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ORIGINAL ARTICLE

Molecular-based diagnosis of respiratory allergic diseases in children from Curitiba, a city in Southern Brazil

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Abstract
Background: Prevalence of respiratory allergic diseases has increased worldwide. Identification of the aeroallergens involved in allergenic sensitisation is important for diagnosis, treatment and prevention.
Objective: To verify the molecular pattern of sensitisation to aeroallergens in patients with allergic respiratory diseases using microarray technique for specific IgE antibody detection.
Methods: Cross-sectional study of 101 children with allergic rhinitis was followed in an outpatient clinic. All patients had positive skin prick tests (SPT) to at least one of the following antigens: *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Blattella germanica*, *Lolium multiflorum*, and dog and cat epithelium. Serum specific IgE antibodies (sIgE) to mites, animal epithelia, fungi, cockroach and pollens components were determined by ImmunoCAP ISAC.
Results: sIgE to group 1 and 2 mite allergens showed higher positive rates: Der p 1 (74.2%), Der p 2 (73.3%), Der f 1 (74.2%), Der f 2 (72.3%). sIgE to animal epithelia were less frequent, Can f 1, Can f 2, Can f 3 in 4.9%, 2.9%, 1.9% respectively and Fel d 1, Fel d 2, Fel d 4 in 16.8%, 0.9% and 1.9%. respectively. Sensitisation to fungi and cockroach were rare, except for Bla g 7, to which 16.8% were positive. There was no significant recognition for tree pollens group. For grass, sIgE were detected to Cyn d 1 in 16.8%, Phl p 1 and Phl p 4 in 14.8% and 12.9%, respectively.
Conclusion: Knowing that the pattern of allergic sensitisation varies according to environment and population, our results reinforce the need for local studies, using molecular-based diagnosis.

Introduction

Epidemiological studies have documented an increase in the prevalence of respiratory allergic diseases in different areas of the world.[1–3] In Brazil, the prevalence of asthma

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and allergic rhinitis has similarly increased in recent years.[4] Identification of the sensitisation profile to aeroallergens is essential for the diagnosis, treatment and prevention of these diseases.[5]

Among the methods available to define allergic sensitisation are skin prick tests (SPT), which detect in vivo the presence of sIgE antibodies and represent the most used diagnostic tool in the field of allergy.[6] They have high sensitivity and specificity, in addition to results that correlate well with in vitro IgE antibody determinations.[7,8]

Allergic sensitisation can also be verified by sIgE measurement in serum. Advances in technology have provided new laboratory methods, in which the allergen to be tested is covalently linked to a solid phase. This confers specificity to the assay. In vitro tests offer numerous advantages such as precise quantification, lack of drug interference, safety and long-term storage of specimens.[7]

The allergenic extracts normally used in vivo and in vitro diagnosis come from defined sources and different extraction procedures. During this process some relevant allergenic proteins can be lost, whereas others can contaminate the extract as they were present in the starting raw material.[5] Although efforts are made to control allergenic extract composition, they are still a mixture of allergenic and non-allergenic compounds.[9]

Recently, new biochemical methods allowed identification of allergenic molecules, the actual primary sensitising agents and triggers of symptoms. Their characterisation has been improved with molecular biology techniques.[9] The antigens used for sIgE determination in microarray system (ISAC® – Immuno Solid-phase Allergen Chip, Thermo Fisher, Uppsala, Sweden) are highly purified molecules, obtained by recombinant DNA methods or natural sources. Allergenic molecules facilitate standardisation and increase the specificity of the test.[5] In contrast to other conventional methods, allergen microarrays allow multiple sensitisation diagnosis in a single assay, with a minimum amount of serum.[5,10,11] In addition, this technique increases the capacity for accurately identifying the allergen components associated with clinical manifestations and may help distinguish true multiple sensitisations from cross-reactions.[12]

In the present study, we mapped the pattern of sensitisation to inhalant components in patients with allergic rhinitis using the ISAC system for molecular-based sIgE detection.

Methods

This cross-sectional study involved outpatients with allergic rhinitis and/or asthma diagnosed respectively according to ARIA (Allergic Rhinitis and its Impact on Asthma)[13] and GINA (Global Initiative for Asthma)[14] guidelines. Patients were recruited at the Pediatric Allergy Division, University Hospital, in Curitiba, Southern Brazil.

We included 101 participants (a sample that was limited by the costs of performing the microarray) with 62 males, mean age 10.7 years, and range 6–15 years, who presented positive skin reaction to at least one of the following common aeroallergens: mites *Dermatophagoides pteronyssinus* and *Blomia tropicalis*, cockroach *Blattella germanica*, pollen *Lolium multiflorum*, and dog and cat dander antigens (IPI-ASAC Brasil®). For skin prick test, a 27 mm needle was introduced through a drop of allergen extract after wiping the forearm's skin with alcohol. A new disposable needle was used for each allergen. Positive (histamine 10 mg/mL) and negative (glycerinated saline) controls were also carried out.[15,16] After 15 min, the mean wheal diameter was measured and considered positive when greater than 3 mm.

To assess the molecular sensitisation pattern of the 101 patients, peripheral blood was collected by venipuncture. Serum samples for sIgE measurement were stored at −80 °C until assayed in Rome, Italy, by ISAC®, a microarray technique for multiplex IgE detection based on allergenic molecules,[9] in which the proteins (purified recombinant or natural allergens) were immobilised in a solid phase and 20 μL of serum were incubated with these proteins under standardised conditions. The antibodies present in the serum were captured by the different allergens and following a washing step to eliminate unbound substances, the antibodies were detected by means of a secondary fluorescent-labelled anti-isotype antibody that was detected by a laser scanner. Results were considered positive if values were greater than 0.3ISU (ISAC standardised units).[11,17] Specific IgE levels were reported as median (range) (Table 2).

We tested 103 allergens coming from natural sources or produced as recombinants: mites, food, animal epithelia, moulds, insects, latex, parasite (*Anisakis simplex)*, tree and grass pollen. This study included mostly inhalant allergen components from house dust mites: Der f 1, Der f 2 (*Dermatophagoides farinae*); Der p 1, Der p 2, Der p 10 (*Dermatophagoides pteronyssinus*); Eur m 2 (*Euroglyphus maynei*); animal epithelia: Can f 1, Can f 2, Can f 3 (*Canis familiaris* – dog); Fel d 1, Fel d 2, Fel d 4 (*Felis domesticus* – cat); insect: Bla g 1, Bla g 2, Bla g 4, Bla g 5, Bla g 7 (*Blattella germanica* – cockroach); moulds: Asp f 1, Asp f 2, Asp f 3, Asp f 4, Asp f 6 (*Aspergillus fumigatus*); Cla h 8 (*Cladosporium herbarum*); tree and grass pollen: Amb a 1 (*Ambrosia artemisiifolia*); Art v 1, Art v 3 (*Artemisia vulgaris*); Bet v 1, Bet v 2, Bet v 4 (*Betula verrucosa*); Cup a 1 (*Cupressus arizonica*); Cyn d 1 (*Cynodon dactylon*); Ole e 1, Ole e 2 (*Olea europaea*); Par j 2 (*Parietaria judaica*); Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 12 (*Phleum pratense*); Pla a 1, Pla a 2 (*Platanus acerifolia*).

Descriptive statistical analysis was performed, using Statistica (Statsoft®, Tulsa, USA) program. This study was approved by the Ethics Committee of Clinical Hospital, Federal University of Parana and all participants provided written informed consent.

Results

Allergic rhinitis was diagnosed in 101 patients, 42.6% were classified as mild and 57.4% moderate/severe depending on the severity of the symptoms. The majority had asthma (89.1%), of which 47.5% were classified as intermittent or mild persistent, 33.7% moderate and 7.9% severe. Allergic conjunctivitis was diagnosed in 67.3% of the subjects and atopic dermatitis in 9.9%.

SPT identified mites as the major allergen source. Eighty-three per cent of the patients showed positive skin reaction to *Dermatophagoides pteronyssinus* and 70.3% to *Blomia*