Pretreatment IgE sensitization patterns determine the molecular profile of the IgG_4 response during updosing of subcutaneous immunotherapy with timothy grass pollen extract

Johannes Martin Schmid, MD,[a] Peter Adler Würtzen, PhD,[b] Ronald Dahl, DrSciMed,[c]* and Hans Jürgen Hoffmann, PhD[a] *Aarhus and Hørsholm, Denmark*

Background: Allergen immunotherapy is an effective treatment of allergic rhinoconjunctivitis. Clinical efficacy is associated with improvement of basophil sensitivity and an increase in allergen-specific immunoglobulin concentration.

Objective: We sought to determine whether changes in allergen component–specific serum IgE and IgG_4 levels during the updosing phase of subcutaneous immunotherapy (SCIT) are biomarkers of the immunologic changes that can lead to treatment efficacy.

Methods: Twenty-four subjects with grass pollen–induced allergic rhinoconjunctivitis were randomized 3:1 to receive SCIT (Alutard SQ) or to an open control group. IgE and IgG_4 concentrations were determined for the major allergens Phl p 1 or Phl p 5 by using ImmunoCAP and for 8 grass pollen molecules by using Immuno Solid-phase Allergy Chip (ISAC) before treatment and after updosing.

Results: Levels of specific IgE against the dominant major allergens Phl p 1 and Phl p 5 increased from a mean of 23.0 to 48.8 kU/L ($P = .01$, n = 18) during the updosing phase in ImmunoCAP measurements but decreased from a median of 4.6 ISAC specific units (ISU) to 2.14 ISU ($P < .0001$, n = 102) when measured by using ISAC against 8 grass allergen components. The updosing phase induced a specific IgG_4 level increase from a median of 0 ISU before treatment to 0.83 ISU after 12 weeks ($P < .0001$, n = 102) but only for allergen molecules to which pretreatment-specific IgE antibodies were detected (Spearman $\sigma = 0.72$, $P < .0001$, n = 102).

Conclusion: Pretreatment allergen component–specific IgE appears to determine the induction of IgG_4 in the updosing phase. Induced IgG_4 seems to suppress IgE levels on ISAC, resulting in a marked decrease in ISAC-measured specific IgE levels after updosing of SCIT. Thus this decrease in ISAC IgE levels can be used to monitor the blocking effect of allergen-specific immunotherapy–induced non-IgE antibodies. (J Allergy Clin Immunol 2016;137:562-70.)

Key words: *Grass pollen allergy, subcutaneous immunotherapy, component-resolved diagnosis, allergen component–specific IgE, allergen component–specific IgG_4, molecular allergy, allergen immunotherapy biomarker*

Hay fever caused by grass pollen allergy[1,2] is a major health problem in industrialized countries. The socioeconomic effect is mainly evident from the loss of productivity at work[3,4] and poorer performance in school.[5]

Allergen-specific immunotherapy (AIT) is the only treatment modifying the natural history of allergic diseases.[6,7] It results in complex cellular and humoral changes.[8] The induction of an allergen-specific IgG_4 response is an early effect of AIT.[9-11]

Traditionally, allergen extracts consisting of a complex and partially characterized mixture of allergen molecules[12] are used for both diagnosis and treatment. The recent development of molecular allergy leads to component-resolved diagnosis (CRD), which characterizes individual sensitizations at a molecular level.[13] To some extent, CRD can demonstrate risk-associated sensitizations and distinguish between primary sensitizations and cross-reactivity.[14]

We explored the performance of biomarkers to monitor treatment efficacy in a trial of subcutaneous immunotherapy (SCIT) compared with an open control group. The primary outcome of this study was a significant decrease in basophil sensitivity with a dominant humoral component.[15]

In the current analysis we examined changes in allergen component–specific IgE and IgG_4 levels measured by using ImmunoCAP and Immuno Solid-phase Allergy Chip (ISAC) before and after updosing to explain this observed decrease in basophil sensitivity and to identify biomarkers for the immunologic changes that might predict treatment effects of AIT.

METHODS

Study population

We recruited 24 subjects with seasonal rhinoconjunctivitis caused by grass pollen allergy in December 2009, recently described in detail.[15] All participants were examined at the baseline visit. A disease history was obtained, and the participants completed a standardized allergy questionnaire, listing all clinical relevant allergies. Sensitization was confirmed by a skin prick test with timothy grass extract (Soluprick SQ *Phleum pratense*; ALK-Abelló, Hørsholm, Denmark) resulting in a wheal diameter of greater

From [a]the Departments of Respiratory Medicine and Allergy, Aarhus University Hospital; [c]the Department of Clinical Medicine, Aarhus University; and [b]Global Research, ALK-Abelló, Hørsholm.

*Professor Dahl is currently affiliated with the Allergy Center, Odense University Hospital, Odense, Denmark.

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Corresponding author: Hans Jürgen Hoffmann, PhD, Department of Respiratory Medicine and Allergy, Aarhus University Hospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark. E-mail: hjh@clin.au.dk.

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Abbreviations used
AIT: Allergen-specific immunotherapy
CRD: Component-resolved diagnosis
FAB: Facilitated Antigen Binding
FAP: Facilitated antigen presentation
IQR: Interquartile range
ISAC: Immuno Solid-phase Allergy Chip
ISU: ISAC specific units
SCIT: Subcutaneous immunotherapy

than 3 mm. Participants retrospectively indicated their maximum symptom score during the grass pollen season preceding treatment when entering the study and reported daily symptom and medication scores during the grass pollen season after updosing of SCIT. We used a symptom score combining 4 nasal and 2 ocular symptoms on a scale from 0 to 3.[16] Participants were randomized 3:1 according to a computer-generated list to receive standard SCIT (n = 18) or to an open control group (n = 6) only receiving reliever medication as needed. All participants provided informed consent when entering the study. The regional ethics committee approved the study (M2009-0121), which meets the CONSORT 2010 guidelines for reporting randomized trials and is registered at ClinicalTrials.gov (NCT01085526).

SCIT

SCIT was initiated with a modified cluster on day 1 (10, 100, and 1000 SQU), followed by injections of increasing doses of timothy grass extract (Alutard SQ *Phleum pratense*, ALK-Abelló) at weekly intervals until a maintenance dose (100,000 SQU) was reached before the 2010 grass pollen season.

Immunoglobulin measurements

Before AIT initiation and after 12 weeks of updosing treatment, allergen-specific IgE and IgG_4 levels were measured by using 2 different standard methods: quantitative levels of Phl p 5–specific IgE or, if less than the detection limit, Phl p 1–specific IgE and corresponding IgG_4 antibodies were measured by using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Specific IgE and IgG_4 antibody levels to Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7 (polcalcin), Phl p 11, and Phl p 12 (profilin) were measured on the semiquantitative 112-allergen ISAC (Thermo Fisher Scientific). Serum samples were frozen at −80°C and processed at the same time.

Data on basophil sensitivity measured by means of flow cytometry and Facilitated Allergen Binding (FAB), an assay measuring the inhibition of allergen binding to B cells used in this analysis, were reported recently.[15]

Statistical analysis

Data are reported as means with 95% CIs or medians with interquartile ranges (IQRs). Normal distribution was verified by QQ-plots and histograms. Normally distributed data were analyzed by using a *t* test for between-group analysis and a paired *t* test for intraindividual in-group analysis. Otherwise, we used a Mann-Whitney-Wilcoxon test to perform between-group analysis and the Wilcoxon signed-rank test to analyze intraindividual in-group differences. We used a multiple regression model with maximum symptom scores before treatment and after the ensuing season as dependent variables and the sum of ISAC grass-specific IgE and IgG_4 levels, basophil sensitivity, and FAB as explanatory variables both before treatment and after updosing. The model was checked by plotting residuals. Correlation coefficients were obtained by using the Spearman rank test. For statistical analysis of the suppressive effect of induced IgG_4 on ISAC IgE measurements, we divided all IgG_4 measurements after updosing into 4 groups. The first had no detectable IgG_4 (<0.03 ISAC-specific units [ISU], n = 56), and the remaining 88 measurements were divided into tertiles: 0.04 to 0.50 ISU (n = 29); 0.56 to 3.19 ISU (n = 30); and 3.22 to 9.98 ISU (n = 29), respectively. We plotted these groups against the changes in IgE concentration between the 2 measurements. The pretreatment IgE measurements were divided into 4 groups according to ISAC classes (not detectable, <0.30 ISU; low, 0.30-0.99 ISU; moderate to high, 1.00-14.99 ISU; and very high, >15.00 ISU) and plotted against changes in IgG_4 concentrations. This is an exploratory study in which a *P* value of .05 or less was considered statistically significant without correction for multiple tests. All data were analyzed with Stata 11.1 software for Windows (www.stata.com).

RESULTS

Baseline characteristics

Median duration of grass pollen–induced hay fever at inclusion was 9.5 years (IQR, 5.5-17.5 years). All study participants were sensitized to at least 1 of the major allergens Phl p 1 and Phl p 5 at baseline (Table I). Ten (41.7%) patients reported hay fever symptoms exclusively caused by grass pollen exposure. Two of these subjects were monosensitized to grass pollen in the pretreatment ISAC measurements. At baseline, participants had detectable IgE against Phl p 2 (54.2%), Phl p 4 (54.2%), Phl p 6 (62.5%), Phl p 11 (50%), Phl p 7 (21%), and Phl p 12 (4%). We measured low concentrations of specific IgG_4 before AIT, which was most prominent for Phl p 4 (median, 0.07 ISU; IQR, 0.0-0.15 ISU) and Phl p 5 (median, 0.0 ISU; IQR, 0.0-0.15 ISU) in 17 and 13 patients, respectively.

Comparison of IgE and IgG_4 levels during SCIT updosing with ImmunoCAP and ISAC

As found in previous studies,[17,18] the mean concentration of allergen-specific IgE against the major allergen Phl p 5 (or Phl p 1 if no Phl p 5 was detected) increased from 23.0 to 48.8 kU/L ($P = .01$, n = 18) when measured by using ImmunoCAP (Fig 1, *A*). In contrast, ISAC measurement of specific IgE to the dominating grass pollen allergen Phl p 1 or Phl p 5 decreased significantly from a mean of 21.4 to 2.5 ISU ($P = .0002$, n = 18; Fig 1, *B*). There was a strong linear correlation between Phl p 1 and Phl p 5 IgE pretreatment measurements by using ImmunoCAP and ISAC ($P < .0001$, n = 18) but not after completed updosing ($P = .20$, n = 18).

The mean concentration of corresponding IgG_4 increased when measured with either ImmunoCAP (0.1-5.2 mg/L, $P = .001$, n = 18; Fig 1, *C*) or ISAC (0.3-5.0 ISU IgG_4, $P = .0001$, n = 18; Fig 1, *D*). There was a strong linear correlation between IgG_4 levels toward Phl p 1 and Phl p 5 measurements both before treatment and after updosing (both $P < .0001$, n = 18). IgE and IgG_4 immunoglobulin levels of the control group did not change.

Single-allergen ISAC measurements during SCIT

We expanded on this observation by assessing IgE and IgG_4 measurements against all 8 available grass pollen molecules found on ISAC (Fig 2). Allergen measurements without detectable IgE or IgG_4 at both time points in a given patient (n = 42) were censored. The measured concentration of IgE for all grass components decreased markedly from a median of 4.60 ISU (IQR, 1.66-13.98 ISU) to 2.14 ISU (IQR, 0.0-4.59 ISU; $P < .0001$; n = 102) during updosing. At the same time, grass-specific IgG_4 concentrations increased from a median of 0.0 ISU (IQR, 0.0-0.11 ISU) to 0.83 ISU (IQR, 0.14-3.35 ISU; $P < .0001$; n = 102).