

Alternaria IgG precipitins and adverse reactions

**William W. Busse, M.D., William W. Storms, M.D.,
Dennis K. Flaherty, Ph.D., Marjorie Crandall, and
Charles E. Reed, M.D. Madison, Wis.**

Late reactions consisting of fever, malaise, and swelling at the site, 4 to 6 hr after injections of Alternaria extract occurred in several patients receiving immunotherapy with Alternaria. These patients had in common serum IgG precipitins and exquisite leukocyte histamine release sensitivity to Alternaria. Such precipitins were 3 times more frequent in patients receiving Alternaria immunotherapy than a control group of patients receiving immunotherapy with other antigens. A prospective study revealed that 5 of 23 Alternaria-sensitive persons had precipitins before immunotherapy and another 6 developed precipitins during therapy. Only one of the 23 experienced a late Alternaria reaction. Thus, precipitins to Alternaria are common and do not seem to be the basis for the late reactions we observed. The finding of precipitins does not contraindicate immunotherapy.

The diagnosis of hypersensitivity pneumonitis to inhaled antigens includes the presence of IgG-precipitating antibody to the suspected antigen.¹ The role played by this precipitin in immunopathology of entities such as farmer's lung, pigeon breeder's lung, or allergic bronchopulmonary aspergillosis remains unclear. In patients with a hypersensitivity pneumonitis, a bronchial challenge with the offending antigen will usually reproduce symptoms and occasionally radiologic changes 4 to 6 hr following the inhalation of the antigen.¹ It has been hypothesized that the IgG-precipitin antibody may be responsible for the pulmonary and systemic reaction; however, the finding of a precipitating antibody in a patient's serum does not necessarily establish the presence of hypersensitivity pneumonitis.² As many as 41% of pigeon breeders have precipitins but no lung disease.³

In the following study, we found a 3-fold increase in the frequency of IgG-precipitating antibodies to *Alternaria* in a group of atopic patients receiving immunotherapy which included *Alternaria* extract. Seven patients with IgG-precipitating antibodies to *Alternaria* experienced chills, fever, arthralgia, and malaise, but no respiratory symptoms, beginning 4 to 6 hr following parenteral administration of the *Alternaria* extract. In these patients it was not known if this precipitating antibody existed prior to the development of this untoward

From the Department of Medicine, University of Wisconsin.

Supported by Grant No. 2-R01-AI-08106.

Received for publication Jan. 13, 1975.

Accepted for publication March 7, 1975.

Reprint requests to: William W. Busse, M.D., 504 N. Walnut St., Madison, Wis. 53705.

TABLE I. The frequency of precipitins to *Alternaria*, *Hormodendrum*, ragweed, grass, and house dust in three patient populations

	Alternaria (10 mg/ml)	Hormo- dendrum (10 mg/ml)	House dust (30 mg/ml)	Ragweed	Grass
Group I: control	8/75* 10.7%	6/75 8%	55/75 73.5%	0/75 0%	0/75 0%
Group II: Atopic patients on immunotherapy not including <i>Alternaria</i>	8/74 12.5%	1/74 1.4%	57/74 77%	0/74 0%	0/75 0%
Group III: Atopic patients on injections including <i>Alternaria</i>	18/57† 31.2%	2/57 3.5%	43/57 75.5%	0/57 0%	0/57 0%

*Number positive/number tested.

†p = 0.02.

response. To clarify the possible significance of the precipitin reaction, a prospective study was done with 23 patients beginning immunotherapy which included *Alternaria*. During the first year of immunotherapy their serums were assayed for IgG-precipitating antibodies to *Alternaria*; leukocyte histamine release studies were also performed, as were serum IgE levels. These findings were analyzed as a possible method for predicting which patients have a potential to develop the late adverse reaction to *Alternaria* immunotherapy.

METHODS AND MATERIALS

Serum samples

To determine the frequency of precipitating antibodies to *Alternaria*, gel-diffusion studies were performed on three patient populations: (1) patients attending a Venereal Disease Clinic in Milwaukee, Wis., (2) patients from the University of Wisconsin Allergy Clinic who were atopic and receiving immunotherapy which did not include *Alternaria*, and (3) atopic patients whose immunotherapy included *Alternaria*.

Gel diffusion techniques

Double immunodiffusion was performed in an agar gel prepared on glass slides. The patient's undiluted serum was placed in the center well; the test antigens (10 mg/ml in 0.1 M borate citrate buffer, pH 8.4) were placed in the peripheral wells.⁴ The gel diffusion slides were incubated at room temperature with high humidity and the reaction was interpreted at 48 hr. In addition to the *Alternaria* antigen, each serum was tested for precipitating antibodies to *Hormodendrum*, house dust, grass, and ragweed.

The *Alternaria* strain was obtained from the ambient air and grown on Czapek's Dox broth (Difco, Detroit, Mich.). After 4 wk of growth at room temperature, the mycelial phase was removed and culture filtrate was dialyzed against distilled water, concentrated, lyophilized, and stored as a powder at -20° C.

Histamine release

Leukocyte histamine release was performed by the method of Lichtenstein and Osler⁵ with modifications by May and associates.⁶ Briefly, leukocytes are suspended in Tris ACM buffer and incubated for 1 hr at 37° C in a water bath with *Alternaria* antigen in concentrations from 3 to 300 µg. Histamine is extracted with n-butanol and the histamine content is determined by the fluorometric reaction with o-phthalaldehyde. The percentage of histamine release is calculated from the total leukocyte histamine content.

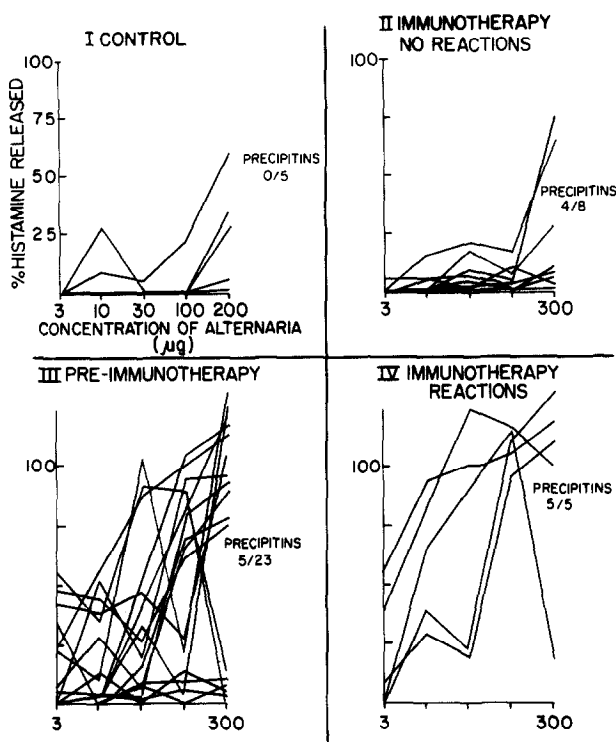


FIG. 1. Leukocyte histamine release in normal nonallergic subjects (I), patients receiving and tolerating immunotherapy including *Alternaria* (II), patients prior to receiving *Alternaria* immunotherapy (III), and patients with late reactions to *Alternaria* (IV). Also indicated is the frequency of precipitins to *Alternaria* in these four groups.

Prospective study patient selection

Patients selected for the prospective evaluation had allergic symptoms during the *Alternaria* season and had immediate skin test reactivity to intradermal challenge with aqueous *Alternaria* extract (100 PNU) (Endocrine Laboratories, Madison, Wis.). Blood samples were obtained for leukocyte histamine release, *Alternaria* precipitins, and IgE levels before initiating immunotherapy, 4 mo later, and again after 1 yr of immunotherapy.

RESULTS

Precipitating antibodies

Precipitating antibodies to *Alternaria* were 3 times more frequent in patients receiving *Alternaria* immunotherapy than in the control group and atopic patients on immunotherapy which did not include *Alternaria* (Table I). Precipitating antibody to *Alternaria* was found in all 7 patients who experienced symptoms of general malaise, low-grade fever, arthralgias, and marked swelling at the injection site. This reaction began 4 to 6 hr following the injection of *Alternaria*. The frequency of precipitating antibodies to the other antigens tested was similar in the three groups.

To exclude a false-positive precipitin test, ie., C-reactive proteins or α -2-macroglobulins, the IgG fractions of 3 sera were isolated by passage through

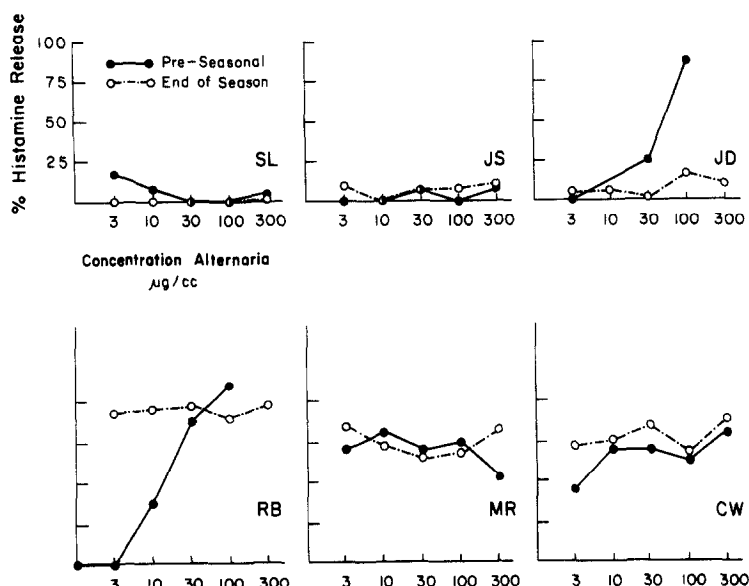


FIG. 2. Leukocyte histamine release in 6 patients who developed precipitins to *Alternaria* while receiving immunotherapy including this antigen.

a DEAE column. The IgG fraction formed a precipitin band with the *Alternaria* extract similar to that formed with the whole serum. No precipitin band was seen when *Alternaria* extract was tested against Fc fragment.

Histamine release

Little leukocyte histamine release occurred with *Alternaria* challenge in the control group and those patients tolerating the *Alternaria* injection, except at high concentrations of *Alternaria* antigen (Fig. 1). Leukocyte histamine release in the prospective group of patients showed a wide variation in cell sensitivity to *Alternaria*. The degree of histamine release tended to reflect the degree of IgE skin test sensitivity and clinical symptomatology. In the patients receiving immunotherapy which included *Alternaria* but who experienced no adverse reactions, histamine release from leukocytes was not much different from that in the control population. This group included 4 subjects with precipitins to *Alternaria*. Leukocyte histamine release reached 100% in all 5 patients tested who experienced late reaction to *Alternaria* immunotherapy.

PROSPECTIVE STUDY

Precipitating IgE antibodies to *Alternaria*

Five of the 23 patients studied prospectively had precipitating antibodies to *Alternaria* before the initiation of immunotherapy with *Alternaria*. An additional 6 patients developed precipitating antibodies to *Alternaria* following 1 yr of injection therapy. Of the total of 11 patients with precipitating antibodies to *Alternaria* only, 1 experienced a reaction 4 to 6 hr after an injection with

Alternaria extract. He had precipitins prior to initiating therapy and reactions followed the first 2 injections. This required discontinuation of immunotherapy with *Alternaria*. No other patients experienced late reactions.

Histamine release

Precipitating antibodies to *Alternaria* developed in patients with varying sensitivities of leukocyte histamine release and were not seen only in those with a high degree of histamine release, as was noted in patients experiencing late reaction (Fig. 2). The 1 patient who experienced a late adverse reaction to *Alternaria* administration released 85% of the total leukocyte histamine at 300 μ g of *Alternaria*.

IgE anti-*Alternaria* antibody

Serum IgE levels were similar in patients whether or not precipitins were present or developed in the course of immunotherapy. Attempts to assay levels of IgE anti-*Alternaria* antibody by the RAST method were difficult to perform and meaningful values were not obtained.

The late reactions originally observed to the *Alternaria* injections were suggestive of immune complex disease. Patients with precipitins to *Alternaria* but not late reactions were sampled for the presence of proteinuria following an *Alternaria* injection. No proteinuria was found. Treatment was terminated for those patients with late reactions. They did not receive challenge doses of antigen so it was not possible to test their urine for proteinuria or determine serum complement levels during the reaction.

DISCUSSION

The frequency of precipitin reactions to house dust and absence of precipitins to ragweed or grass pollen extracts in all populations sampled confirms an earlier observation.⁷ The frequency of precipitins to *Alternaria* was noted to increase during the course of immunotherapy; whether this is related to the development of IgG-blocking antibody is not resolved since we have no evidence that the antigen reacting with the IgG precipitin is the same antigen reacting with IgE.

The 7 patients who developed an adverse reaction 4 to 6 hr after the administration of an aqueous extract of *Alternaria* had received immunotherapy for 1-4 yr and previously experienced no difficulty. With the elimination of the *Alternaria* extract from the immunotherapy mixture, these patients did not experience late reactions.

The time sequence and character of the symptoms were indeed suggestive of an immune complex reaction. The finding of an IgG-precipitating antibody appeared to add credence to this belief; however, many other patients had precipitins without late adverse reactions. In addition, precipitins to *Alternaria* were found in 5 of 23 patients prior to initiation of injection therapy and 6 more patients developed precipitins in the course of therapy. In only 1 of this group did a late reaction occur and in this case the adverse response followed the initial injection and did not occur after prolonged therapy as seen in the

original 7 patients. This would appear to indicate that the presence of precipitating antibody to *Alternaria* was not solely a consequence of immunotherapy with this antigen. Precipitins are not a sufficient cause of the late reaction, since many patients with precipitins tolerate immunotherapy without difficulty. If precipitins are involved at all, there must be additional, as yet unidentified, factors operating.

The other consistent abnormal finding in the patients with a late reaction to *Alternaria* injections was a leukocyte histamine release of 100%. It is of interest to speculate that for the clinical appearance of a late reaction to *Alternaria* immunotherapy, at least 2 conditions must be present: IgG-precipitating antibody to *Alternaria* and high leukocyte histamine release with an *Alternaria* challenge. The large release of histamine may then predispose to the deposition of an IgG-*Alternaria* complex with subsequent initiation of symptoms. This would parallel observations on the importance of histamine and other vasoactive amines in initiating immune-complex diseases, as discussed by Kniker and Cochrane.⁸ A third possibility is that the late reactions are due to large amounts of IgE antibody, as has been suggested as the mechanism of the late reaction in aspergillosis by Dolovich and associates.⁹

In summary, we have observed that late reactions to *Alternaria* may occur during the course of immunotherapy. These patients had IgG-precipitating antibody to *Alternaria* and 100% histamine release following incubation with *Alternaria*. Precipitating antibodies were found in some individuals prior to immunotherapy, however, and in others they developed during *Alternaria* immunotherapy. Their presence did not appear to be a contraindication to either initiation or continuation of therapy unless late reactions occurred with *Alternaria* administration. In addition, from our prospective studies, the occurrence of late reactions was not common.

REFERENCES

- 1 Pepys, J.: Hypersensitivity disease of the lungs due to fungi and organic dusts, in Monographs in allergy, New York, 1969, S. Karger, vol. 4, pp. 1-19.
- 2 Salvaggio, J.: Diagnostic significance of serum precipitins in hypersensitivity pneumonitis, *Chest* 62: 242, 1972.
- 3 Fink, J. N., Barboriak, J. J., Sosman, A. J., Bukosky, R. J., and Arkins, J. S.: Antibodies against pigeon serum proteins in pigeon breeders, *J. Lab. Clin. Med.* 71: 20, 1968.
- 4 Flaherty, D. F., Barboriak, J., Emanuel, D., Marx, J., Moore, V., Reed, C. E., and Roberts, R.: Multilaboratory comparison of three immunodiffusion methods used for the detection of precipitating antibodies in hypersensitivity pneumonitis, *J. Lab. Clin. Med.* 84: 298, 1974.
- 5 Lichtenstein, L. M., and Osler, A. G.: Histamine release from human leukocytes by ragweed pollen antigen, *J. Exp. Med.* 120: 507, 1964.
- 6 May, C. D., Lyman, M., Alberto, R., and Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volume of blood, *J. ALLERGY* 46: 12, 1970.
- 7 Kawai, T., Arguembourg, P., Salvaggio, J., and Marsh, D.: Detection of anti-house dust precipitins in man, *J. ALLERGY CLIN. IMMUNOL.* 49: 134 (a), 1972.
- 8 Kniker, W. T., and Cochrane, C. G.: The localization of circulating immune complexes in experimental serum sickness, *J. Exp. Med.* 127: 119, 1968.
- 9 Dolovich, J., Hargreave, F. E., Chalmers, R., Shier, K. J., Gauldie, J., and Bienenstock, J.: Late cutaneous allergic responses in isolated IgE-dependent reactions, *J. ALLERGY CLIN. IMMUNOL.* 52: 38, 1973.