Sensitization to Alternaria Allergens: Characterization of the IgE Specificities and Relative Reactivities of Skin Test-Positive Individuals from Multiple U.S. Locations

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Abstract

Alternaria alternata spores and mycelia are prevalent sources of airborne allergens in many areas of the United States, and a major contributor to severe asthma. Differences between the Alternaria allergens recognized by allergic patients and those contained in commercial extracts can result in inaccurate or inconsistent diagnoses and unfavorable immunotherapy outcomes. Although several allergens have been identified in Alternaria cultures or fractions, the specific regions or three-dimensional protein structures responsible for allergic reactions in most states or geographic regions remain largely unknown.

In this study, sera obtained from 70 patients with positive Alternaria skin tests from allergy clinics in two locations (WI, AR) were analyzed for IgE-binding specificity patterns and relative reactivities with commercial and non-commercial Alternaria extracts. Ten different profiles were identified by immunoblotting, with all but two showing strong reactions to the prominent allergenic protein Alt a 1. Blot reactivity patterns with individual extracts were consistent for most patient sera. Differences in reactions to 60-100 kilodalton proteins were apparent for many patients but were less prevalent overall relative to Alt a 1. Patients with negative Alternaria ImmunoCAP reactivities displayed only weak IgE interactions with proteins larger than 100 kilodaltons in size. Inhibitions of immunoblot and ImmunoCAP reactions were near-complete for some commercial extracts but only partial to very low for others. Dose-response curves for most extracts were consistent and parallel, providing strong evidence for the suitability of Alternaria extract standardization based on the cumulative IgE-binding properties of multiple allergenic components in these products.

The observed patterns of Alternaria reactivity were mostly similar for a majority of study patients from the two clinic locations. Further studies examining other geographic regions and allergen extracts from distinct *A. alternata* cultures or strains are warranted to determine the optimal compositions and properties of Alternaria products for most U.S. locations.

Materials and Methods

The Alternaria extracts employed for these studies were 1:10-1:20 w/v, 50% glycerin products from 7 FDA-regulated U.S. allergen manufacturers (Greer Labs, Lenoir, NC; ALK Abello, Port Washington, NY; Allergy Labs, Oklahoma City, OK; Allermed Labs, San Diego, CA; Antigen Labs, Liberty, MO; Hollister-Stier Labs, Spokane, WA; and Nelco Labs, Deer Park NY). These products are designated as A-G in no particular order. Cellular extracts and culture filtrates from four different *A. alternata* strains cultured at Greer (shown as 1-4 and 1f-4f) were also analyzed.

Blood samples were obtained at two allergy clinics from patients displaying positive skin prick tests to Alternaria. Skin test devices, extracts, controls and criteria for positive reactions were identical to those currently used in each clinical practice. Both wheal and erythema reactions were measured and recorded as diameters (mm) or class scores. Serum was isolated from the blood samples by centrifugation, stored at -20°C, and then shipped to Greer for immunochemical analyses. Proper informed consent and HIPAA guidelines were followed by each clinic, and all study activities were approved by investigational review boards.

SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was performed using 12% acrylamide gels and 0.1-0.5 µg loads of Alternaria extract protein using Bio-Rad Mini-Protean II. Separated gel proteins were then transferred to PVDF membranes (Millipore Immobilon-P^{SQ}) and blocked with Tween-20. Standard protein markers at known kilodalton (kDa) values were visualized with colloidal gold. Alternaria blots were incubated with individual patient sera (1:20-1:400 v:v dilutions), and IgE antibodies detected using goat anti-human IgE-AP conjugate and BCIP-NBT substrate. Immunoblot inhibitions included pre-incubation of patient sera and each Alternaria extract (1:10 v:v dilutions, 2 hours at 20-25°C) prior to addition to the Alternaria blots.

ImmunoCAP100 analyses (Phadia, Portage, MI) were conducted at Greer using Phadia reagents and assay conditions. Inhibitions of human IgE interactions with Alternaria (m6) ImmunoCAP allergens were performed by pre-incubation (2 hours, 20-25°C) of patient sera (1:2-1:40 v:v dilutions) with 5-fold serial dilutions of Alternaria extracts (1:10-1:6250 v:v final). Percent inhibition values for extract dilutions were calculated based on the reactivities of uninhibited (positive control) samples. IgE-binding potencies from % inhibition vs. log dilution dose-response curves were determined by parallel line bioassay analysis and expressed relative to the data obtained for Alternaria extract G.

IgE Specificity Patterns/Groups

Immunoblotting of 70 serum samples from Alternaria skin test-positive patients revealed IgE-positive proteins in multiple commercial and non-commercial Alternaria extracts ranging from 21 to greater than 80 kDa in size. Three distinct low molecular weight components were detected, including the 30-40 kDa bands identified as the prominent Alternaria allergen Alt a 1. Seven high molecular weight (> 80 kDa) IgE band patterns were also observed.

Based on these reactions, one negative (N) and nine positive (P1-P9) specificity groups were identified, including selective reactions with four Alternaria extracts (F, 3, 3f and 4).

Serum Group	Molecular weights of IgE+ bands (kDa)									# of Sera	
	>80	>80	>80 (3f)	>80 (3)	>80 (F)	>80 (4)	>80	30-40	24	21	WI	AR
N											4	8
P1											17	12
P2											11	5
P3											2	0
P4											1	2
P5											1	0
P6											2	1
P7											1	0
P8											0	2
P9											1	0
% or Total	36	1	7	1	13	9	4	81	9	3	40	30

Twelve patient sera (17% of total) produced negative immunoblot results (group N). Group P1 (29 sera, 41%) displayed specific IgE reactions to Alt a 1. All other positive groups except P9 exhibited IgE interactions with Alt a 1 and some high molecular weight components. The group P9 serum (1%) recognized high molecular weight allergens but not Alt a 1. Bands shown above in lighter shading varied in intensity for several sera in groups P3, P4 and P6.

Immunoblot band patterns and specific extract inhibition profiles for sera from IgE specificity groups P3, P6 and P9 are illustrated in the next column. Sera from groups P3 (WI) and P6 (AR) contained the highest Alternaria-positive IgE levels among all samples from the two clinics based on both immunoblot and Alternaria ImmunoCAP assay reactivities. Inhibitory activities of the Alternaria extracts were near-complete with most serum groups (B, D, F, G, 3f) or consistently low with all serum groups (A, C, E).

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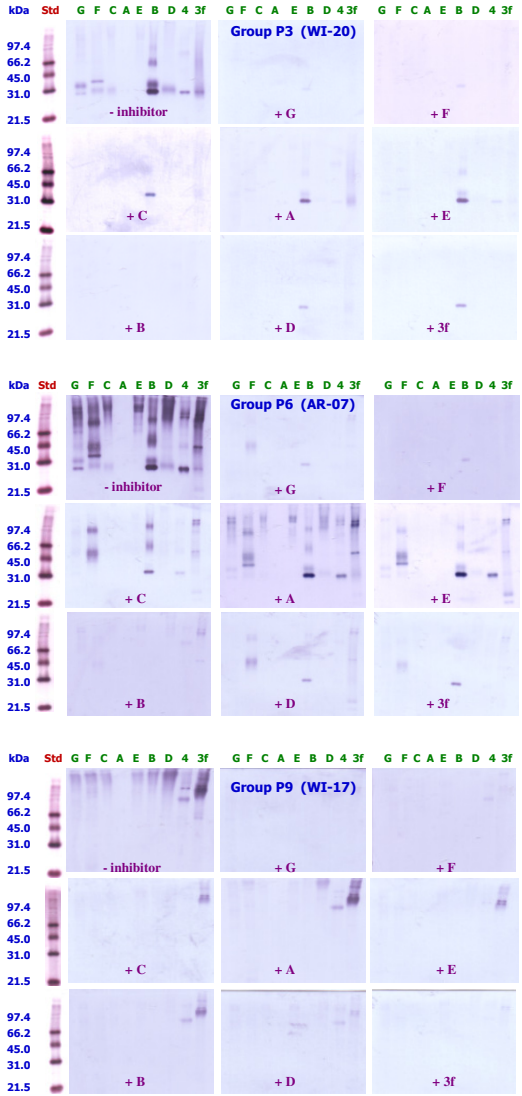
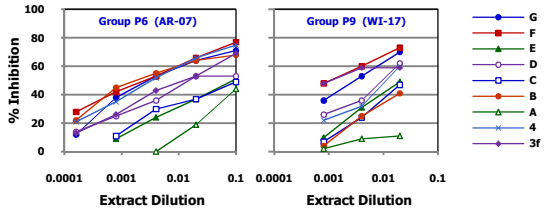
Immunoblot Inhibition Patterns

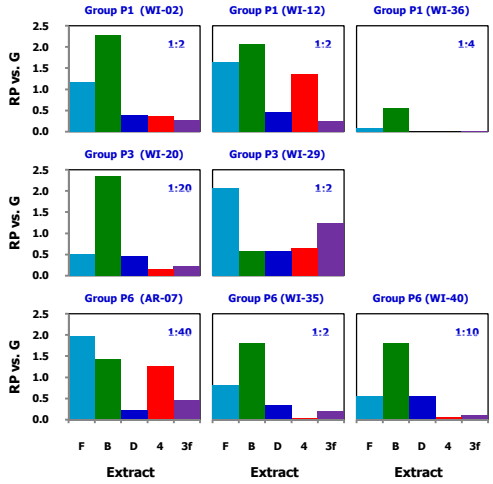


ImmunoCAP Inhibition Patterns

Alternaria (m6) ImmunoCAP reactivities for sera from all 10 groups were consistent with their immunoblot intensities. Concentration-dependent inhibitions of m6 ImmunoCAP reactions by the various Alternaria extracts produced parallel dose-response curves for most serum-extract combinations.



The relative IgE-binding potencies of the Alternaria extracts varied noticeably for many sera within the same group in addition to those assigned to different groups.



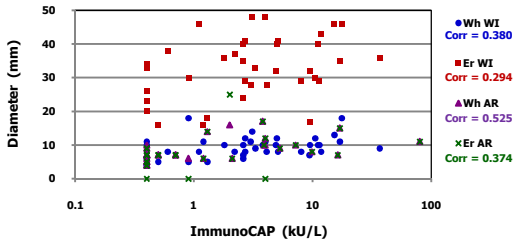
These results demonstrate that sera possessing similar qualitative IgE specificity patterns can be differentiated further based on quantitative competitive inhibitions with Alternaria extracts containing varying levels of the specific IgE epitopes recognized by these skin test-positive patients.

MPN 0907H011

Skin Test Results

The diameters of the skin test wheal (Wh) or erythema (Er) reactions produced by the Alternaria extracts used at the two clinics (A, F, G) did not correlate closely with Alternaria-specific IgE reactivities or concentrations determined in immunoblot or m6 ImmunoCAP analyses.

No apparent differences in skin sensitivity were observed for patients exhibiting different IgE specificity patterns.



Conclusions

Alternaria-sensitive patients from allergy clinics in different geographic regions (WI, AR) displayed a variety of complex IgE specificity patterns to multiple extract components, with most patients exhibiting moderate or strong reactions to the major Alternaria allergen Alt a 1. A diverse group of commercial and non-commercial Alternaria extracts blocked IgE binding to immobilized Alternaria allergens to varying degrees, even with numerous sera possessing similar IgE specificities. Immunoblot and ImmunoCAP inhibition analyses with individual patient sera provided detailed qualitative and quantitative assessments of extract composition (allergen profiles), similarity (parallelism) and relative reactivity (degrees of inhibition at defined extract strengths).

Based on these data, the standardization of Alternaria extracts to multiple allergenic components is feasible for several (but not all) of the current commercial Alternaria products available in the U.S.. Continued investigations focusing on the allergen compositions of Alternaria (or other fungal) extracts and the IgE epitope specificities of sensitive patients across the U.S. are essential to define the optimal compositions of single-strain extracts or multi-strain mixtures formulated to diagnose and treat fungal allergies.