

# A double monoclonal antibody assay for the *Alternaria* allergen GP70

Jay Portnoy, MD; Freddy Pacheco; Bala Upadrashta; and Charles Barnes, PhD

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*A double monoclonal antibody-based assay for the 70-kD Alternaria allergen GP70 is described. The assay is sensitive to GP70 concentrations as low as 0.2 µg/mL and is highly specific for this allergen. The glycoprotein detected by this assay is shown to bind to human IgE from Alternaria-sensitive patients, proving that it is an allergen. Two of three commercial Alternaria preparations tested contained no detectable GP70. Several related fungi were shown to contain detectable concentrations of GP70, but the most abundant source was a commercial preparation of house dust. Further measurement of GP70 in biologic materials should increase our knowledge of the distribution and importance of this Alternaria allergen in the environment and in the development of allergic diseases.*

## INTRODUCTION

Fungal extracts derived from spores and mycelia of members from the genus *Alternaria* are complex mixtures of proteins and carbohydrates.<sup>1</sup> Standardization of such extracts has been a significant problem, in part due to the extreme variability of eluted materials. This is true even when growth and extraction conditions are held constant. A consistent finding by investigators to date is that multiple allergens, or at least different molecular weight moieties, are involved in *Alternaria* sensitivity.<sup>2-4</sup>

Recently, considerable progress has been made towards standardization of several allergen extracts. Techniques used have involved purification of major allergens and production of assays for measuring specific concentrations in unknown extracts. Such assays have been reported for *Der p1* and *Der f1* from house dust mite<sup>5</sup>; selected major allergens from mouse, rat, and rabbit<sup>6</sup>; *Fel d1* in cat<sup>7</sup>; and a major

allergen from cedar pollen.<sup>8</sup> Immunoassays based on monoclonal antibodies have been shown to be particularly useful due to their specificity and reproducibility.<sup>9</sup>

We now describe a monoclonal antibody-based ELISA that permits measurement of concentrations of a previously described 70-kD glycoprotein from *Alternaria*.<sup>10</sup> We have used this assay to measure the concentration of this allergenic glycoprotein in three commercial preparations of *Alternaria*, selected other fungi in the family Deuteromycetes, and in other nonfungal extracts. The intrinsic limitations of this type of assay with respect to fungal extracts is also discussed.

## MATERIALS AND METHODS

### Human Studies

All clinical studies were performed after obtaining approval from the pediatrics institutional review board of the University of Missouri-Kansas City. Written consent was obtained before any experimental procedure was performed. Volunteers were tested percutaneously using three commercial *Alternaria* preparations (Greer Laboratories, Lenoir, NC; Center Laboratories, Port Washington, NY; and Hollister-Stier, Spokane, WA). Serum was obtained from those with 5-mm or greater wheals to one or more of the extracts and screened for anti-*Alternaria* IgE by direct ELISA. Samples

with significant specific IgE (greater than two times the background) were pooled. The final serum pool consisted of equal amounts of sera from 13 patients.

### Preparation of Extracts

A strain of *Alternaria* previously designated AL 001 was grown at 24 °C for 2 weeks in a potato dextrose broth. This *Alternaria* has been submitted to American Type Culture Collection (Rockville, MD) with acceptance pending. To produce a mycelial extract, fungal mats containing both spores and mycelia by light microscopy were briefly washed with distilled water, air dried for six hours at 37 °C, and powdered with a mortar and pestle. The powdered material was extracted at 4 °C in deionized water at a concentration of 10 g of dry weight per 100 mL for 24 hours. The slurries were centrifuged and all of the supernate removed. For production of the culture medium extract, spent culture medium was decanted and filtered through a Whatman 5 filter. Both extracts were filtered through a 0.45-µm filter (milipore), dialyzed overnight against 50 mM ammonium bicarbonate at 4 °C, and lyophilized. The resultant preparations were stored desiccated at -20 °C until used. This combination of strain, extraction, and growth conditions has previously been found to produce large quantities of

From the Section of Allergy/Immunology, The Children's Mercy Hospital, Kansas City, Missouri.

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GP70.<sup>11</sup> Lyophilized powders were reconstituted at concentrations of 10 mg dry weight per mL in TBS for use in purification procedures and for assays.

#### *Electrophoresis*

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 10% vertical slab gels (BioRAD, Richmond, CA) using the method of Laemmli<sup>12</sup> using prestained molecular weight markers (BioRad) with each run. Lyophilized samples were reconstituted in deionized water at a concentration of 1 mg/mL and 10  $\mu$ L of each sample diluted in 30  $\mu$ L of TRIS-SDS (pH 6.8) with mercaptoethanol and 25% glycerol were applied to each lane. SDS-PAGE gels were stained for glycoprotein using a silver stain according to the method of Wray.<sup>13</sup>

#### *IgE Immunoblot*

Proteins from SDS-PAGE gels were transferred electrophoretically to nitrocellulose membranes (BioRAD TransBlot) in 25 mM TRIS and 192 mM glycine buffer, pH 8.8 containing 20% methanol at 30 V overnight.<sup>14</sup> After blocking overnight with 5% nonfat dry milk (Carnation Co., Los Angeles, CA) in TBS, the blots were probed overnight with the 1:10 pooled human serum. The blots were next incubated for two hours with 1:1000 peroxidase-conjugated rabbit anti-human IgE (Sigma, St. Louis, MO), washed with TBS, and visualized with a Web membrane that is an immobilized horse radish peroxidase substrate on a plastic matrix (IBI, New Haven, CT).<sup>15</sup>

#### *Coating of Microtiter Plates*

Polystyrene microtiter plates (Immulon-2, Dynatech Laboratories Inc., Chantilly, VA) were coated by dissolving lyophilized materials in a 0.15 M bicarbonate buffer (pH 9.6) and adding 200  $\mu$ L per well overnight at room temperature. For direct ELISA with *Alternaria* in solid phase, a concentration of 10  $\mu$ g dry weight/mL was used. The optimal

binding concentration for each of the monoclonal antibodies was empirically determined to be 10  $\mu$ g dry weight/mL.

#### *Production and Purification of Antibodies*

Monoclonal antibodies directed to GP70 were produced as described previously.<sup>10</sup> Aqueous whole *Alternaria* extracts were used for immunization in the initial fusions. To produce a monoclonal antibody directed towards a separate epitope, affinity purified GP70 was emulsified in incomplete Freund's adjuvant and mice were immunized with this material. All polyclonal sera and monoclonal antibody-containing ascites fluids were purified with protein A prior to use and were tested for anti-*Alternaria* activity by direct ELISA.

#### *Production of F(ab)'<sub>2</sub>*

Purified monoclonal antibodies were applied to a column containing pepsin conjugated to sepharose (Pierce, Rockford, IL) at a concentration of 10 mg dry weight/mL and continuously rotated. After incubating for two hours, the column was washed with TBS using a syringe with a filter and the eluted material was applied to a protein A-sepharose column. The unbound material, which represented the F(ab)'<sub>2</sub> fragment, was dialyzed against 50 mM ammonium bicarbonate overnight and lyophilized. Activity of the F(ab)'<sub>2</sub> fragments was confirmed by direct ELISA with *Alternaria* in solid phase using alkaline phosphatase-conjugated antimouse F(ab)'<sub>2</sub> as the detecting antibody.

#### *Purification of GP70*

The 70-kD glycoprotein GP70 was purified by affinity chromatography using a monoclonal antibody (F8F11) in solid phase as described previously.<sup>10</sup> Purity was confirmed by SDS-PAGE. The unbound extract was subsequently reapplied to the F8F11 column until no further GP70 was elutable. This residual extract was then lyophilized, reconstituted in TBS at a concentration of

10 mg dry weight/mL, and is what we refer to as the GP70-depleted extract.

#### *Biotinylation of the Purified GP70 and Monoclonal Antibodies*

For detection of human IgE, an anti-human IgE monoclonal antibody (ATCC HB 141) was labeled with biotin. Two milligrams of purified lyophilized monoclonal antibody was dissolved in 200  $\mu$ L of 0.05 M bicarbonate buffer (pH 7.5) and incubated at 4 °C for one hour with 3  $\mu$ L of N-hydroxysuccinimide biotin (BioRAD). At the end of this time, the antibody was dialyzed at 4 °C overnight against 0.1 M TBS and stored at -20 °C in 50% glycerol at a concentration of 5 mg dry weight/mL. GP70 was biotinylated by the same procedure starting with 2.0 mg of purified GP70. The activities of the biotinylated antibody and biotinylated GP70 were confirmed by direct ELISA using either 1:1000 avidin-alkaline phosphatase (Sigma) or a GP70-specific monoclonal antibody (F8F11) to detect them.

#### *Choosing Monoclonal Antibodies for the Assay*

To develop a sandwich ELISA, it was necessary to identify two monoclonal antibodies that recognize different antigenic determinants on GP70. Twenty monoclonal antibodies were evaluated for this purpose. To determine whether different epitopes were recognized by the monoclonal antibodies, separate microtiter plates were coated with each of four monoclonal antibodies with the highest titers (D6B3, D6B4, F8F11, and P2G8). Serial dilutions of each of the 20 monoclonal antibodies were next added to aliquots of 10  $\mu$ g/mL biotinylated GP70 and allowed to bind for two hours. These mixtures were then incubated in antibody-coated wells for two hours, washed, and detected with 100  $\mu$ L perwell 1:1000 alkaline phosphatase-conjugated avidin (Sigma). Two monoclonal antibodies recognizing different epitopes gave a high

OD405 and conversely monoclonal antibodies recognizing the same epitope gave substantial inhibition.

#### *Double Monoclonal Antibody Sandwich ELISA*

Microtiter plates were coated overnight with the F(ab)<sub>2</sub> fragments of the capturing monoclonal antibody (P2G8) as described above. Coated plates were washed and blocked with 0.5% gelatin (Fisher) at 37 °C for one hour to reduce the background. Serial dilutions of 100 µL per well of samples containing GP70 were next added to each well and incubated for two hours. After washing, the plates were incubated with 100 µL per well of a 10 µg/mL secondary monoclonal antibody (F8F11) for two hours. The plates were again washed and incubated for one hour with 100 µL per well of 1:1000 alkaline phosphatase-conjugated protein A (Sigma). The protein A did not bind to the F(ab)<sub>2</sub> used for coating the plate because the coating antibody had been absorbed against protein A. After washing and the addition of 1 mg/mL *p*-nitrophenyl phosphate, the OD405 was measured with a Dynatech MR7000 plate reader.

#### *Human IgE Sandwich ELISA*

For the human IgE sandwich ELISA, microtiter plates were again coated overnight with the F(ab)<sub>2</sub> fragments of the capturing monoclonal antibody (P2G8). After washing and blocking with 0.5% gelatin (Fisher) at 37 °C for one hour, serial dilutions of samples containing GP70 were added to each well as above and incubated for two hours. The plates were next incubated with 100 µL per well of pooled human IgE anti-*Alternaria*-containing serum diluted 1:10 in phosphate buffered saline with 0.1% gelatin for two hours. The plates were washed and incubated for one hour with 100 µL per well of 10 µg/mL biotinylated monoclonal anti-human IgE. The biotinylated anti-IgE antibody was detected with 10 µg/mL alkaline phosphatase-conjugated avidin

(Sigma, St. Louis, MO). The OD405 was then read as described above.

#### *Standardization of the Assay*

Since accurate standardization of an assay is critical, the concentration of purified GP70 was measured both in dry weight and in milligrams protein per milliliter by two different methods: Lowry<sup>16,17</sup> and BioRad dye binding.<sup>18</sup> The BioRad assay is based on the color change of Coomassie Blue G250 in response to binding to basic and aromatic amino acid residues. This assay will give different results depending on the hydrophobicity of the protein being measured. The Lowry method relies on binding of a copper reagent to the N-terminal peptide bonds of a protein and subsequent reaction with a folin-phenol reagent. The results of the assay can therefore be expressed either as milligram dry weight or mg protein/mL. Carbohydrate moieties were identified and their molar ratios measured by high pressure liquid chromatography using a carboPac PA1<sup>TM</sup> column and a pulsed amperometric detector (Dionex Corp, Sunnyvale, CA).

The immunoassays were performed using serial dilutions of the purified GP70 to generate a standard curve. The log of the known GP70 concentrations vs OD405 was analyzed by linear regression and compared to GP70 concentrations in simultaneously run unknown preparations.

## RESULTS

#### *Identification of Monoclonal Antibodies Directed to Separate Epitopes*

Table 1 shows the percent inhibitions of 10 µg dry weight/mL solutions of four of the inhibiting monoclonal antibodies. The first three gave virtually identical inhibition curves to the other 17 initially screened with this procedure. They inhibited each other by between 60% to 75%, again suggesting recognition of a common epitope. For this reason, we performed another

fusion using mice immunized with affinity purified GP70. The resulting monoclonal antibody (P2G8) was also directed to GP70 by direct ELISA but appeared to recognize a different epitope in that it inhibited and was inhibited by the first three monoclonal antibodies by between 5% to 38%. This was used as the capturing monoclonal antibody. Since F8F11 had been used to affinity purify the GP70, it was used as the detecting monoclonal antibody in the interest of consistency.

#### *Protein Determinations of the Standard*

The purified GP70 was dissolved in deionized PBS at a concentration of 10 mg dry weight/mL and assayed for protein concentration by two methods as described above. Though it is not possible to determine whether all of the dry powder did dissolve in solution, the resulting aliquot was visually clear after shaking. The two protein determination methods gave quite different results with the Lowry method giving significantly higher protein values (1.48 mg protein/mL) than the BioRad method (0.25 mg protein/mL). Complicating factors such as detergents, basic buffers, and glycerol that could have interfered with these assays were not present in this preparation. This difference between two methodologies has been observed by other investigators as well.<sup>19</sup> The measurements were performed in duplicate and were repeated when the discrepant values were observed. For the rest of this discussion, protein values will represent those determined by the Lowry method. GP70 concentrations per total protein concentrations (ie, parts per thousand) were the same when measured by either method probably because the differences between the two methods cancelled each other out.

Since GP70 is a glycoprotein, measurement of carbohydrate could be another method for standardization of this assay. Characterization

Table 1. Percent Inhibition by Inhibiting Monoclonal Antibodies of Binding Between Biotinylated GP70 and Capturing Monoclonal Antibodies

| Inhibiting Monoclonal Antibody | Capturing Monoclonal Antibody |      |       |      |
|--------------------------------|-------------------------------|------|-------|------|
|                                | D6B4                          | D6B3 | F8F11 | P2G8 |
| D6B4                           | 60%                           | 65%  | 75%   | 40%  |
| D6B3                           | 50%                           | 65%  | 60%   | 35%  |
| F8F11                          | 60%                           | 80%  | 65%   | 25%  |
| P2G8                           | 5%                            | 20%  | 38%   | 75%  |

of the carbohydrate moieties of GP70 is shown in Table 2. The major sugar components of the glycoprotein are glucose, mannose, and galactose with smaller proportions of others. Three other N-acetyl sugars are also present, but have not yet been identified. The actual sequence of the side chains also has not yet been determined. We did find that deglycosylation with TMSF reduced the molecular weight of the glycoprotein to 63 kD as determined by SDS-PAGE (not shown), suggesting that it consists of about 10% carbohydrate.

Figure 1 shows the SDS-PAGE protein and IgE immunoblot patterns of the whole *Alternaria* extracts, purified GP70, and the GP70-depleted preparation remaining from the culture medium. The GP70 lane demonstrates the relative purity of the glycoprotein as it eluted from the F8F11 column. We frequently see two bands at around 70 kD in purified fractions and speculate that this is due to differences in degrees of glycosylation, though the true explanation is unknown. Three other previously described al-

lergens can also be seen at 45, 31, and 18 kD in the whole *Alternaria* extracts and very faintly in the IgE immunoblot of the culture medium.

Figure 2 shows the OD405 of the double monoclonal antibody-based sandwich immunoassay versus reciprocal dilution of the mycelial and culture medium *Alternaria* preparations, purified GP70, and GP70-depleted extract. Since the purified GP70 was determined to have a concentration of 1480  $\mu$ g protein/mL, its observed OD405 at a starting dilution of 1:10 gives a calculated concentration of 148  $\mu$ g/mL in the well and decreases linearly with the log of the dilution ( $r^2 = .99$ ) until it merges with the background signal at a dilution of 1:640. This gives an assay sensitivity of about 200 ng/mL. The calculated GP70 concentration in the whole culture medium extract is 210  $\mu$ g/mL in the initial solution. The mycelial extract had no detectable GP70 activity.

The GP70-depleted sample also gave no signal indicating that the assay is specific for this 70 kD glycoprotein.

Figure 3 shows the OD405 of GP70 captured by a monoclonal antibody and detected by human IgE. The curves are very similar to those seen in the double monoclonal antibody assay. In particular, the *Alternaria* culture medium preparation and GP70 curves are linear and have similar slopes and intercepts. The GP70 standard curve merges into the background at a dilution of 1:300 giving approximately the same assay sensitivity as seen with the double monoclonal antibody assay. Again, the GP70-depleted extract gave no reaction.

The double monoclonal antibody assay was next used to measure GP70 activity in three commercial extracts of *Alternaria*, various fungi, house dust, and mite (Hollister-Stier, Spokane, WA). Dilution curves of extracts containing detectable GP70 are shown in Figure 4. Two of the three commercial *Alternaria* preparations contained no detectable GP70. GP70 activity was detected in extracts of *Rhizopus*, *Cladosporium*, *Ustilago*, and house dust (Table 3). Although the house dust extract tested contained GP70 activity, no measurable GP70 was present in extracts of other allergens

Table 2. Carbohydrate Analysis of Purified GP70 Expressed as Molar Ratio of Detectable Moieties as Measured by HPLC

| Carbohydrate             | Molar Ratio |
|--------------------------|-------------|
| Galactose                | 1.0         |
| Glucose                  | 2.1         |
| Sucrose                  | 0.2         |
| Mannose                  | 1.7         |
| Fructose                 | 0.4         |
| N-Acetyl glucosamine     | 0.4         |
| N-Acetyl neuraminic acid | 0.8         |

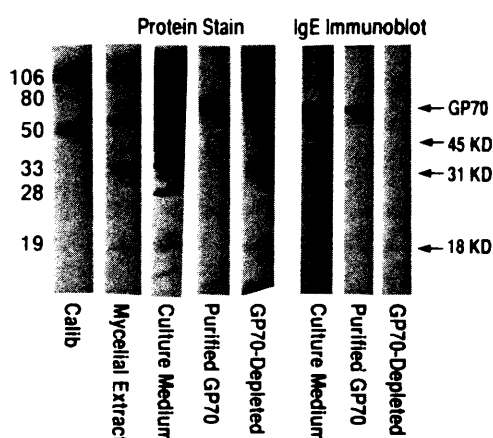


Figure 1. Silver stain and IgE immunoblot of *Alternaria*, purified GP70, and the GP70-depleted preparations used in this study.

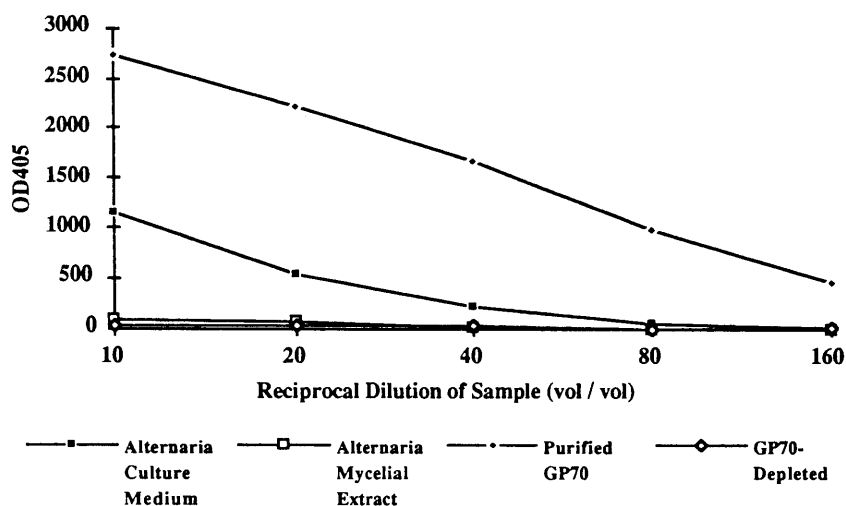


Figure 2. OD405 of the double monoclonal antibody-based sandwich immunoassay versus reciprocal dilution of two whole *Alternaria* preparations, purified GP70, and GP70-depleted extract.

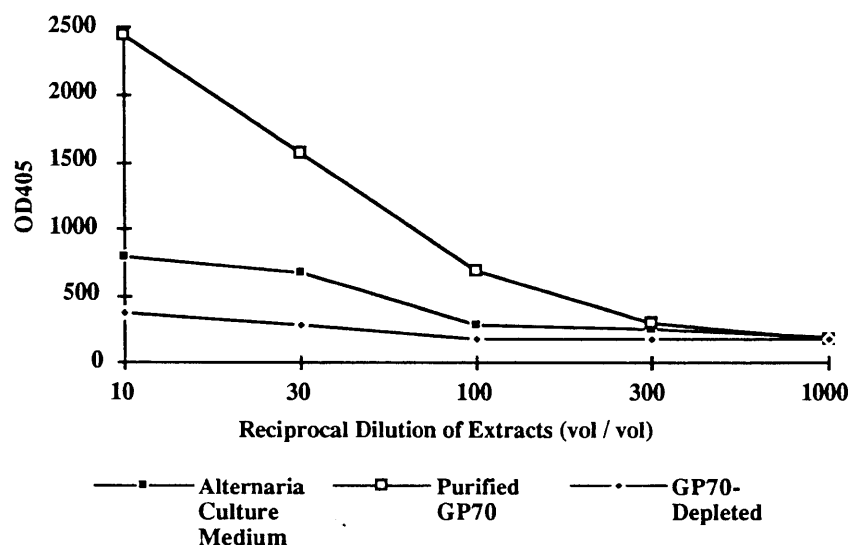


Figure 3. OD405 of reciprocal dilutions of GP70 captured by a monoclonal antibody (P2G8) and detected by human IgE.

(mite, cockroach, cat, mouse) reported to be present in some house dust extracts.

## DISCUSSION

Although most commercial mold extracts are prepared from mycelial mats, the culture medium turned out to be a richer source of GP70 for this strain of *Alternaria*. This is similar to results we have reported

previously.<sup>20,21</sup> We do know that GP70-depleted extracts have residual IgE activity from a previous study.<sup>10</sup> Since the double monoclonal antibody assay did not detect GP70 activity in the depleted extract, we conclude that the assay is specific for this one glycoprotein with respect to allergens. In addition, since human IgE does detect captured GP70, we conclude that

the glycoprotein is an allergen in that it has at least one IgE-recognized epitope.

As mentioned above, the GP70 concentration in whole culture medium extract was approximately 210  $\mu\text{g/mL}$ . Since the culture medium extract had a protein concentration of 1.48  $\text{mg/mL}$ , we conclude that GP70 accounts for 14% of the total protein in this particular solution. This value is virtually identical to the 13% reported by our group previously using a different extract and method for GP70 measurement.<sup>10</sup> Other strains of *Alternaria* grown under different conditions will probably make less GP70 than this because we have optimized our procedures for its production.

Two of the commercial *Alternaria* preparations tested did not contain detectable GP70 and the third contained a concentration near the lower end of the detection range. One explanation for this may be related to the dilution of the extracts. Since it is difficult to determine the dry weight/mL of commercial extracts described as 1:20 (wt/vol), we really do not know the actual concentrations of these preparations. Another explanation may be related to differences in methods used for extract production. Most companies use mycelial based extracts. It is unlikely that such products will include large amounts of GP70 if it is primarily secreted into the culture medium as we suspect.

The high GP70 content in one sample of house dust is interesting but requires further study before any conclusions can be made about this amorphous material. It is possible that our assay detects cross-reacting proteins in house dust that are derived from nonfungal sources or there may indeed be fungi in dust as suggested by one report.<sup>22</sup> Until the GP70 activity is purified from dust and subjected to analysis, its nature will remain unclear.

Standardization of this assay is somewhat arbitrary since we could have expressed concentrations in

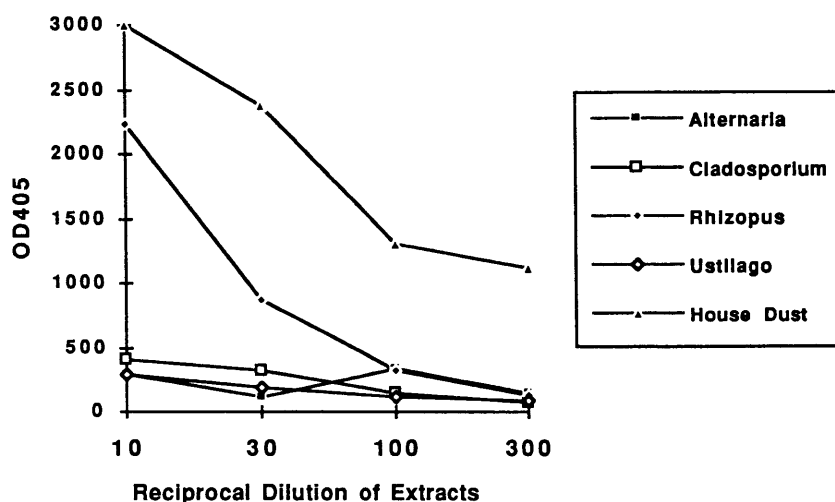


Figure 4. OD405 versus reciprocal dilutions of commercial extracts (1:20 wt/vol) in 50% glycerol measured with the double monoclonal antibody-based GP70 assay. The preparations shown are those with detectable amounts of GP70.

Table 3. GP70 Concentrations in Various Commercial Allergenic Extracts\*

| Antigen                      | GP70, $\mu\text{g/mL}$<br>in 1:20 (wt/vol) | Antigen                 | GP70, $\mu\text{g/mL}$<br>in 1:20 (wt/vol) |
|------------------------------|--------------------------------------------|-------------------------|--------------------------------------------|
| Commercial Alternaria 1      | 236                                        | Aureobasidium           | 14                                         |
| Commercial Alternaria 2      | <14                                        | Rhizopus                | 139                                        |
| Commercial Alternaria 3      | <14                                        | Rodotorula              | <14                                        |
| <i>Aspergillus fumigatus</i> | <14                                        | Ustilago                | 24                                         |
| <i>Aspergillus mix</i> †     | <14                                        | Stemphyllium            | <14                                        |
| <i>Fusarium</i>              | <14                                        | Cat                     | <14                                        |
| <i>Epicoccum</i>             | <14                                        | <i>D. pteronyssinus</i> | <14                                        |
| <i>Penicillium</i> ‡         | <14                                        | <i>D. farinae</i>       | <14                                        |
| <i>Cladosporium</i>          | 72                                         | House dust              | 335                                        |
| <i>Helminthosporium</i>      | <14                                        | Cockroach               | <14                                        |
| <i>Mucor</i>                 | <14                                        | Mouse                   | <14                                        |

\* The three *Alternaria* preparations were obtained from Hollister-Stier (Spokane, WA), Center Labs (Port Washington, NY), and Greer Labs (Lenoir, NC). The others were obtained from Hollister-Stier. These are the concentrations in extracts labelled 1:20 and stored in 50% glycerine.

† Equal volumes of *A. fumigatus*, *terreus*, *niger*, and *nidulans*.

‡ Equal volumes of *P. digitatum*, *expansum*, *glaucum*, *roseum*, and *notatum*.

terms of dry weight or protein/mL by at least two different methods. Other investigators have expressed their standards as  $\mu\text{g}$  dry weight and Lowry protein,<sup>23</sup> inhibitor concentration in mg dry weight and PNU's,<sup>24</sup>  $\mu\text{g}$  dye binding protein/mL,<sup>25</sup> unspecified  $\mu\text{g/mL}$ ,<sup>26</sup> and arbitrary units compared with a reference preparation.<sup>27</sup> This lack of consistency with respect to measurements is an issue that should be addressed by investigators in the field of allergen standardization. At the very least, the method used for

measurement of allergen concentrations should be clearly specified in any study where allergens are measured.

Standardization might be improved with an amino acid analysis of an ultra pure or recombinant protein. This would permit the concentrations of allergens to be expressed in molar equivalents per liter and would reduce potential variability introduced by the source material and by the different molecular weights of allergens. We do not feel that use of carbohydrate for stand-

ardization of fungal allergens is appropriate at the present time because it is not known whether the set of molar ratios described in Table 2 will be the same for GP70 prepared from other strains of *Alternaria*.

Tests for specific allergens as described in this study are having an important impact on the area of allergy diagnosis and immunotherapy. For example, monoclonal antibody-based assays could eventually lead to the development of separate assays for all of the significant allergens in *Alternaria*. This series of tests could then be used for extract standardization. Specific allergen immunoassays may also lead to the study of cross reactivity of allergens between related species of mold. We have begun this process here by identifying GP70 activity in taxonomically related allergy-causing fungi. If the cross-reacting proteins can be purified and shown to also be allergens that are in some way similar to GP70, a family-wide identification system for fungal allergens may be possible. Such a system could potentially extend our basic understanding of mold classification and taxonomy.

Health care workers have begun to establish standards for indoor foreign protein exposure with the aid of assays for specific environmental allergens.<sup>28</sup> As the number of assays for specific allergenic material grows, our understanding of the relationship between exposure to different allergens and development of allergic disease will increase. The feasibility of home testing also should increase. Eventually, this type of research should have a major impact on our understanding the nature of the allergic process.

## CONCLUSIONS

We have described a double monoclonal antibody-based assay for the 70-kD *Alternaria* allergen, GP70, which is both sensitive and specific. Commercial *Alternaria* preparations tend to have little GP70; most

likely because GP70 is secreted into the culture medium and commercial preparations are made from mycelia. Several related fungi contain detectable concentrations of GP70, but the most abundant source of GP70 identified in this study was a sample of house dust. Further measurement of GP70 in biologic materials should help to delineate its distribution in the environment and determine its relative importance in the development of allergic diseases.

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*Request for reprints should be addressed to:*  
 Jay Portnoy, MD  
 Section Allergy/Immunology  
 The Children's Mercy Hospital  
 Kansas City, MO 64108