Diversity of Protein Compositions and Immunochemical Reactivities in Alternaria Extracts from Multiple Sources or Cultures

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Abstract

Fungal organisms express a complex variety of allergenic and non-allergenic macromolecules in response to the carbon source, host species or distinct genetic characteristics of a particular strain. Qualitative differences may also result from culturing organisms on defined media in a laboratory setting compared to more diverse substrates in nature. Extracts manufactured using different cultures, growth conditions or extraction methods can thus produce differences in composition or concentrations of important allergens that may deviate from those recognized by allergic patients.

In this study, Alternaria extracts prepared by seven U.S. manufacturers were assessed using laboratory methods sensitive to differences in product compositions or biological activities. Alternaria products manufactured in 2003 and 2005 were examined for total protein and carbohydrate levels, specific protein (Alt a 1) concentrations and antibody-binding activities along with Greer extracts prepared using four experimental (non-licensed) *A. alternata* strains. Protein, carbohydrate and Alt a 1 concentrations varied widely among the commercial products. Experimental extracts displayed similar protein concentrations but variable Alt a 1 levels. SDS-PAGE and immunoblot band patterns were distinct for products from different sources or strains. Alt a 1 immunoblots revealed the presence of IgE and IgG epitopes on proteins at different molecular weights in these products. Human IgE ELISA inhibition analyses produced parallel dose-response curves for most extract combinations, an indicator of significant similarities in the allergenic components of these products. Cross-wise immunoblot inhibition experiments confirmed that several extracts contained allergen structures and levels sufficient to block human IgE or rabbit IgG binding to proteins from all other sources and strains included in these analyses.

These results indicate that, in spite of considerable quantitative and qualitative variations in extract compositions, similar allergenic or antigenic epitope structures may be present in Alternaria products from different commercial sources or *A. alternata* strains. Subsequent studies will define the IgE specificities of Alternaria-sensitive patient populations and the degrees of shared and distinct allergens among these individuals.

Materials and Methods

Glycerinated Alternaria extract concentrates (1:10-1:20 w/v) were obtained from all 7 FDA-regulated U.S. allergen manufacturers in 2003 and 2005. Products from these companies (listed below) are designated as A-G in no specific order. Cellular extracts and culture filtrates from five different *A. alternata* strains cultured at Greer (designated as 1-5 and 1f-5f) were also examined.

Greer	Lenoir, NC	1:20 w/v
ALK Abello	Port Washington, NY	1:10 w/v
Allergy Laboratories	Oklahoma City, OK	1:20 w/v
Allermed Laboratories	San Diego, CA	1:10 w/v
Antigen Laboratories	Liberty, MO	1:10 w/v
Hollister-Stier Laboratories	Spokane, WA	1:10 w/v
Nelco Labs	Deer Park, NY	1:10 w/v

Two product lots from each company were analyzed for total protein (Bradford method), total reducing carbohydrate (phenol-sulfuric acid method) and specific allergen Alt a 1 (two-site monoclonal antibody ELISA) concentrations. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12% acrylamide gels in a Bio-Rad Mini-Protean II slab cell (0.5 µg protein per lane). Gels were visualized by silver staining (Invitrogen SilverXPress) or immunoblotting with specific antibodies or serum samples after transfers to polyvinylidene difluoride (PVDF) membranes (Millipore Immobilon-P^{SQ}).

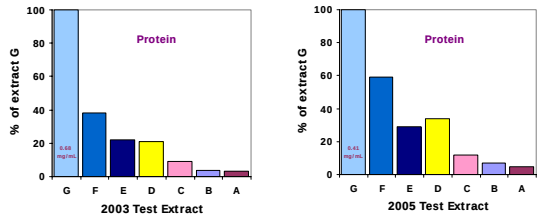
Enzyme-linked immunosorbent assay (ELISA) inhibition experiments were conducted in 2003 and 2006 with extract lots obtained within the previous year. Specific pairs of Alternaria extracts were evaluated directly, with one extract serving as coating and reference extract and the other as test extract. Three-fold serial dilutions of reference and test extracts were co-incubated with a human serum pool containing Alternaria-positive IgE, then probed for IgE binding using validated detection reagents (biotinylated anti-human IgE, avidin-alkaline phosphatase conjugate, para-nitrophenyl phosphate chromogenic substrate). Percent inhibitions and relative IgE-binding potencies were calculated using a parallel line bioassay spreadsheet macro.

Immunoblot inhibitions were performed by co-incubating specific Alternaria extracts with an allergic human serum pool, and detecting changes in human IgE binding patterns after developing blots with appropriate probe reagents (goat anti-human IgE-alkaline phosphatase conjugate, bromo-chloroindolyl phosphate/nitro-blue tetrazolium precipitating substrate formulation).

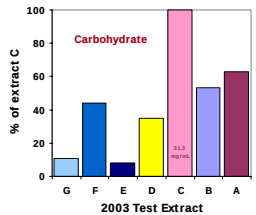
Compositional Analyses

The relative protein, carbohydrate and Alt a 1 levels in Alternaria extracts from 7 commercial sources are illustrated below.

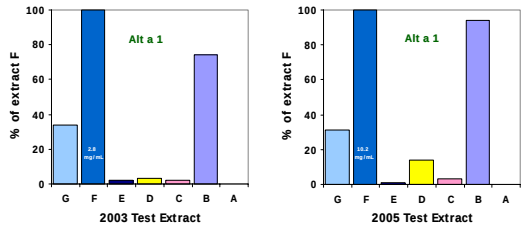
Total protein levels ranged from 17 to 683 µg/mL (26-fold range), with product lots from a single source exhibiting values within 10% in most cases.



Total carbohydrate concentrations varied up to 12-fold with no consistent correlations to protein content.



Relative (company-company) Alt a 1 reactivities were similar for extracts obtained in 2003 and 2005; however, absolute values varied up to five-fold due to lot-to-lot differences in the characteristics of critical ELISA reagents (monoclonal capture and probe antibody, recombinant Alt a 1 reference).



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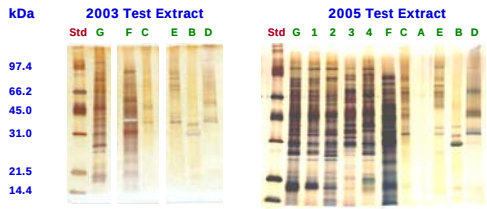
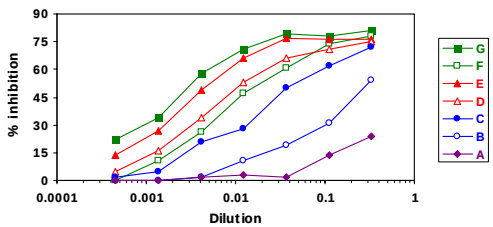
SDS-PAGE Profiles

Alternaria extracts from different U.S. manufacturers (A-G) displayed considerable variations in SDS-PAGE band patterns and staining intensities. Glycerinated extract lots obtained from individual companies in 2003 and 2005 possessed very similar protein molecular weight profiles. Cellular extracts prepared at Greer using four biochemically-diverse *A. alternata* strains (1-4) contained both shared and distinct protein bands.



ELISA Inhibition Potencies

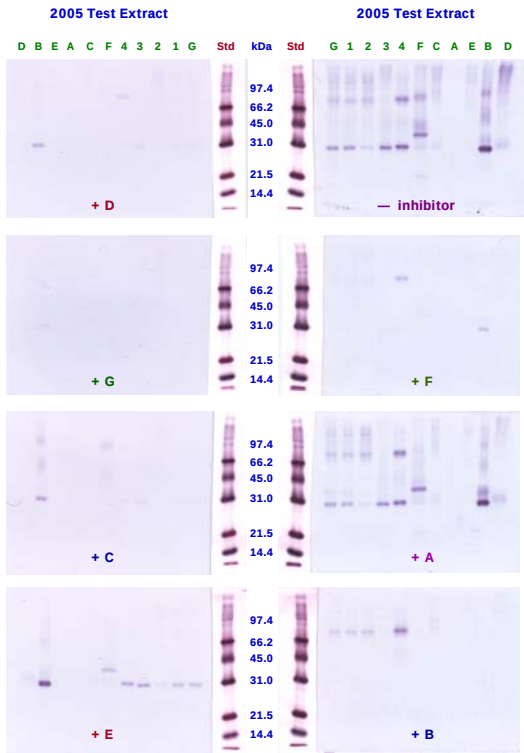
Human IgE ELISA inhibition analyses of Alternaria extracts from manufacturers A-G produced parallel dose-response curves for most product combinations. Achievement of parallelism is indicative of significant conservation of allergenic structures among these products. The relative potencies of the Alternaria products were highest for extracts D, E and G (0.6-1.7) and lowest for extracts A and B (< 0.1) when tested with extracts D, F and G as references. Extracts with high Alt a 1 levels (B and F) displayed lower ELISA inhibition reactivities to multiple Alternaria allergens.



Immunoblot Inhibition Patterns

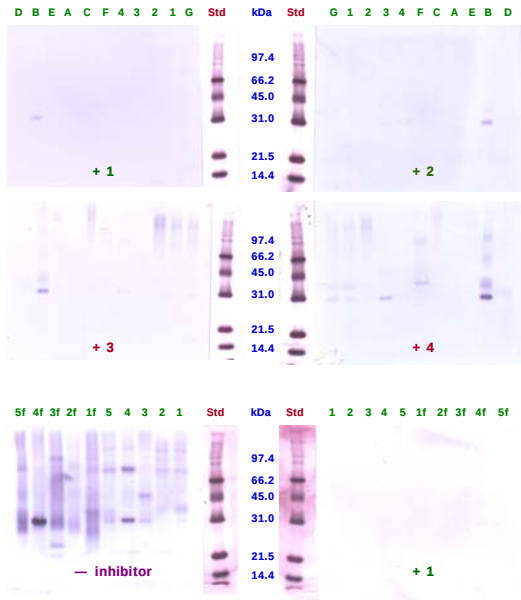
Alternaria extracts from different sources (at 1:10 dilutions) also inhibited human IgE binding to commercial and non-commercial Alternaria products on non-reducing SDS-PAGE immunoblots. Several extracts produced complete (G) or near-complete (C, D and F) inhibition under these conditions. Others (B and E) demonstrated partial or selective inhibitions of Alternaria allergens, resulting in distinct patterns of residual (uninhibited) allergen activities. One extract (A) yielded very low inhibition of most prominent immunoblot bands (reactivities similar to inhibitor-free control).

Alternaria extracts 3-4 produced much lower and more selective IgE-blocking activities compared to extracts 1-2.



Immunoblot Inhibition Patterns

Extract 1 strongly inhibited IgE binding to all major allergens present in cellular extracts and culture filtrates of the five *A. alternata* strains examined to date in the Greer R&D lab.



Conclusions

Alternaria extracts from different sources contain a complex mixture of common and distinct allergenic proteins at variable concentrations. Some (but not all) Alternaria products possess compositions sufficient to completely inhibit IgE binding to many other Alternaria extracts. The presence of differing patterns of human IgE binding and inhibition activity infers that selection of Alternaria extracts that most closely match the IgE specificities of allergic patients may lead to improved clinical benefits. Studies are underway to screen allergens from multiple *A. alternata* strains with sera from allergic patients across the U.S.