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Longitudinal profiles of serum specific IgE and IgG4 to *Dermatophagoides pteronyssinus* allergen and its major components during allergen immunotherapy in a cohort of southern Chinese children

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ABSTRACT

Longitudinal data on serum specific sIgE and sIgG4 to allergen component of *Dermatophagoides pteronyssinus* (Der p) during allergen immunotherapy (AIT) are limited in Chinese populations. We serially followed up serum sIgE and sIgG4 to Der p and its components (Der p 1 and 2) in 51 Der p-sensitized children receiving guideline-based medications alone and additional 36-month AIT. The the Der p 1 and Der p 2 sIgE levels were elevated at 6 months and progressively declined from 12 months; the sIgG4 levels for Der p, Der p 1 and Der p 2 were increasing during the first year and reached a plateau thereafter; the sIgE/sIgG4 ratios for Der p 1 and Der p 2 decreased continuously from 6 through 24 months of AIT. Subgroup analysis showed that younger children (≤8 years) experienced a greater increase in sIgG4 levels for Der p, Der p 1 and Der p 2 during AIT compared with older children (9–16 years). In summary, sIgE and sIgG4 to Der p 1 and Der p 2 may be more useful than those to Der p in reflecting the change in immunological reactivity during AIT. Earlier delivery of AIT may yield greater increase in sIgG4 after 36-month treatment than given later in life.

1. Introduction

House dust mite (HDM) is a source of perennial allergen and a well-recognized cause of allergic rhinitis and asthma. Globally, the prevalence of HDM sensitization ranges from 64 to 130 million in the general population, and nearly half of patients with asthma are HDM-sensitized (Calderon et al., 2015; Li et al., 2009; Ma et al., 2015; Huang et al., 2015). Attempts have been made to avoid environmental exposure to HDM allergens but unfortunately with limited success. Medications with histamines do alleviate symptoms in allergic rhinitis and asthma, however, allergen immunotherapy (AIT) so far remains to be the only treatment option that definitely offers disease-modifying effects for the patients (Biagtan et al., 2014). In addition, outcomes of AIT may vary depending on the type of HDM allergen (whole allergen vs components) being used (Guimaraes Junqueir de Queiros et al., 2008).

Dermatophagoides pteronyssinus (Der p) is an important HDM species in southern China (Sun et al., 2014a,b). Twenty-four Der p allergen components have been identified (Biagtan et al., 2014), yet only a minority of them are being investigated for clinical purposes (Pajno et al., 2001). Sensitization to Der p 1 and Der p 2, two major Der p allergen components, has been reported in over 90% of the HDM-allergic patients (Weghofer et al., 2013). Several studies have demonstrated the change in serum specific immunoglobulin E (sIgE) or immunoglobulin G4 (sIgG4) to Der p during AIT (Thomas, 2010; Lai et al., 2013). However, these studies focused on the whole Der p allergen in crude extracts, but not on allergen components of Der p, such as Der p 1 and/or Der p 2. Furthermore, longitudinal

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alterations rather than cross-sectional profiles in the serum sIgE and sIgG4 to Der p allergen components over time during AIT in Chinese population have rarely been delineated. Data seem even scarcer regarding whether such changes differ across age groups, and interaction between sIgE and sIgG4 in patients on AIT. To shed light on these aspects, we present our observational data from a pediatric series undergoing treatment for mild to moderate rhinitis with or without asthma in a tertiary teaching hospital in southern China.

2. Methods and materials

2.1. Ethics statement

The study protocol was approved by the Ethics Committee of First Affiliated Hospital, Guangzhou Medical University (GYFYY-2008-02-23). All experiments were performed in accordance with relevant guidelines and regulations of the Ethics Committee of First Affiliated Hospital of Guangzhou Medical University. Written informed consent was obtained from legal guardians of all participants. This study was reported in consistent with Transparent Reporting of Evaluations with Non-randomized Designs (TREND) (Des Jarlais et al., 2004). The study was registered in the Chinese Clinical Trial Registry (http://www.chictr.org/cn/, registration number: ChiCTR-DDT-13003728).

2.2. Patients

This was a prospective observational study by using data from the Allergy Registry at China State Key Laboratory of Respiratory Disease. The Allergy Registry collects so far the largest dataset of allergic patients referred to our center for allergen tests from over 50 hospitals in Guangzhou, the capital city of Guangdong Province in southern China (Sun et al., 2014c; Zeng et al., 2015). Review of these registered entries from August 2008 to August 2013 identified a cohort of children who suffered from mild to moderate rhinitis with or without asthma, and were monitored on a regular basis for serum levels of sIgE and sIgG4 to Der p, Der p 1 and Der p 2 throughout their AIT for HDM atopy. The inclusion criteria were: (1) patients aged 5–16 years; (2) a history of atopy to HDM and a physician-diagnosis of mild to moderate rhinitis with or without asthma based on *Allergic rhinitis and its impact on asthma* (ARIA) (Allergic Rhinitis and Its Impact on Asthma (ARIA), 2015) and *Global initiative for asthma* (GINA) (Global Initiative for Asthma (GINA), 2015) guidelines; and (3) sensitization to Der p as indicated by skin prick test (SPT) and confirmed by serum sIgE test at our institution (sIgE $\geq$ 3.5 kU/L, see below).

The AIT for the children was decided and entirely delivered by their referring doctors after a thorough communication with the parents. Finally, our observational study included 51 subjects (38 boys and 13 girls) who received AIT via subcutaneous injection.

2.3. Skin prick test

All subjects had undergone skin prick test (SPT) as the first level of diagnosis. The SPT was performed by using a standard panel of aeroallergens on the medial surface of the right forearm at least five days after withdrawal of any prescribed antihistamines. The test panel included crude HDM extracts (*D. pteronyssinus and Dermatophagoides farinae*), 50% glycerinated saline (as negative control) and 10 mg/mL histamine (as positive control). The wheal diameter was recorded for each child. A positive response was indicated by a wheal measuring >3 mm in diameter larger than the negative control at 15 min of SPT (Heinzerling et al., 2005).

2.4. Laboratory measurements of serum Der p, Der p 1, and Der p 2-specific antibodies

Peripheral blood samples (5 ml) were collected and centrifuged at 3000 rpm for 10 min. The supernatant was decanted and used for detection. For each subject, serum sIgE and sIgG4 levels for Der p, Der p 1, and Der p 2 were measured by using ImmunoCAP system (Phadia 1000, ThermoFisher Scientific Inc., California, USA) according to the manufacturer's instructions. The sIgE levels were listed in kilounits per liter (kU/L), equilibrated against the World Health Organization standard (75/702) for IgE (WHO, 1981), and the sIgG4 levels were reported in mass units (mg/L) as proposed by Klein et al. (1985). The detection range was from 0 to 100 kU/L for sIgE, and 0–30 mg/L for sIgG4. Where sIgE levels may be higher than 100 kU/L or sIgG4 levels higher than 30 mg/L, the blood sample was tested after tenfold dilution.

2.5. Treatments delivered by the referring physicians for the subjects

All subjects received medications according to the ARIA and/or GINA guidelines, including inhaled corticosteroids and antihistamines. The drug doses were adjusted according to these guidelines when necessary. As-needed use of short-acting bronchodilators for relieving asthma symptoms was also allowed.

In addition to these medications, the patients received AIT. The AIT in these children followed a conventional schedule in the 15-week initial phase with a commercial product of Der p extract (Alutard, ALK-Abelló, Hørsholm, Demark) according to the manufacturer's instructions, then ensued the maintenance injection of Alutard every 4–6 weeks for up to 36 months. The therapy doses were recorded in the Immunotherapy Unit Management software (INMUNOWIN, ALK-Abelló, Hørsholm, Demark) (Tabar et al., 2011). The patients were monitored for blood pressure and pulse rate within at least 30 min after each dose of AIT. Patient compliance to treatments was ensured with regular interview over the telephone by their physicians, and recorded in the INMUNOWIN program.

2.6. Measures of the observational study

The patients were referred to our laboratory to be measured for serum levels of sIgE and sIgG4 to Der p, Der p 1, and Der p 2 at baseline (immediately prior to treatment) and during the treatment. sIgE/sIgG4 ratio was computed to reflect the sIgE-sIgG4 interaction throughout the treatments. In cases where an individual sIgG4 value was below the detection limit, the sIgE/sIgG4 ratio was calculated by arbitrarily replacing the sIgG4 value by 0.01 mg/L.

2.7. Statistical analysis

Intention-to-treat analysis was used in the study. All data were processed with SPSS version 13.0. Kolmogorov-Smirnov test was used to evaluate the data normality. Normally-distributed quantitative data were expressed as mean and standard deviation (SD), and those in non-normal distribution as median and interquartile range (IQR). Student's *t*-test and non-parametric Wilcoxon paired test were used for comparisons of data in normal and non-normal distributions, respectively. Spearman correlation test was used for correlation analysis. Comparison of qualitative data was completed with Chi-square test. $P < 0.05$ was considered statistically significant.