

Clinical Testing of Recombinant Allergens of the Mold *Alternaria alternata*

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Key Words

Alternaria alternata · Allergen, recombinant · Nonfusion protein · Skin test

Allergens from fungal mycelia and spores are less well known than allergens from pollen, foodstuffs, insects and animal dander. Depending on geographical and climate conditions the incidence of allergy to a single mold like *Alternaria alternata*, *Cladosporium herbarum* and *Aspergillus fumigatus* may be as low as 1% of all allergic patients. But, for instance, in the south of the USA, allergies to all molds together may be as high as 30% [1].

Skin testing with commercial mold extracts sometimes shows poor specificity and sensitivity. Specific immunotherapy with mold extracts was often infrequently used because it leads to relatively severe side effects. To improve this situation, recombinant mold allergens might be very useful. To our knowledge, the study presented here is the first study of skin testing with recombinant *A. alternata* allergens.

We prepared a cDNA expression library [2, 3] in the lambda vector Uni-ZAP XR. A complete cDNA clone coding for Alt a 1, the major allergen of *A. alternata*, was obtained from this library by means of an oligonucleotide derived from an N-terminal amino acid sequence of Alt a 5 [4, 5]. A comparison of the nucleotide sequence with the N-terminal

sequence of the mature natural protein showed that Alt a 1 contains a classical N-terminal ER targeting sequence and that the mature protein starts at Asp 26. Testing of the IgE reactivity of the recombinant monomeric and dimeric form (under reducing and nonreducing conditions, respectively) revealed that Alt a 1 was recognized by 80% of all *A. alternata* allergic patients [5].

Enolase (Alt a 2) [3] is the second most important allergen of *A. alternata*. It is a well-known cytoplasmic enzyme active in glycolysis. A cDNA coding for enolase was cloned by screening the *A. alternata* library with a *C. herbarum* enolase DNA probe. The recombinant enzyme was expressed as a nonfusion protein in *Escherichia coli* and was recognized in immunoblots of 22% of our patients allergic to *A. alternata*. The recombinant protein showed the expected enzymatic activity. Enolase is an important allergen in many allergenic fungal species studied so far [3].

YCP4 is a protein of unknown function that is cytoplasmic and highly conserved in fungal species. A cDNA clone coding for YCP4 was isolated from our library by immunological screening with patients' sera. In IgE immunoblots about 20% of the *A. alternata*-allergic patients recognized this recombinant protein.

We present here the production and purification to homogeneity of the three *A. alternata* proteins. The proteins were used for skin prick testing and intradermal testing of 10 controls (5 non-allergics and 5 allergics that were not sensitized to *A. alternata*) and 7 patients (selected according to a positive coated allergen particle (CAP) test and a clinical

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cal history of late summer rhinoconjunctivitis and/or asthma). Skin prick testing and intradermal testing were performed according to standard clinical protocols. Recombinant allergen concentrations for the prick tests were between 0.1 and 100 µg/ml and for intradermal tests between 1 ng and 1 µg/ml. Histamine and saline were used as a positive and negative control, respectively. The recombinant proteins could correctly diagnose all 7 patients. In detail 6/7 patients showed positive skin reactions to recombinant Alt a 1, 2/7 patients showed positive skin reactions to enolase and no patient reacted with YCP4. We conclude that probably by using just two *A. alternata* recombinant allergens (Alt a 1, and enolase), a reliable diagnostic test can be performed. None of the controls showed unspecific reactivity towards the recombinant proteins although the recombinant proteins strongly reacted with patients' sera in IgE immunoblots.

In contrast to the above results, commercially available *A. alternata* extracts (ALK-Abello-Spain, ALK, Horsholm, Denmark and Stallergen, Fresnes, France) in 2/10 cases failed to correctly diagnose the patients. False-positive results were obtained with the control individuals in 2/10 cas-

es. Only the extract manufactured by ALK-Abello was tested in this case. All extracts were used according to the manufacturers' recommendations.

These data show that presently available commercial extracts of *A. alternata* are less than perfect for diagnostic purposes, which might be due to strain variabilities and the phenomenon of batch-to-batch variations. Further, among the many unknown substances in such extracts some might interfere with the positive in vivo reaction or the concentration of a specific allergen in the extract might be too low in some lots of these extracts.

As the results presented above clearly show, recombinant *A. alternata* allergens (Alt a 1 and enolase) are superior to commercial raw extracts of this mold both with respect to positive and negative predictability in *Alternaria* patients and in controls. It is now suggested that these pure recombinant allergens are also used for therapeutic purposes.

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