

IgE and IgG antibody responses to recombinant Alt a 1 as a marker of sensitization to *Alternaria* in asthma and atopic dermatitis

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Summary

Background Sensitization to *Alternaria alternata* is a risk factor for the development of wheezing and asthma. Alt a 1 is the major *Alternaria* allergen causing sensitization in asthmatics. Some atopic dermatitis (AD) patients have very high immunoglobulin (Ig)E antibody (ab) to *Alternaria* as analysed by Pharmacia CAP, however, it is not clear whether these are specific responses or whether Alt a 1 is involved in disease symptoms.

Objective The aim of this study was to analyse specific IgE and IgG ab responses to recombinant Alt a 1 in asthmatic and AD patients and to compare these results to IgE ab against *Alternaria* measured by CAP.

Methods Sera from individuals who were IgE positive to *Alternaria* by CAP were obtained from 58 patients with asthma/rhinitis, 19 patients with AD, and 20 patients with cystic fibrosis (CF) who were included as specificity controls. IgE and IgG ab to recombinant Alt a 1 were measured by radioimmunoassay (RIA).

Results Of 43 asthma/rhinitis patients having an *Alternaria* CAP score > 2, a high percentage (93%) had both IgE and IgG ab to Alt a 1, emphasizing its importance as a major allergen. Only, 47% of AD patients with CAP score greater than 2 had ab to Alt a 1, and their levels were low when compared to the asthmatics. For CF controls, 75% of these patients had no IgE ab to Alt a 1, and those which were positive to Alt a 1 by RIA were also positive by CAP. Overall, patients with a low CAP (1–2) had a low prevalence (20–30%) of IgE or IgG ab to Alt a 1.

Conclusion IgE and IgG ab to Alt a 1 in asthmatics are good markers for sensitization to *Alternaria*. Although AD patients gave high *Alternaria* CAP scores, they had low or undetectable levels of IgE to Alt a 1, suggesting that other *Alternaria* allergens may be important in AD or that the CAP results are non-specific. Recombinant allergens may provide more specific measures of sensitization to fungi.

Keywords: *Alternaria*, atopic dermatitis, antibody response, asthma

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Introduction

Sensitization to *Alternaria* allergens is an independent risk factor for the development of wheezing and asthma in

children and young adults and has been associated with severe cases of asthma and respiratory arrest [1,2]. Previous studies have shown Alt a 1 to be the major *Alternaria* allergen with greater than 80% of sensitized individuals having Immunoglobulin E antibody (ab) against Alt a 1 [3–5]. Although a high proportion of patients with atopic

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dermatitis (AD) have elevated serum IgE ab against *Alternaria*, the strongest association with AD is sensitization to the skin colonizing fungus *Malassezia furfur* (formerly *Pityrosporum orbiculare*) [6]. The clinical relevance of high specific IgE to *Alternaria* in patients with AD is not clear [7,8].

The Pharmacia CAP system was recently reported to be useful for assessing sensitization to *Alternaria* in asthma and AD patients [7,8]. Some AD patients appear to give non-specific results in the CAP system which makes it difficult to assess whether or not they are truly sensitized to *Alternaria* [8]. The objective of this study was to use recombinant (r)Alt a 1 to assess IgE and IgG ab responses to *Alternaria* and compare the results for IgE ab with those obtained using the CAP system.

Methods

Serum samples

Serum was obtained from a panel of patients who were IgE positive to *Alternaria* by the Pharmacia CAP System (score 1–6) (Pharmacia Biotech, Piscataway, NJ, USA). The groups consisted of 19 AD patients from the University of Virginia Allergy Clinic, and 58 asthma or rhinitis individuals from studies conducted in Atlanta, Charlottesville and Boston [7,8]. Serum from 20 Cystic Fibrosis (CF) patients, obtained from Dr Bob Selden of the University of Virginia, having high IgG ab to Asp f 1, a major allergen from the fungus *Aspergillus fumigatus*, were used as controls.

IgG and IgE ab measurements

Human antibody binding radioimmunoassays (RIAs) were performed on all sera using rAlt a 1 (BIOMAY, Vienna, Austria) labelled with ^{125}I by chloramine-T (20 μg rAlt a 1 was labelled with 0.5 mCi ^{125}I). For IgE ab assays, serum was diluted 1/2 and 1/10 in PBS-Tween containing 1% bovine serum albumin (BSA), followed by the addition of 100 μL human IgE myeloma carrier serum (diluted 1/200) and 110 000 cpm/100 μL ^{125}I rAlt a 1. Samples were incubated for 4 h at room temperature (25°C) before precipitating antigen/antibody complexes overnight at 4 °C with 50 μL sheep anti-human IgE (The Binding Site; Birmingham, UK). Precipitates were washed three times in borate buffered saline and counted in a gamma counter. Human IgG ab assays used 1/12.5 and 1/50 dilutions of serum and were precipitated with 100 μL of a 1/4 dilution of goat anti-human IgG/Fc (HTI Bio-Products Inc; Ramona, CA, USA). The assay backgrounds for both RIAs were based on counts for three non-allergic control sera which were included in all assays.

Statistical analysis

Antibody binding to rAlt a 1 in different patient groups having *Alternaria* CAP scores either greater than or less than 2 was analysed by Pearson Chi square.

Results

Previous studies in our laboratory found that natural and recombinant Alt a 1 gave parallel binding curves in ELISA [9]. Therefore, the recombinant allergen was used to analyse allergen-specific IgE and IgG ab responses among patients with asthma/rhinitis or AD, and these results were compared with IgE ab to *Alternaria* antigens measured using the CAP system (Fig. 1). Most asthma patients (13/15, 87%) with a weak CAP score (class 1–2) had undetectable or low levels of IgE and IgG ab to rAlt a 1. Almost all asthma patients with a moderate CAP score (class 3–4) had detectable IgE and IgG ab to rAlt a 1 (40/43, 93%) and these patients had the highest levels of Alt a 1-specific ab (Fig. 1).

In contrast, 62% (8/13) of AD patients with a CAP class 3–4 to *Alternaria* had no detectable ab to rAlt a 1. Four AD patients showing the highest CAP score to *Alternaria* (class 5–6) had low or undetectable IgE and IgG ab to rAlt a 1. Overall, AD patients had weak responses to Alt a 1 and 10/19 AD sera (53%) which were IgE positive by CAP had no IgE to rAlt a 1 in the binding assay. Those CF patients who had IgE ab to Alt a 1 were also positive to *Alternaria* by CAP, indicating that ab responses in the binding assay were specific for Alt a 1. Two of the CF patients had a CAP score of 3–4 and had no IgE ab to Alt a 1.

The ab data is summarized in Table 1. Among patients with a CAP score greater than 2 (> 3.5 IU IgE/mL), 93% of asthma/rhinitis individuals had both IgE and IgG ab to rAlt a 1. In contrast, AD patients were significantly different with only 47% of individuals having either IgE or IgG ab against rAlt a 1 ($P < 0.001$). Sera from CF patients with *Alternaria* CAP > 2 , were similar to AD patients in that only 60% had IgE ab to rAlt a 1 (significantly different than asthmatics, $P = 0.024$), however, 80% had IgG ab. As a whole, patients with a CAP score of 1–2 (< 3.5 IU/mL) had a very low prevalence of both IgE and IgG ab to rAlt a 1 which were significantly less when compared to the asthma/rhinitis patients with CAP score > 2 ($P < 0.001$).

Discussion

The results indicate that IgE ab to rAlt a 1 are a good marker for sensitization to *Alternaria* in patients with asthma and rhinitis. Greater than 90% of patients in this group with a CAP score above 2 had IgE ab to rAlt a 1,

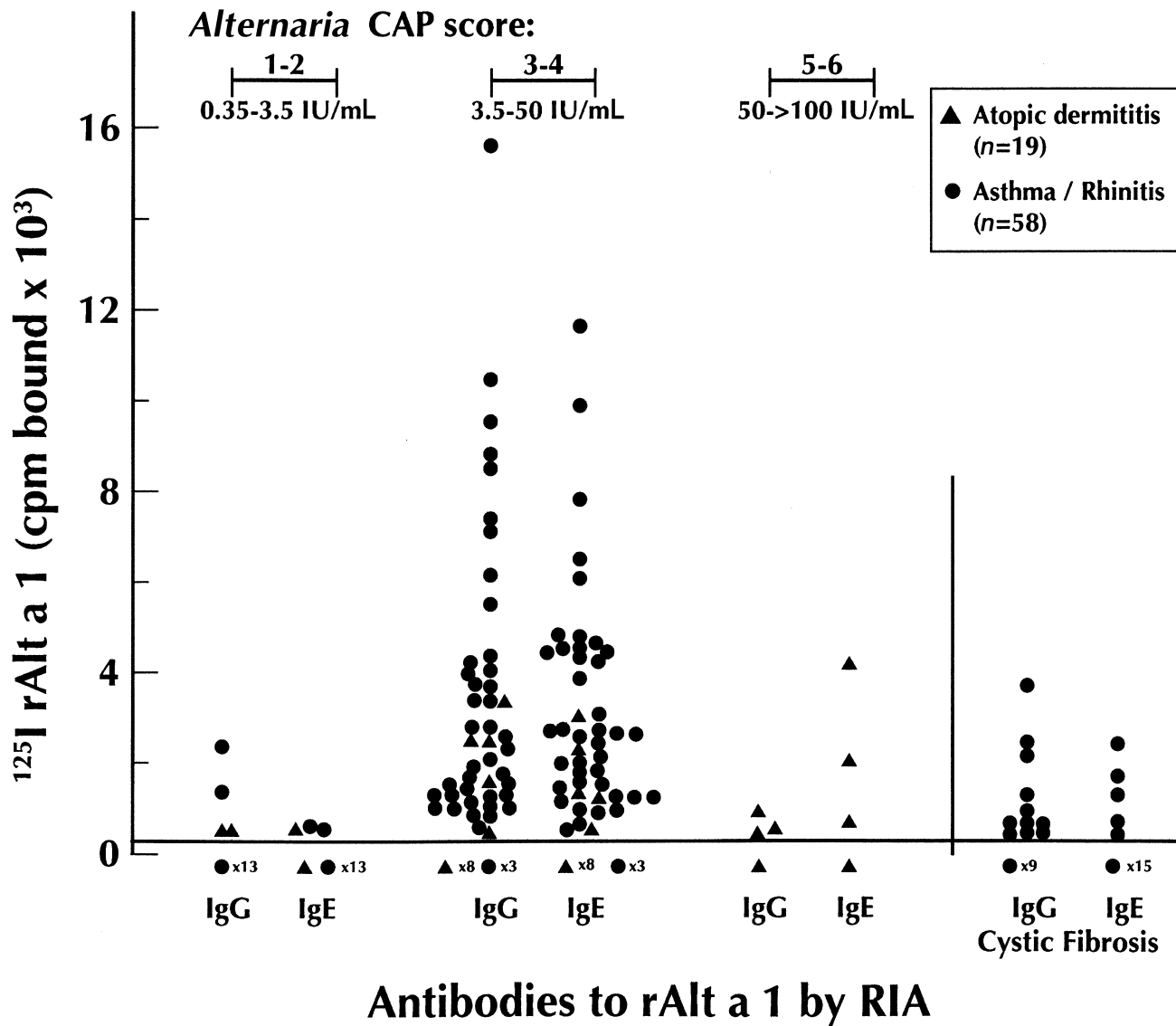


Fig. 1. IgG and IgE antibody responses to recombinant Alt a 1. Antibodies to rAlt a 1 were measured by antigen binding assay and patient results were classified by *Alternaria* CAP score. Values above 200 cpm were considered positive (> 2 SD above background of three non-allergic control sera). Cystic fibrosis sera having IgE ab to rAlt a 1 were CAP class 2–3.

further supporting previous studies that Alt a 1 is the major allergen recognized by individuals sensitized to *Alternaria* [4,5]. Patients showed strong IgE and IgG binding to the recombinant allergen, with low background and high specificity, as indicated by the results for CF patients. These individuals were included as specificity controls and were selected because they had high IgG to another common fungal allergen, Asp f 1 (156–16 250 units/mL anti-Asp f 1 IgG). Indeed, 75% of these patients had no detectable IgE against Alt a 1 in the binding assay, and those CF sera which did have IgE binding to rAlt a 1 also had IgE ab to *Alternaria* by CAP.

Results of sensitivity to *Alternaria* for AD patients are not as clear. Although 90% of the patients selected had CAP scores greater than 2, only 47% had IgE or IgG ab to rAlt a 1. By classification standards, Alt a 1 would still be considered an important allergen for AD patients, which is in contrast to results reported for the major *Aspergillus* allergen, Asp f 1, against which AD patients were reported not to have detectable IgE ab [10]. The prevalence and magnitude of IgE responses to Alt a 1 among AD patients were much lower than those for the asthma/rhinitis group. These results may have several explanations including that a different *Alternaria* allergen is important in AD; that the

Table 1. Prevalence of antibodies to *Alternaria* allergens in different patient groups

Patient Group	<i>Alternaria</i> CAP Score > 2	rAlt 1 a bound† IgG	IgE
Asthma/Rhinitis	43/58 (74%)	40/43 (93%)	40/43 (93%)
Atopic Dermatitis	17/19 (90%)	8/17 (47%)**	8/17 (47%)**
Cystic Fibrosis	5/20 (25%)	4/5 (80%)*	3/5 (60%)*
All Groups	CAP-1–2	7/22 (32%)**	5/22 (23%)**

†> 2 SD above background of three non-allergic control sera in RIA.

* $P = 0.024$, ** < 0.001 ; when compared to asthma/rhinitis group.

high total IgE associated with AD binds somewhat non-specifically to the CAP; that the IgE ab is of low affinity; or that AD sera recognize a cross-reactive fungal allergen on the CAP. Scalabrin *et al.* found that by CAP analysis, AD patients had IgE ab to a number of fungi, and there was significant correlation between specific IgE to these fungi which may suggest the presence of a cross-reacting allergen [7]. In fact, enolase has been found to be a conserved allergen among some fungi [11]. Those CF patients who had positive CAP score with no IgE ab to Alt a 1 may also be recognizing a cross-reactive allergen. Another possibility, is that for some AD patients, the IgE cross-reactivity may be non-specific against glycoproteins or other contaminating proteins found in fungal extracts [8,12]. There was a slightly significant correlation between total IgE and *Alternaria* CAP score among AD patients ($R = 0.51$, $P = 0.03$; total IgE range 716–67 507) which was not present for the asthma/rhinitis group ($R = 0.01$, $P = 0.9$; total IgE range 3–3000). Neither group showed a significant correlation between total IgE and Alt a 1 specific IgE (data not shown). The weak correlation between total IgE and CAP score may suggest non-specific binding to the CAP among some AD patients having very high IgE levels.

Overall low prevalence of IgE binding to rAlt a 1 among asthma patients having a CAP score of 1–2, suggests that these patients are weakly allergic to *Alternaria*. However, the fact that IgE and IgG ab to rAlt a 1 were distinguished by RIA in some patients from this group may indicate that the recombinant allergen is a more sensitive *in vitro* assay. Given the problems associated with making fungal extracts (interstrain differences, variable allergen content, and variations due to extraction procedures), the use of recombinant allergens as markers for assessing sensitivity is particularly useful [13,14]. Our data supports that of others, suggesting that by including one or two additional recombinant *Alternaria* allergens in a cocktail with rAlt a 1,

it might be possible to diagnose >95% of individuals sensitized to *Alternaria* in IgE ab assays [15,16]. Recombinant allergens would allow more accurate skin testing using defined concentrations of allergen and would aid in determining the clinical relevance of *Alternaria* allergens in asthma and atopic dermatitis.

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