

Studies on *Alternaria* allergens

IV. Biologic activity of a purified *Alternaria* fraction (Alt-I)

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We have previously isolated a purified Alternaria fraction (Alt-I) by means of immunochemical (RAST) assays to document allergenicity. In the present study we investigated the allergenicity of Alt-I by in vivo and in vitro biologic tests in a selected group of 16 nonimmunized Alternaria-sensitive patients, all of whom had positive skin tests to crude Alternaria extract; a control group of eight nonallergic individuals was also studied. Intradermal skin-test titration endpoints to Alt-I ranged from 60 pg/ml to 60 ng/ml in the Alternaria-sensitive patients. Alt-I induced >30% in vitro leukocyte histamine in all Alternaria-sensitive patients at submicrogram concentrations. Alt-I did not produce positive skin tests or induce significant leukocyte histamine release in nonallergic individuals. In 14 of 16 Alternaria-sensitive patients bronchoprovocation testing produced >20% fall in forced expiratory volume in one second at Alt-I test concentrations of 0.6 to 60 µg/ml. In a separate prospective study of 100 unselected individuals, concordant skin-test reactions were noted to crude Alternaria extract and Alt-I in 96 instances. These results confirm that the Alt-I fraction is a biologically active, major allergenic component of crude Alternaria extract. (J ALLERGY CLIN IMMUNOL 71:36, 1983.)

Alternaria is a major cause of fungus-induced allergic rhinitis and extrinsic (IgE-mediated) asthma in humans. By a combination of ammonium-sulfate precipitation, anion-exchange chromatography, preparative electrofocusing, and gel filtration, we have isolated from crude *Alternaria* extract a major allergenic fraction designated Alt-I.¹ During these separation procedures, the RAST was utilized to monitor the allergenicity of fractions produced. In the same study we demonstrated significant elevations in IgE antibody to Alt-I in 21/25 patients having positive skin tests to crude *Alternaria* extract, but not in 15 patients with intrinsic (non-IgE-mediated) asthma.

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The present studies were undertaken to document the biologic activity of Alt-I in *Alternaria*-sensitive subjects, as measured by skin testing, LHR assay, and inhalation provocation testing.

MATERIALS AND METHODS

Patients

We studied 16 allergic patients, ages 10 to 30 yr. All patients had either rhinitis alone ($n = 7$) or rhinitis plus asthma ($n = 9$) coinciding with the *Alternaria* sporulation season (July to October). All patients had positive skin tests to crude *Alternaria* extract (see below). Although 11 of the 16 patients had coexisting sensitivity to short ragweed pollen as documented by skin testing, *Alternaria* was the predominant allergen by clinical history. None of the patients had received *Alternaria* immunotherapy in the preceding 7 yr. The control group comprised eight normal adults who had no history of allergic symptoms. Written informed consent was obtained for all procedures.

Antigens

Lyophilized crude *Alternaria tenuis* extract (Greer Laboratories, Inc., Lenoir, N.C., Lot LMI-116) was diluted with S-HSA.² Alt-I fraction was prepared in our laboratory by the method previously described¹ and was also diluted in S-HSA. Fresh solutions of each concentration being used for testing were made at 30 day intervals.

Abbreviations used

RAST:	Radioallergosorbent test
S-HSA:	Sorenson's phosphate buffer with 0.5% human serum albumin
LHR:	Leukocyte histamine release
FEV ₁ :	Forced expiratory volume in one second
PD ₂₀ :	Dose of nebulized allergen required to produce a 20% fall in FEV ₁
PNU:	Protein nitrogen unit

Skin tests

Patients and controls had titration intradermal skin tests to crude *Alternaria* at 10-fold concentrations from 5 to 500 PNU/ml and to Alt-I at 10-fold concentrations from 6 pg/ml to 60 ng/ml. Intradermal skin tests were performed and graded by the method of Norman.³ Puncture skin tests were done in a prospective study of 100 unselected patients presenting for diagnostic allergy tests at Mayo Clinic. These patients were tested with crude *Alternaria* extract at a single 1:5 w:v concentration and with serial dilutions of Alt-I at concentrations of 1.0, 0.1, and 0.01 mg/ml. Puncture tests were considered positive when the diameter of the resulting wheal was equal to or greater than the diameter of a wheal produced by a histamine phosphate solution (1 mg/ml).

LHR

LHR was measured by the method of May et al.⁴ Leukocytes were incubated with 10-fold dilutions of crude *Alternaria* extract (0.005 to 500 PNU/ml) and Alt-I (5 ng/ml to 500 µg/ml), as well as with anti-human IgE. Histamine released was measured by a photofluorometric technique.

IgE antibodies

Specific IgE antibodies to crude *Alternaria* and Alt-I were measured by the mini-RAST method described by Gleich et al.,⁵ using allergens that had been linked to microcrystalline cellulose particles. RAST results were expressed as a percent of the binding produced by serum from a nonallergic individual.

Bronchial inhalation challenge

The initial antigen concentration for bronchial challenge was usually that which produced a 2+ intradermal skin test. Test concentrations of crude *Alternaria* extract ranged from 0.5 to 5000 PNU/ml, while concentrations of Alt-I ranged from 6 pg/ml to 60 µg/ml, both in S-HSA. The procedure used was that recommended by Chai et al.⁶ A DeVilbiss No. 42 nebulizer containing 1 ml of allergen solution was powered by the Rosenthal Dosimeter, which was preset at 20 psi; the nebulization duration was 0.6 sec. The same nebulizer was used throughout the study on all patients. After challenge with S-HSA alone to establish baseline pulmonary function values, the patient inhaled four breaths of the allergen solution. After 10 min a flow-volume curve was obtained by means of a wedge spirometer and a storage

TABLE I. Puncture skin-test results with crude *Alternaria* and Alt-I in 100 unselected patients

	Alt-I						Totals
	0.01 mg/ml		0.1 mg/ml		1 mg/ml		
	+	-	+	-	+	-	
Crude <i>Alternaria</i> extract (1:5 w:v)							
+	3	9	6	6	10	2	12
-	0	88	0	88	2	86	88
Totals	3	97	6	94	12	88	100

oscilloscope. A 20% or greater fall below baseline in FEV₁ was considered a positive test. Results were expressed as the PD₂₀, the allergen dose in breath units calculated to produce a 20% fall in FEV₁. One breath unit was defined as one inhalation of an allergen solution containing 100 PNU/ml or 1 mg/ml.⁶

RESULTS

Skin test

All 16 allergic patients showed positive intradermal skin tests (2+ reaction or greater) to both crude *Alternaria* and Alt-I, with endpoints ranging from <5 to 500 PNU/ml and from 60 pg/ml to 60 ng/ml, respectively. No subjects of the control group had positive skin tests to either crude *Alternaria* or Alt-I.

Prospective skin testing of 100 additional patients by the prick technique to both crude *Alternaria* and Alt-I (Table I) showed 91 concordant reactions at Alt-I concentrations of 0.01 mg/ml, 94 concordant reactions at 0.1 mg/ml, and 96 concordant reactions at 1 mg/ml. Thus the 1 mg/ml concentration proved to be the most discriminating prick test dose for Alt-I skin testing. This concentration of Alt-I was not a skin irritant, as shown by the 86 concordant negative reactions. In 12 patients who were skin-test positive to crude *Alternaria*, 10 (83%) were also skin-test positive to Alt-I at the concentration of 1 mg/ml.

LHR

Leukocytes from all 16 allergic patients released 30% or more of total cellular histamine to crude *Alternaria*, Alt-I, and anti-IgE. Ten of 16 patients released more than 50% histamine with anti-IgE; in 11 of 16 patients there was greater than 50% release with both *Alternaria* allergens. The quantity of crude *Alternaria* and Alt-I required to produce 50% histamine release correlated significantly ($r = +0.64$, $F_{1,9} = 6.30$, $p < 0.05$; one-way analysis of variance) as shown in Fig. 1. Leukocytes from five of the eight

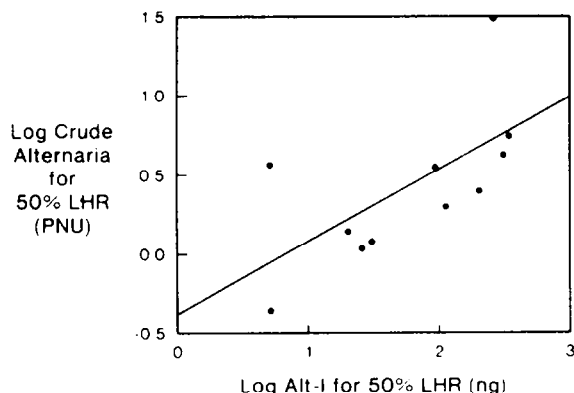


FIG. 1. Correlation between the quantity of crude *Alternaria* extract (PNU) and Alt-I (ng) required to release 50% total cellular histamine using leukocytes from 11 *Alternaria*-sensitive patients ($r = +0.64$, $p < 0.05$).

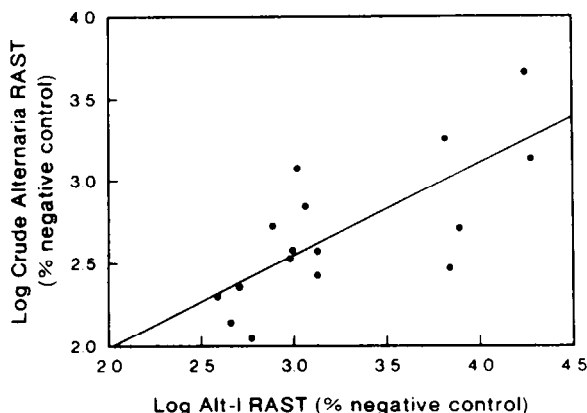


FIG. 2. Correlation between RAST binding values to crude *Alternaria* extract and Alt-I in 16 *Alternaria*-sensitive patients ($r = +0.74$, $p < 0.005$).

nonallergic individuals released more than 50% histamine with anti-IgE, but neither *Alternaria* allergen induced $>20\%$ histamine release in these individuals. The mean percent histamine released by anti-IgE (59%) was identical in the allergic and the control groups.

Specific IgE antibodies to *Alternaria*

In *Alternaria*-sensitive individuals the RAST binding to crude *Alternaria* ranged from 115% to 4600% negative control, while the RAST binding to Alt-I ranged from 400% to 19,000% negative control. Fig. 2 is a graph of the specific IgE antibody levels to both allergens. The RAST binding values were significantly correlated ($r = +0.74$, $F_{1,14} = 16.46$, $p < 0.005$). All individuals in the control group had RAST binding values below 200% negative control to both crude *Alternaria* and Alt-I.

Bronchial inhalation challenge

Positive bronchoprovocation challenges ($>20\%$ decrease in FEV_1) were noted in 13 of 16 patients with crude *Alternaria* extract, with the PD_{20} ranging from 3.5 to >822 breath units. Fourteen of 16 patients had positive challenges to Alt-I, with the PD_{20} ranging from 0.006 to >0.257 breath units. The patients with negative bronchoprovocation tests were all patients who had a history of having rhinitis only. The PD_{20} dosages for crude *Alternaria* extract and Alt-I were not significantly correlated.

DISCUSSION

Alternaria, an ubiquitous mold, has long been incriminated as an important airborne allergen.⁷ The isolation of a major allergenic fraction, Alt-I, from crude *Alternaria* has been reported previously by our group.¹ The purpose of this study was to document the biologic allergenic activity of Alt-I by both in vitro and in vivo procedures and to compare this activity with that of crude *Alternaria* extract. Few, if any, previous studies involving purified fungal allergens have involved in vivo documentation of their allergenicity.*

All 16 *Alternaria*-sensitive patients showed positive intradermal skin tests ($\geq 2+$) and significant LHR ($>30\%$) to Alt-I at picogram per milliliter or nanogram per milliliter test concentrations. Normal control individuals were not reactive by skin testing or LHR to these Alt-I concentrations. In the prospective skin testing of 100 additional unselected patients by the prick technique, we showed concordant reactions between crude *Alternaria* and Alt-I. In 12 of these patients who were skin-test positive to crude *Alternaria*, 10 (83%) were also positive to Alt-I at the prick test concentration of 1 mg/ml. This skin-test reactivity rate (percent of individuals reacting to crude *Alternaria* who also react to Alt-I) is virtually identical to the reactivity rate calculated by RAST in our previous study.¹ These data suggest that although Alt-I is a major allergenic fraction in crude *Alternaria* extract, it does not contain the only allergen.

Of the 16 *Alternaria*-sensitive patients, 11 released 50% or more of their total leukocyte histamine with both *Alternaria* antigens. None of the control individuals released histamine from their peripheral leukocytes after challenge with either *Alternaria* antigen. The leukocytes from all individuals studied released histamine after challenge with antihuman IgE.

The sensitive patients who did not react to both crude *Alternaria* and Alt-I in bronchoprovocation challenge testing at the selected concentrations were patients who had histories of having rhinitis and no

asthma during the *Alternaria* season. Had we used fivefold instead of 10-fold increases in the concentration of the solutions and increased the test concentrations somewhat, we may have been able to titrate the endpoint more precisely in a greater number of patients. None of the control group individuals showed a positive bronchial reaction to either crude *Alternaria* extract or Alt-I.

Our data confirm that the Alt-I fraction is a biologically active allergenic component of crude *Alternaria* extract, as measured by skin test, LHR, and bronchoprovocation tests. However, there are probably other allergenic components in crude *Alternaria* extracts.

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