

Allergic bronchopulmonary aspergillosis: Characterization of antibodies and results of treatment

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Two antibodies, one skin sensitizing and the other precipitating, are thought to play important roles in the pathogenesis of the syndrome of allergic bronchopulmonary aspergillosis. Further characterization of the antibodies in this study reveals the ability of the skin-sensitizing antibody to passively transfer systemic sensitivity to the monkey and the classification of the precipitating antibody as an IgG immunoglobulin. Two patients with the syndrome of allergic bronchopulmonary aspergillosis were treated with oral corticosteroids and amphotericin B aerosol. The treatment resulted in marked improvement including clearing of pulmonary infiltrates, decrease in eosinophilia, weight gain, increase in vital capacity, eventual negative sputum culture for Aspergillus, and disappearance of precipitating antibody.

Allergic bronchopulmonary aspergillosis, first described in 1952 by Hinson and associates¹ in England and later extensively studied by Pepys² and others,^{3, 4} has been reported only rarely in the United States.⁵ Recently we reported instances of this syndrome in an adult⁶ and in a 9-year-old child, the youngest yet reported.⁷ Both patients were residents of the United States and fulfilled all of the clinical and laboratory criteria for this disease, namely, low-grade fever with wheezing, expectoration of plugs containing mycelia, repeated culture of *Aspergillus* from sputum, transient pulmonary infiltrates, eosinophilia of blood and sputum, positive immediate skin tests to *Aspergillus*, and positive precipitating serum antibody to *Aspergillus*.

We have previously demonstrated some characteristics of two distinct antibodies against *Aspergillus*. The immediate skin-sensitizing antibody to *Aspergillus fumigatus* showed cross-reactivity to other species of *Aspergillus* and passive transfer by the Prausnitz-Küstner technique to the skin of both man and

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monkey. Positive responses to intrabronchial aerosolization of *Aspergillus* could be elicited.^{6, 7} The precipitating antibody was in the gamma globulin range, caused a "late" direct skin reaction, and resulted in a successful reverse passive Arthus reaction in a guinea pig.⁶ Comparison of the serum of our two patients on double gel diffusion showed a reaction of identity between two bands and a spurring when compared with serum of a guinea pig immunized with *Aspergillus*.⁷

We now report further characterization of the two antibodies to *Aspergillus* and the clinical and immunologic results of successful treatment of this disease.

METHODS AND MATERIALS

Passive transfer of systemic sensitivity to the monkey

A male and female *Macaca mulatta* monkey served as recipients of human serum. On Day 1 the male received 30 ml. of normal human serum intravenously and the female 30 ml. of serum, intravenously, from a patient with allergic aspergillosis. On Day 2 the same amounts of the same sera were again injected. On Days 3 and 4 both monkeys received 5 minutes' aerosolization of a 5 per cent aqueous solution of *Aspergillus fumigatus** via an ultrasonic tent.

Characterization of the immunoglobulin class to which the precipitating antibody belongs

Sera from both patients were run through a Sephadex G-200 column, 5 cm. by 85 cm. with 1M acetate buffer, pH 4.1, and the major IgG and IgM fractions separated. Ordinary double gel diffusion⁷ was performed with the two fractions against *Aspergillus fumigatus* extract.

RESULTS

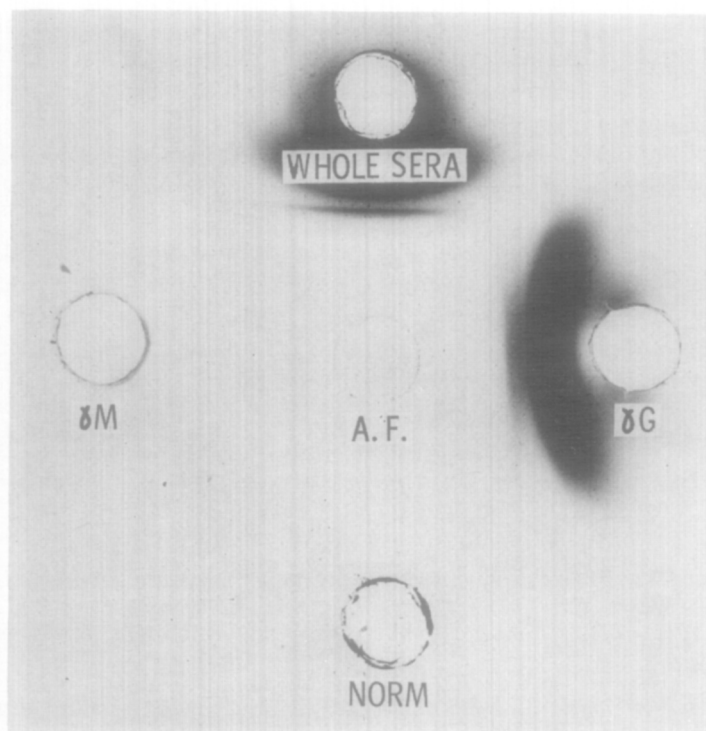
Passive transfer of systemic sensitivity to the monkey

On Day 3, the male monkey who received two injections of normal human serum appeared perfectly normal after *Aspergillus* aerosolization. In contrast, the female monkey who received serum from a patient with allergic aspergillosis developed marked signs and symptoms after several minutes of aerosolization. She became quite cyanotic, restless, and dyspneic. Respirations were labored, and tachypnea of 40 per minute was present. Auscultation revealed scattered expiratory rhonchi and wheezes. On Day 4, shortly after initiation of aerosolization, the same symptoms and signs returned in the female, while the male remained normal.

Characterization of the precipitating antibody

Fig. 1 illustrates the double gel diffusion of the Sephadex-separated fractions of one patient's serum against *Aspergillus fumigatus*. Both precipitin bands were found between the IgG fraction and the *Aspergillus* extract. The other patient's precipitating antibody was also an IgG.

*Lot No. 812-3308, Abbott Laboratories, North Chicago, Ill.

**Fig. 1**

Double gel diffusion plate showing precipitin bands between *Aspergillus* extract, patient's serum, and the IgG fraction of the patient's serum. *A. F.*: *Aspergillus fumigatus*, 20 mg. per milliliter.

Clinical and immunologic results of treatment

Table I indicates the effect of successful treatment on several clinical and immunologic parameters of allergic bronchopulmonary aspergillosis in a 9-year-old girl. Treatment with amphotericin B aerosol and oral corticosteroids resulted in remarkable clearing of the chest x-ray, drop in peripheral blood eosinophilia, increase in weight and vital capacity, and eventually negative sputum cultures for *Aspergillus*.

Fig. 2 demonstrates the gradual decrease with treatment in precipitating antibody against *Aspergillus* in the patient's serum. The adult patient also showed a gradual decline in serum precipitating antibody. Skin-sensitizing antibody in both patients remained the same.

DISCUSSION

Characterization of the two distinct antibodies against *Aspergillus* in patients with allergic aspergillosis is especially important, since the antibodies are thought to play vital roles in the pathogenesis of the disease and to be related to the clinical manifestations. It has been suggested^{2, 6} that the nonprecipitating skin-sensitizing antibody is responsible for the immediate skin reaction, the eosino-

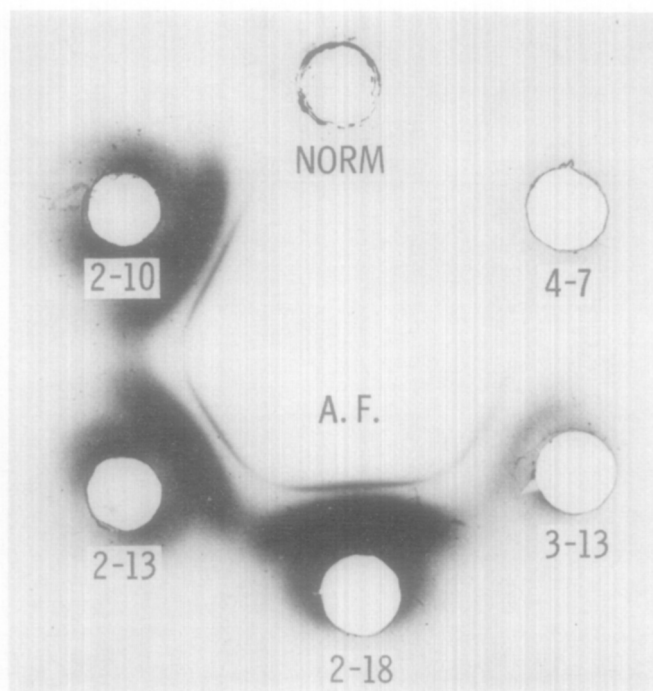
Table 1. *Clinical and immunologic findings during treatment of allergic bronchopulmonary aspergillosis in a 9-year-old girl*

<i>Date</i>	<i>Therapy</i>	<i>Chest x-ray</i>	<i>Peripheral eosinophilia (%)</i>	<i>Vital capacity (% normal)</i>	<i>Sputum culture (for aspergillosis)</i>	<i>Weight (pounds)</i>	<i>Precipitating antibody</i>
2-17	Amphotericin B aerosol	Atelectasis left lung; infiltrate right upper and middle lobes	49	25	Heavy growth	47	Strong
2-20	Amphotericin B aerosol		7				
2-25	Amphotericin B + prednisone, 25 mg. a day	Area of radiolucence above left diaphragm	4	36	Moderate growth	47	
3-3	Amphotericin B + prednisone, 25 mg. a day	Marked clearing on left	1	38	Moderate growth	46	
3-10	Amphotericin B + prednisone, 20 mg. a day	Additional clearing bilaterally	2			50	
3-17	Amphotericin B + prednisone, 10 mg. a day	Only slightly bilateral infiltrates	0	59	Late scanty growth	53	Mild
3-24	Amphotericin B aerosol		6		Negative	56	
3-31	Amphotericin B aerosol	Further improvement	1		Negative	58	
4-7	Amphotericin B aerosol	Right clear; left small infiltrate posterior segment left upper lobe	1	74	Negative	60	Negative

philia, and the clinical feature of asthma. The precipitating antibody is thought to account for the late skin reaction and the pulmonary infiltrates, both on the basis of Arthus reactions.

We^{6, 7} have previously shown that the skin-sensitizing antibody was cross-reactive with other species of *Aspergillus*, could be passively transferred to the skin of both man and monkey, and was probably responsible for the positive intrabronchial challenge with aerosolized *Aspergillus*. In the present study we have shown that, in addition to local transfer, systemic transfer to the monkey of sensitivity to *Aspergillus* is also possible. While the immediate manifestation of asthma was clearly demonstrated, we could not produce pulmonary infiltrates on the chest x-ray. The chest films of the monkey remained negative.

Precipitating antibody to *Aspergillus* has been characterized in a number of ways, as mentioned previously. This study now clearly demonstrates the precipitating antibody of both of our patients to be in the IgG class.

**Fig. 2**

Progressive decrease in patient's precipitating antibody to *Aspergillus* with successful treatment of allergic aspergillosis. A. F.: *Aspergillus fumigatus*, 20 mg. per milliliter.

The treatment of pulmonary aspergillosis has frequently been ineffective. We chose the therapeutic program of amphotericin B by aerosol and oral corticosteroids for several reasons. In both patients, tube dilution sensitivity studies showed the *Aspergillus* to be sensitive to amphotericin B. Because of the well-known complications of intravenous amphotericin B and the reports of its safety and efficacy intrabronchially,^{9, 10} it was decided to use the drug by aerosol. In both patients the amphotericin was diluted in 2 ml. of sterile water and delivered over a 10 minute period by intermittent positive pressure breathing twice a day. The initial dose was 5 μ g, the second dose 50 μ g, the third dose 0.5 mg., and the fourth dose 5 mg. This final concentration was used for the remainder of the treatments. The first patient, who was an adult, tolerated the aerosolization quite well. The second patient, a 9-year-old girl, developed mild nausea and vomiting, chills, fever, anorexia, malaise, and headache minutes or hours after the aerosol therapy. After two weeks, these symptoms disappeared. On one occasion blood was drawn 1, 2, and 4 hours after aerosolization, and no detectable serum levels of amphotericin were noted.¹¹ Renal function, as determined by urinalysis and serum creatinine, remained normal. One explanation for the systemic effects seen early during aerosolization therapy is a type of Herxheimer reaction due to release of protein secondary to death of fungal organisms. In

both cases, corticosteroids were begun after the initiation of amphotericin B therapy. Improvement in the chest x-rays and decrease in peripheral blood eosinophilia while on amphotericin B only would point to the efficacy of this agent by aerosol. An alternate explanation for the rapid reduction in the eosinophilia might be the pyrogenic effect of the amphotericin on the eosinophilia rather than the elimination of the *Aspergillus*. The advantages of aerosolization of amphotericin B over intravenous administration are obvious in terms of safety and convenience.

Corticosteroids have been shown to decrease the late skin reaction and the pulmonary infiltrates of allergic aspergillosis,² and others¹² have used corticosteroids in this disease with a view to lessening the allergic reaction and reducing airway obstruction so as to facilitate expectoration of remaining antigen. The improvement in both patients seemed to be more rapid after initiation of corticosteroids, and amphotericin B and corticosteroids were discontinued after 2 months of treatment. Marked clinical improvement occurred along with disappearance of the precipitating antibody to *Aspergillus*. Skin-sensitizing antibody remained the same. In the case of the 9-year-old girl, a 2 week course of corticosteroids 3 months after cessation of treatment resulted in complete disappearance of a small pulmonary infiltrate.

The concomitant use of corticosteroids and amphotericin B in the treatment of allergic aspergillosis seems to offer distinct advantages over other modes of therapy. When corticosteroids are used alone, there is a high incidence of recurrence of the disease. In instances when the *Aspergillus* organism is sensitive to amphotericin B, this drug can be used to great effect by aerosolization.

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