

EDITORIAL

Clinical & Experimental Allergy

The problem of cross-reactivity in the diagnosis of fungal allergy

This editorial discusses the findings of the paper in this issue by Postigo et al. [7] pp. 443–451.

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Type I fungal allergy, although recognized as a major health risk for humans [1], should still be considered an unsolved problem due to the limited power of the diagnostic tools available [2]. The extent of the problem becomes particularly evident if we consider that crude estimates about the incidence of fungal allergy range from 6% to 24% in the general population increasing to about 44% and 80% among atopic individuals and asthmatic patients, respectively [3]. The reasons for a lack of reliable diagnostic tools for fungal allergic diseases are manifold. The most important ones are (i) the lack of validated, standardized extracts recognized by the US Food and Drug Administration, (ii) the large number of different fungal species able to induce allergic reactions, (iii) the time- and culture condition-dependent production of allergenic molecules, (iv) the complex number of different allergen structures produced by fungi and (v) the presence of phylogenetically conserved highly cross-reactive allergens. To complicate the picture even more, a high proportion of patients allergic to fungi are sensitized to more than one fungal species and might also be sensitized to other environmental allergen sources like pollens, mites or even food allergens [4]. Although a major problem related to the diagnosis of fungal allergy in daily clinical practice is related to the lack of standardized extracts, as even commercially available products show high variations in the allergen content [5], another equally important problem is the presence of cross-reactive allergens throughout the fungal kingdom [6]. Therefore, the question of whether recombinant allergens can improve the diagnosis of fungal allergy, although not new, is still of

pivotal importance. In this issue, Postigo et al. [7] present a study that investigates the *in vitro* diagnostic value of the two recombinant allergens Alt a 1 and Alt a 6 (enolase) from *Alternaria alternata* for the diagnosis of sensitization to Pleosporaceae. Not surprisingly, the authors conclude that these two allergens are not sufficient for a highly sensitive diagnosis of *A. alternata* allergy. Although there is no doubt that Alt a 1 constitutes a major allergen of this fungus, whereas Alt a 6 is clearly a minor allergen, it is also known for many years that *A. alternata* has the capability to produce a whole array of allergens [8]. At present, 10 *A. alternata* allergens are registered in the official list of allergens administrated by the IUIS Allergen Nomenclature Subcommittee (<http://www.allergen.org>). Obviously, because the immune system can only mount an immune response against allergens to which the atopic individual is exposed, and because it cannot be expected that every patient will be exposed to all allergens, the recognition patterns of allergens resulting from exposure to complex allergenic sources always be patient specific [9, 10]. In general, it is widely recognized that the diagnosis of allergic diseases based on recombinant allergens is superior to extract-based diagnosis in terms of specificity both, *in vitro* [11] and *in vivo* [12]. The technological achievements in producing recombinant proteins allow the reconstitution of the allergenicity of extracts by mixing up panels of single, perfectly standardized recombinant allergens. However, the big challenge in the development of diagnostic and therapeutic tools based on recombinant allergens will be to estimate the size of the repertoires needed to substitute allergen extracts, and to clarify the role of cross-reactive structures in order to reduce the number of allergens required to achieve a universal diagnosis of allergy. As clearly shown by Postigo et al. [7] by inhibition experiments using an *A. alternata* extract coated to solid phase, IgE binding of serum from patients sensitized to this mould can be efficiently inhibited not only by extracts from other Pleosporaceae (*Curvularia lunata*, *Stemphylium botryosum*, *Ulocladium botrytis*) but also to

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70% and 55%, respectively, with extracts of fungi belonging to other orders like *Cladosporium herbarum* (Capnodiales) or *Aspergillus fumigatus* (Eurotiales). Because *C. herbarum* and *A. fumigatus* do not produce allergens cross-reacting with Alt a 1 as demonstrated by the lack of inhibition of serum IgE binding to solid-phase coated Alt a 1 with extracts of these two fungi [7], the inhibition must result from other allergen structures sharing common epitopes produced by the fungal species investigated. It is not surprising at all that the authors identified manganese-dependent superoxide dismutase (MnSOD) as a so far unidentified allergen produced by *A. alternata* [7]. MnSOD [13] is, together with cytophylin, thioredoxin and P₂ acidic ribosomal protein, one of the best-characterized cross-reactive fungal structures and, interestingly, all these phylogenetically conserved allergen structures are also involved in IgE-mediated autoreactivity to the corresponding human proteins [14, 15]. MnSOD, first described as an allergen produced by *A. fumigatus* [13], is not the only cross-reactive allergen produced by *A. alternata* and other fungi [3] and therefore allergen cross-reactivity represents an even greater problem than thought previously, and not only in fungal allergy [16]. Bioinformatics analyses based on structural motifs and BLAST similarity search methods involving 101 602 and 135 850 protein entries deposited in the Swissprot database predicted 4093 and 4768 different potential allergen structures, respectively [17, 18]. Therefore, one can assume that the size of the allergen repertoire involved in eliciting allergic symptoms is in the range of 5000 different structures. For a universal diagnosis of fungal allergy, however, the number of allergen structures required will be much lower. At present, 101 fungal allergens, 22 from Basidiomycota and 79 from Ascomycota are listed by the official allergen nomenclature list (<http://www.allergen.org>), but 48 of these allergens represent highly homologous proteins. Because homologous proteins sharing more than 50% sequence identity can be expected to cross-react [19], the maximal number of unique fungal allergen structures currently available for a component-resolved diagnosis of fungal allergy is about 50 and might not be sufficient for a highly sensitive diagnosis of fungal allergy. To achieve this goal, it will be necessary to identify and produce all species-specific fungal allergens only found in particular genera, and

to identify orthologous allergens widespread throughout the fungal kingdom. The fast progress in genome sequencing of a variety of fungi provides the opportunity to assess the occurrence of allergen orthologues across the fungal kingdom for the rapid identification of potentially cross-reactive structures [20]. Although the production of allergen chips covering a large panel of fungal allergens would not be a major problem [10, 21], the results obtained by screening such a chip with serum IgE of sensitized patients might be difficult to interpret in terms of determining the primary fungal species causing the allergy. It has to be noted, however, that the most important allergenic fungi like *A. alternata*, *C. herbarum*, *Malassezia sympodialis* or *A. fumigatus* express major allergens that are specific to species or at least genus [20]. Such marker allergens provide a species- or genus-specific diagnostic tool and are already used in some cases [22]. These marker allergens allow a highly specific diagnosis of fungal allergy but, although representing major allergens of the single species or genera, do not reach the sensitivity obtained with fungal extracts because they are only recognized by 60–90% of the atopics sensitized to the corresponding fungus. Here, a microarray containing all major species- or genera-specific fungal allergens complemented with the known cross-reactive structures might allow reaching or even surpassing the *in vitro* diagnostic specificity obtained with fungal extracts. We can assume that recombinant allergens will contribute to a better diagnosis of fungal allergy in the near future. However, it is questionable whether they can contribute to an improvement of allergen-specific immunotherapy of fungal diseases, which so far is not established [23] due to the large number of sensitizing allergens produced by fungi and to the high costs related to the development of allergy vaccines under the current regulations [24].

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