Alt a 1. New approach for the diagnosis and treatment of allergy to Alternaria alternata

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More than 3 billion people worldwide are infected with parasitic worms called helminthes or suffer allergic disorders such as asthma, allergic rhinitis, food allergies and eczema. A common feature of these infectious or inflammatory conditions is the called allergic or "type 2" immune response. Type 2 immune responses are induced by and confer protection against helminthes, but can also play pathologic roles, promoting acute and chronic inflammatory responses against a miryad of allergens[1]

Allergens derived from fungi, especially Alternaria alternata, Cladosporium herbarum, Aspergillus spp, and Penicillium spp, are well-known causes of type I allergy and depending on geographic and climate conditions[2]*. A recent study showed that 19% of the allergic population reacted to at least one fungal extract, as determined by means of skin tests, and more than 66% of these fungal sensitized patients reacted to A. alternata extract*[3]

Alternaria is considered one of the most aeroallergens in Europe. Moreover, Alternaria sensitization was an independent risk factor associated with the development of wheezing and asthma in children and young adults, and skin test sensitivity to Alternaria was significantly associated with increased bronchial responsiveness[2]*. One of the major complications for allergen-specific diagnostic and immunotherapy (SIT) with fungal extract is the diversity and variability of the strain used, the diverse culture conditions and the extraction processes, with the subsequent difference in composition and allergenic potency of commercial available extracts*[4]*.*

Until recently available extracts of allergenic molds were not reliable tools for diagnostic purposes due to the difficulty of identifying related species based on morphological criteria, genetic variation and strain variabilities, the lack of consensus on production and quality control procedures, and the phenomenon of batch to-batch variation[5-8]*. The intraspecific variability of A. alternata allergenic extracts was recognized for a number of years and strain variability has also been extensively described for this species in terms of allergologic or immunologic and biochemical data* [9-14]*, as well as the yield of production processes. However, less data was available on the intraspecific variability of individual allergens from A. alternata.*[15,16]

Rosenthal suggested that variability in allergen content is the result of posttranslational events. Alt a 1 is continuously released into the medium of A. alternata cultures, where it accumulates, and culture filtrates constitute the optimum source of raw material for the production of allergenic extracts[16]*.*

A goal of standardization is to ensure the lot-to-lot consistency of allergen products. Strict adherence to established manufacturing and quality controls for monitoring consistency can reduce product variability. The establishment of a well characterized reference preparation with a validated potency assay could facilitate allergen standardization efforts[17]*.*

Currently, the quality of commercial fungal extracts in Europe is variable. Thus purified mold allergens could be of great interest because purified allergens can be produced in suitable purity and batch consistency and hence are a perfectly standardized diagnostic material. Diagnostic studies with allergenic molecule–based approaches for skin testing have been performed. The use of purified proteins for in vitro assays has been more widely used because some pollen allergens are available as commercial reagents, allowing a component-resolved

diagnosis approach for some of them or evaluation immunotherapy by means of determination of specific antibodies toward purified allergens. 11 allergens have been described in A. alternata extracts so far, although only 1 of them is the more prevalence allergen.

Alt a 1 is the major allergen, reacting with the serum IgE of more than 90 % of A. alternata–sensitized patients. Alt a 1 is a heat-stable dimer of 28 kDa, which dissociates into 14.5 kDa and 16 kDa subunits under reducing conditions. Alt a 2 is recognized by IgE antibodies of 16 (61%) of 26 individuals allergic to A. alternata. Minor A. alternata allergens, such as Alt a 11 Alt a 3, Alt a 4, Alt a 6 (the highly conserved fungal allergen enolase), Alt a 7, Alt a 10, Alt a 12, and nuclear transport factor 2, have been also reported[3].

Therefore, diagnostic and immunotherapy with Alt a 1 alone may well suffice to detect a putative sensitization and improve manifestations of sensitization to the entire allergen composition of A. alternata.

The mechanism of action of SIT is not definitively established, but it may consequence of treatment-induced changes of the immunological mechanism (figure 1). Our group determined the safety of SIT with purified natural Alt a 1 and to evaluate its effects on the immunological response

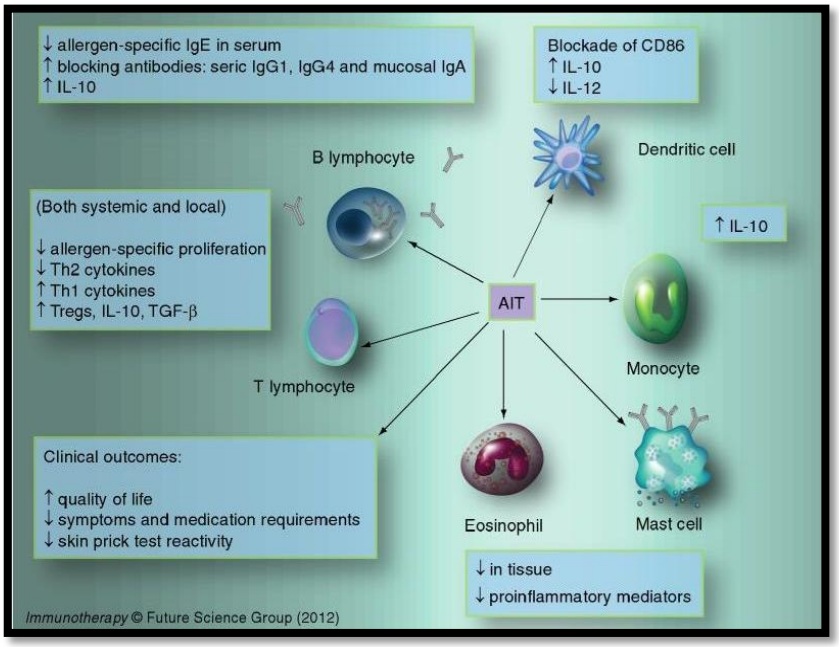

Figure 1. Mechanism of action of SIT. WHO Position Paper: Allergy 1998; 44(53): 5-42

For this purpose[4]*, male and no pregnant female subjects among 9-60 years with allergic rhinitis, with or without mild/moderate asthma, skin sensitization to A. alternate and Alt a 1 (10 µg/mL) and specific IgE levels up to class 2 were recruited from the allergy service of Hospital Dr Peset of Valencia, Spain.*

All asthmatic subjects were well-controlled for at least 3 months by treatment with inhaled β2 agonist on demand or with a daily dose of beclomethasone dipropionate ≤ 1000 µg or equivalent. All patients were nonsmokers and none had history of chronic bronquitis, emphysema, or respiratory tract infection during 4 weeks before the study.

The study was a single-center, randomized, double blind, placebo controlled, parallel-group study. Patients were randomized to receive either active treatment consisting of increasing doses of purified natural Alt a 1given subcutaneously or placebo.

Specific IgG4 levels to rAlt a 1 were evaluated according instructions of ImmunoCAP Specific IgG4 (Phadia Ab, Uppsala, Sweden)

Alternaria alternata extract were produced from raw material containing spores and mycelia from a specific strain. Alt a 1 was purified from A. alternata extract by three chromatographic steps and a HPLC MS/MS analysis was performed to verify the presence of the purified Alt a 1.

SIT was administered with a cluster schedule that made it possible to reach the maintenance dose in 4 weeks. Maintenance injections were administered every 4 weeks for 1 year.

Forty-two patients were enrolled, and 40 were assigned randomized treatment sets and included in the safety evaluation, but finally 35 finished the study (17 in the actively treated group and 18 in the placebo group. Baseline characteristics were comparable for the two treatments groups. Active treatment induced strong IgG4 responses against the Alt a 1 allergen. IgG4 concentrations increased approximately 17-fold after 6 months of treatment and 23-fold after 12 months of treatment. Comparison between the groups showed statistically significant different at all-time points after the initiate of treatment.

SIT with Alt a 1 was well tolerated, with no life-threatening reactions. The overall incidence of adverse events was comparable between the treatment groups. There were 33 local adverse events, 17 and 16 in the active and placebo group, respectively. Thirty-one adverse events were classified as systemic, with 15 and 16 in the active and placebo group, respectively. Most were episodes of rhinoconjuntivitis, asthma exacerbation or common cold. All these systemic adverse events were considered for the clinical investigator as not related with the study treatment.

It has been hypothesized that SIT results is a deviation in the T lymphocyte response and a modified TH2 response. An increase in T-regulatory cells contributes to this process, and their production of IL-10 and TGF-β favor the suppression of IgE production and the increase in IgG4 antibodies. Additionally, it has been suggested that allergen-specific IgG4 antibodies have the potential to reduce early responses to allergen by blocking Fcε-dependent mast cell activation and release of performed mediators. The results of our study clearly demonstrate that SIT with purified Alt a 1 is associated with a highly significant increase in allergen-specific IgG4 level.

This is the first clinical study of immunotherapy using a purified natural Alt a 1 for the treatment of allergic rhinitis with or without asthma demonstrated the good tolerance, with the induction of strong allergen-specific IgG4 antibody responses[4].

Other challenges that we are leading is the ortologic feature of Alt a 1.

There is a general consensus regarding the scarce cross-reactivity existing between Alternaria alternata and others allergenic moulds suchs as Aspegillus fumigatus, Penicillium notatumor Cladosporium notatum . However Alternaria alernata has been shown a very significant level os allergenic cross-reactivity with other fungi belonging to the Pleosporaceae family. To date, we are studying the contribution of major allergen Alt a 1 to the cross-reactivity shown in these molds, specially of Ulocladium botrytis, Stemphylium botryosum, Curvularia lunata and Epicoccum nigrum.

Our most advance studies has been performed with Ulocladium Botrytis in order to characterize its reactivity in a sensitized population, mainly focusing in the ortologic feature of the major allergen Ulo b 1, cross-reacting with Alt a 1.

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