induce Arthus skin reactivity. When evidence of such reactivity was obtained together with that of strong precipitin production, the animals were aerosol-challenged with that antigen. Indeed, using significant depressions in arterial oxygen tension as an indicator of pulmonary damage, such immunization was shown to result in pulmonary abnormalities following aerosol challenge. Such abnormalities were never seen in any of 16 unimmunized controls following similar aerosol challenge, which also indicated that neither the repeated bleeding procedure nor the chlorpromazine altered the Pa_{O_2} from the normal conditions. Especially interesting was the observation that the rabbits responded in different patterns, i.e., early, late, and continuous responses, reminiscent of the same kind of changes described in humans following provocation testing.[1] Thus it initially appeared that this animal model was quite relevant for providing information about the spontaneous human disease and seemingly it strongly suggested but did not prove that precipitins were directly involved in the etiology of the disease. However, humans do not become sensitized in such an artificial manner (intradermal injections every other day), nor has HSA even been catalogued in the ever-growing list of agents known to cause human hypersensitivity pneumonitis.

With this in mind, attention was turned to a more relevant antigen, *A. fumigatus* spores, but it was first necessary to perform control inhalation chal-

lenges on unimmunized animals. Surprisingly, such control animals were found to respond frequently with depressed arterial oxygen tensions even though they had received no known immunization against these fungi. It can be argued that these animals had received natural immunization from sources such as contaminated litter, and indeed an occasional single precipitin line was found on a few of the sera from these rabbits, but there were many more animals that responded with depressed arterial oxygen tensions than produced precipitins. Further, all animals were used at 3 mo of age, at a time rather insufficient to be exposed to much of the antigenic environment, and their cages were provided with daily changes of synthetic litter mats. Of even greater importance, the likelihood that rabbits can produce significant antibodies against casual contact with fungi is very remote because extensive experience from these laboratories showed that it is difficult to purposefully immunize rabbits with fungal antigens.[38-40]

The ability of the spores to induce decreased arterial oxygen tensions appeared to be not only dose-related but also species-related. When the number of spores was increased 10-fold, many more responders were evident, and it was also shown that *A. terreus* was capable of inducing more reactions than *A. fumigatus*. Perhaps *A. terreus* is to rabbits as the latter is to humans.

Since the presence of precipitins could not be used to explain the decreased Pa_{O_2} values, an alternate mechanism of reaction was sought. The total hemolytic activity of peripheral complement was evaluated on the identical samples as those used for blood gas analysis. Consistent and dramatic decreases in CH_{50} were seen in unimmunized rabbits in response to inhalation challenge with 10X *A. terreus* spores, although occasional decreases were also seen with *A. fumigatus*. Further support was obtained when no Pa_{O_2} decreases could be demonstrated following such 10X *A. terreus* challenge in animals whose complement had been depleted by cobra venom factor inactivation. Since the CH_{50} levels were depressed prior to challenge and remained so at 24 hr after challenge, it can be inferred that complement activation was necessary to induce tissue damage leading to alteration of gas exchange.

These observations lend in vivo confirmation to the observations of Marx and Flaherty,[19] who demonstrated that many fungi associated with hypersensitivity pneumonitis can activate complement in vitro. These results also provide experimental evidence for the clinical observations of Edwards, Baker, and Davies,[17] who described complement activation in farmer's lung patients with precipitin-negative sera. Perhaps the pulmonary mycotoxicosis described by Emanuel, Wenzel and Lawton[20] in patients experiencing massive inhalation of fungal spores was due to this same mechanism.

In conclusion, results obtained from animals exposed to these two antigens, HSA and fungal spores, show that possibly two mechanisms were operating. Perhaps with soluble protein antigens, precipitin production might be important in disease etiology, but in the case of fungal spores, such precipitins may not be important. Rather, the nonspecific activation of complement in the absence of antibody may be an important contributing cause of this disease. Whatever the mechanism, the establishment of an experimental model that is capable of being monitored by methods analogous to those used to assess human hypersensitivity

pneumonitis has produced data that either indicate that precipitins are not important in the etiology of human disease due to inhalation of fungal spores, or else conclusions from physiologic lung function testing following provocation testing in humans are erroneous with respect to immunologic etiology in human disease. It is suggested that data obtained from experimental models using classical soluble protein antigens such as HSA may be providing misleading data, since almost invariably human disease is caused by the inhalation of particulate antigens.

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