Molecular profiling of allergen-specific antibody responses may enhance success of specific immunotherapy

Azahara Rodríguez-Domínguez, MSc,[a] Margot Berings, MD, PhD,[b] Alexander Rohrbach,[c] Huey-Jy Huang, PhD,[a] Mirela Curin, PhD,[a] Philippe Gevaert, MD, PhD,[b] Paolo M. Matricardi, MD,[c] Rudolf Valenta, MD,[a,d,e,f] and Susanne Vrtala, PhD[a] *Vienna and Krems, Austria; Ghent, Belgium; Berlin, Germany; and Moscow, Russia*

Background: House dust mites (HDMs) are among the most important allergen sources containing many different allergenic molecules. Analysis of patients from a double-blind, placebo-controlled allergen-specific immunotherapy (AIT) study indicated that patients may benefit from AIT to different extents depending on their molecular sensitization profiles.
Objective: Our aim was to investigate in a real-life setting whether stratification of patients with HDM allergy according to molecular analysis may enhance AIT success.
Methods: Serum and nasal secretion samples from patients with HDM allergy (n = 24) (at baseline, 7, 15, 33, and 52 weeks) who had received 1 year of treatment with a well-defined subcutaneous AIT form (Alutard SQ 510) were tested for IgE and IgG reactivity to 15 microarrayed HDM allergen molecules with ImmunoCAP Immuno-solid-phase Allergen Chip technology. IgG subclass levels to allergens and peptides were determined by ELISA, and IgG blocking was assessed by basophil activation. *In vitro* parameters were related to reduction of symptoms determined by combined symptom medication score and visual analog scale score.
Results: Alutard SQ 510 induced protective IgG mainly against *Dermatophagoides pteronyssinus* (Der p) 1 and Der p 2 and to a lesser extent to Der p 23, but not to the other important allergens such as Der p 5, Der p 7, and Der p 21, showing better clinical efficacy in patients sensitized only to Der p 1 and/or Der p 2 as compared with patients having additional IgE specificities.
Conclusion: Stratification of patients with HDM allergy according to molecular sensitization profiles and molecular monitoring of AIT-induced IgG responses may enhance the success of AIT. (J Allergy Clin Immunol 2020;146:1097-108.)

Key words: *House dust mite, immunotherapy, recombinant allergens, basophil activation, IgE, IgG, IgG_1, IgG_2, IgG_3, IgG_4, IgG subclass, allergen microarray*

Allergen-specific immunotherapy (AIT) is the only disease-modifying treatment for patients with allergy that has long-lasting effects.[1,2] AIT seems to be even more effective than pharmacotherapy in reducing symptoms,[3,4] and it prevents the progression from mild forms of allergy (rhinoconjunctivitis) to severe forms (asthma).[5] However, several factors, such as long duration of treatment with multiple applications and potential side effects, lead to poor compliance of patients and thus limit utilization of AIT.[6,7] Another important problem relates to difficulties in obtaining high-quality allergen preparations for the formulation of AIT vaccines.[8] The latter problem is particularly relevant for house dust mites (HDMs), which contain a variety of different allergen molecules.[9,10] HDMs are among the most important allergen sources worldwide, and they are responsible for severe forms of respiratory and cutaneous allergic manifestations.[11] Because HDMs are rich in proteases and immunomodulatory components,[12] it is difficult to obtain high-quality allergen extracts. In the HDM, *Dermatophagoides pteronyssinus* (Der p) 1, Der p 2, Der p 5, Der p 7, Der p 21, and Der p 23 seem to represent the most clinically relevant allergens[13,14]; however, most allergen extracts prepared from natural allergen sources contain mainly Der p 1 and Der p 2, whereas the other allergens occur in small amounts or are missing. In this context, it has been demonstrated that important allergens were missing from HDM allergen extracts used for skin testing.[15]

Although several studies have demonstrated clinical efficacy of subcutaneous AIT (SCIT) for the treatment of HDM allergy[16-18] it

From [a]the Department of Pathophysiology and Allergy Research, Division of Immunopathology, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna; [b]the Upper Airways Research Laboratory, Ghent University, Ghent; [c]the Department of Pediatric Pulmonology, Immunology and Intensive Care Medicine, Charité-University Medicine Berlin; [d]NRC Institute of Immunology, Federal Biomedical Agency of Russia, Moscow; [e]the Department of Clinical Immunology and Allergy, Sechenov First State Medical University, Moscow; and [f]Karl Landsteiner University of Health Sciences, Krems.

Funded by the Austrian Science Fund (grants DK W 1248-B13, F4602, and F4605), the Medical University of Vienna, and the Flemish Scientific Research Foundation (Flanders, Belgium). M. Berings receives funds from the Flemish Scientific Research Foundation (grants FWO.3F0.2013.0004.01 and FWO.3F0.2013.0004.02). R. Valenta is the recipient of a megagrant of the Government of the Russian Federation (grant 14.W03.31.0024). P. Gevaert receives a grant as a senior clinical investigator from the Flemish Scientific Research Foundation. The opinions, results, and conclusions reported in this article are those of the authors and are independent of the funding sources.

Disclosure of potential conflict of interest: R. Valenta has received research grants from Viravaxx, Vienna, Austria, and serves as a consultant for the company. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication December 12, 2019; revised February 28, 2020; accepted for publication March 26, 2020.

Available online April 13, 2020.

Corresponding author: Susanne Vrtala, PhD, Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria. E-mail: susanne.vrtala@meduniwien.ac.at.

0091-6749

https://doi.org/10.1016/j.jaci.2020.03.029

Abbreviations used
AIT: Allergen-specific immunotherapy
CSMS: Combined symptom and medication score
Der f: *Dermatophagoides farinae*
Der p: *Dermatophagoides pteronyssinus*
HDM: House dust mite
ISAC: Immuno-solid-phase Allergen Chip
Phl p: *Phleum pratense*
rDer p: Recombinant *Dermatophagoides pteronyssinus*
SCIT: Subcutaneous immunotherapy
SPT: Skin prick test
VAS: Visual analog scale

seems that SCIT for HDM allergy is less effective than SCIT for seasonal allergies such as grass pollen allergy when vaccines of the same type are compared (ie, when SCIT is compared with the aluminium hydroxide–adsorbed allergen extract Alutard).[19-22] In fact, SCIT with the aluminium hydroxide–adsorbed HDM allergen extract Alutard has been studied quite extensively in clinical studies in adults and children.[23,24] Analysis of the underlying immunologic mechanisms indicated that the induction of allergen-specific IgG_4 antibodies not only served as a marker for the immunologic response but was also partly associated with clinical efficacy.[23] Accordingly, the induction of allergen-specific IgG, and the induction of allergen-specific IgG_4 in particular, is currently considered an important biomarker for effects and, in part, efficacy of AIT.[25,26] Further support for the importance of allergen-specific IgG antibodies in reducing allergic symptoms comes from a recent study demonstrating that passive immunization with allergen-specific IgG mAbs potently suppressed allergic symptoms in a clinical study.[27] However, analysis of the induction of allergen-specific IgG_4 and the IgE-blocking activity with crude HDM allergen extracts showed modest correlations between clinical efficacy and IgE-blocking activity of the therapy-induced IgG_4 response.[23] Because HDMs (unlike cat, birch pollen, or grass pollen) contain several different important allergens that may not be well represented in natural allergen extracts, it is tempting to speculate that patients with different profiles of IgE reactivity to HDM allergens may respond in a different manner depending on presence of the individual HDM allergens in the vaccine. In fact, the *post hoc* analysis of IgE and IgG responses of sera from patients undergoing a clinical trial with HDM SCIT in which clinical effects were recorded in an allergen challenge chamber indicated that the reduction of total nasal symptom scores may be related to the patients' IgE recognition profiles and development of allergen-specific IgG.[28]

Here we have performed the first real-life study using a large panel of purified HDM allergen molecules and analyzed the molecular IgE sensitization profiles and development of allergen-specific IgG antibodies in patients with HDM allergy who were receiving SCIT with Alutard and in a parallel control group without SCIT. In particular, we performed a detailed analysis of allergen-specific IgG subclass responses. The ability of treatment-induced IgG to inhibit allergen-induced basophil degranulation was studied with multiple allergens, and immunologic parameters were related to clinical outcome. The results of our study indicate that molecular diagnosis and molecular monitoring of AIT-induced IgG antibodies may be useful to predict and monitor efficacy of HDM AIT in real life.

METHODS

Subjects and treatment

We analyzed serum samples from 24 patients with HDM allergy who had been treated with conventional HDM SCIT (Alutard-SQ 510, ALK-Abelló, The Netherlands) (a group of patients with HDM allergy who received immunotherapy [referred to as the HDMIT group]), a group of 9 patients with HDM allergy without immunotherapy (referred to as the HDMA group), and a group of nonallergic subjects.[29] Patients with HDM allergy had a positive skin prick test (SPT) result with HDM, a level of Der p–specific IgE antibodies greater than 0.35 kUA/L determined by ImmunoCAP (Phadia AB/ThermoFisher, Uppsala, Sweden), and a clinical history of symptoms of allergy to HDM. AIT was conducted for a 3-year period, and monitoring and sample collection were performed for the first 12 months. One group of patients with allergy (the HDMIT group) received weekly subcutaneous injections for 15 weeks, with monthly injections for 1 year afterward. The dosage was an escalation regimen from 100, 1,000, and 10,000 to 100,000 standardized quality units per milliliter, which was reached at the end of the initiation (15 weeks) and maintained for the remaining year (Fig 1). All patients were treated at the Ghent University Hospital, in Ghent, Belgium; all data and samples were collected prospectively. At each visit—at baseline (at visit 0 [V0], halfway through initiation (7 weeks [V1]), at the end of initiation (15 weeks [V2]), after 8 months of maintenance (V3), and after 1 year of treatment (V4) (Fig 1)—patients filled out questionnaires, underwent SPTs, had their clinical symptoms assessed (with use of visual analog scale [VAS] scores and combined symptom medication scores [CSMSs]), and had blood and nasal secretions collected as described.[30] During the same period, serum samples and nasal secretions were obtained from the parallel HDMA group (n = 9) and from the group of nonallergic subjects (n = 9). The study was approved by the local ethical committee of Ghent University Hospital (EC 2013/672). Written informed consent was obtained from each patient, including permission to investigate their blood samples. Patients' baseline characteristics and immunologic parameters are displayed in Table I. The anonymized serum samples were analyzed with permission from the ethics committee of the Medical University of Vienna (EK 1641/2014).

ImmunoCAP ISAC assay

Serum samples from the HDMIT group (n = 24), the HDMA group (n = 9), and the group of nonallergic subjects (n = 9) that had been collected at every visit (V0-V4) were analyzed for IgE and IgG reactivity to multiple microarrayed allergen molecules, including 15 HDM allergens (ie, Der p 1, *Dermatophagoides farinae* 1 [Der f 1], Der p 2, Der f 2, Der p 4, Der p 5, Der p 7, Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, Der p 21, Der p 23, and Der p 37). The samples were analyzed by using the MeDALL chip, which is based on Immuno-solid-phase Allergen Chip (ISAC) microarray technology (Phadia Multiplexing; ThermoFisher Scientific) as described.[31] For IgE measurement, serum samples were used undiluted, whereas for IgG measurement, sera were diluted 1:50. Results were evaluated by using Phadia Microarray Image Analysis software, and levels of allergen-specific IgE and IgG antibodies were reported in ISAC standardized quality units for IgE antibodies or ISAC standardized quality units for IgG antibodies, with a cutoff of 0.3 ISAC standardized quality units.

HDM allergens and peptides

Natural purified Der p 1 was obtained from Professor W. R. Thomas.[32] Recombinant Der p (rDer p) 2, rDer p 23, rDer p 5, and rDer p 21 were expressed in *Escherichia coli* and purified as described.[33-35] The expression and purification of rDer p 7 are described in the Online Repository.

Peptides of Der p 1, Der p 2, and Der p 23 with a length between 29 and 43 amino acids, covering the sequences of the allergens (see Table E1 in this article's Online Repository at www.jacionline.org), were synthesized by using a 9-fluorenylmethoxycarbonyl strategy as described elsewhere.[36] The peptides were identified by mass spectrometry and purified by preparative HPLC to a purity greater than 90%.[37] The sequences and numbers of amino acids of each of the peptides, their location in the corresponding allergen, and the ability of peptide-specific IgG antibodies to inhibit IgE binding in patients with allergy, as determined in earlier studies, are shown in Table E1.

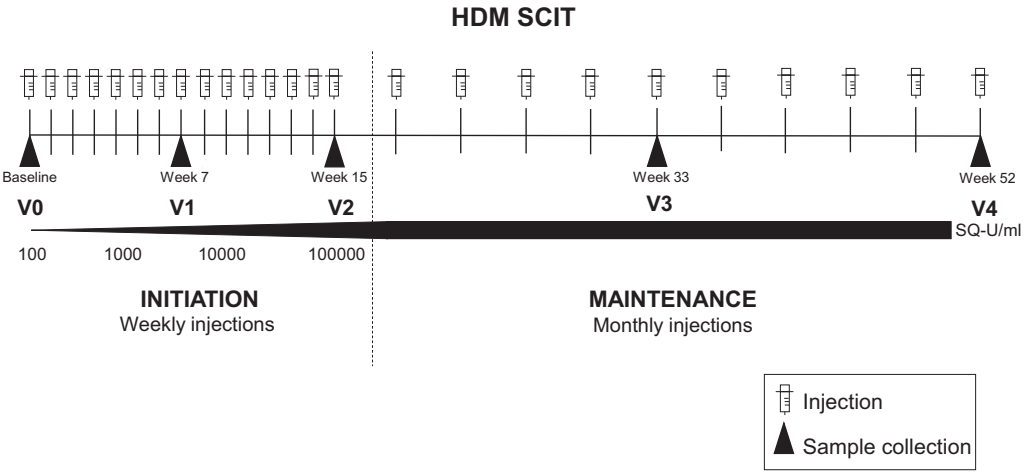


FIG 1. Scheme of AIT treatment and sample collection. Patients received subcutaneous AIT consisting of a 15-week updosing period with 15 weekly injections (initiation) and monthly maintenance injections for 1 year (maintenance). Blood and nasal secretions were collected at visits 0 to 4, as indicated. *SQ-U*, Standardized quality units.

TABLE I. Demographic and clinical characteristics of subjects included in the study

Group	HDMIT group	HDMA group	Patients without allergy
No.	24	9	9
Median age, y	31.44	27.73	26.87
Sex (male/female)	9/15	4/5	3/6
Median length (cm)	170	170	168
Median weight (kg)	63.5	73	64
Median BMI (kg/m^2)	21.65	22.84	23.71
Asthma (%)	46.67	11.11	na
Rhinitis (%)	100	100	na
Median total IgE, kU/L (range)	134.74 (16.45-1975.84)	80.06 (27.67-374.30)	13.81 (3.15-107.89)
Median *D pteronyssinus* sIgE level, kUA/L (range)	0.05-200.91 (19.43)	0.39-69.37 (4.87)	na
Median *D farinae* sIgE level, kUA/L (range)	0.05-188.20 (12.78)	0.21-49.61 (5.35)	na
Monosensitized to HDM, n (%)	6 (25.00)	4 (44.44)	na
Cosensitizations, n (%)	18 (75.00)	5 (55.55)	na
Grass, n (%)	16 (66.66)	4 (44.44)	na
Tree, n (%)	9 (37.50)	1 (11.11)	na
Weed, n (%)	3 (12.50)	na	na
Mold, n (%)	4 (16.66)	1 (11.11)	na
Cat/dog, n (%)	15 (62.50)	2 (33.33)	na

HDMA, Patients with HDM allergy who did not receive immunotherapy; *HDMIT*, patients with HDM allergy who received immunotherapy; *na*, not applicable.

ELISA assays

Allergen- and peptide-specific human IgG_1, IgG_2, IgG_3, and IgG_4 subclass responses were measured by ELISA.[38,39] For this purpose, serum samples were diluted 1:50 and nasal secretions were diluted 1:4 in PBS containing 0.5% Tween 20. OD values were recorded on a FLUOstar Omega Microplate Reader (BMG Labtech, Ortenberg, Germany). All determinations were performed in duplicate, and the results were displayed as means of the OD values with deviations less than 5%. OD values from different plates were harmonized by including standards on each plate.

Quantitative IgG_1 and IgG_4 ELISA measurements were performed by using 96-well ELISA plates (Nunc Maxisorp, Roskilde, Denmark). Each well was coated with 100 μL of natural Der p 1, rDer p 2, or rDer p 23 (2 μg/mL in 100 mM carbonate buffer [pH 9.6]), after which the plates were incubated for 5 hours at room temperature. After being washed with 0.05% vol/vol Tween-20 (Sigma-Aldrich, St Louis, Mo) in PBS and being blocked with 2% wt/vol BSA in PBS/0.05% Tween 20 overnight, the plates were incubated with patients' sera (diluted 1:50 and 1:100 in PBS/0.05% Tween-20/0.5% BSA) for 2 hours at 37°C and 1 hour at room temperature. Standard curves for IgG_1 and IgG_4 were established by coating plates with 2 μg/mL of *Phleum pratense* (Phl p) 2 and Phl p 5 and incubating them with a human IgG_1 mAb specific for Phl p 2[40] or with a human IgG_4 mAb specific for Phl p 5[41] with mAb concentrations of 50, 150, 450, 900, and 1350 ng/mL diluted in PBS/0.05% Tween-20/2% BSA in triplicate for 2 hours at 37°C and 1 hour at room temperature. Bound antibodies were detected with biotinylated subclass-specific antibodies (mouse anti-human IgG_1 mAb (clone G17-1) or IgG_4 (clone G17-4) (BD, San Diego, Calif) (diluted 1:1000 in PBS/0.05% Tween-20/0.5% BSA) mixed with horseradish peroxidase–conjugated streptavidin (BD Pharmingen, catalog no. 554066) (diluted 1:2500 in PBS/0.05% Tween-20/0.5% BSA) overnight at 4°C. The substrate 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma-Aldrich) was added at a rate of 100 μL per well, and the ODs were measured by using an ELISA Reader (Tecan Infinite F50, Männedorf, Switzerland) at 405/490 nm. Concentrations of allergen-specific IgG_1 and IgG_4 were calculated according to the standard curve of OD values obtained for known concentrations.

Basophil activation and inhibition of basophil activation with blocking IgG

Rat basophilic leukemia RBL cells (clone RS-ATL8)[42] transfected with the cDNA coding for the human high-affinity IgE receptor were maintained in minimum essential medium (MEM) with Earle salt supplemented with 10% FBS, 2mM L-glutamine (ThermoFisher Scientific), 100 U/mL of penicillin, and 100 μg/mL of streptomycin, 200 μg/mL of Geneticin (ThermoFisher Scientific), and 200 μg/mL of Hygromycin B (ThermoFisher Scientific) at 37°C. Cells were plated in 96-well sterile tissue culture plates (50 μL per well) with the sera (1:20 dilution in supplemented MEM) obtained from the treated patients (n = 9) before immunotherapy at baseline (ie, at visit V0) and incubated overnight at 37°C and 7% CO_2. For control purposes, cells were incubated only with supplemented MEM as a negative control. The next day, serum samples that had been obtained from the same patients before and after AIT were incubated for 1.5 hours at 56°C to inactivate IgE antibodies. Thereafter, IgE-inactivated sera diluted 1:20 in Tyrode buffer (ie, Tyrode salts dissolved in water according to the manufacturer (Sigma Aldrich), 0.02M $NaHCO_3$, 1% wt/vol BSA, and 50% vol/vol D_2O [7.2]) were incubated with different concentrations of purified allergens (1, 10, and 100 ng/mL) for 2 hours at 37°C and added to the cells, which had been washed 3 times with washing buffer (Tyrode salts, 0.02M $NaHCO_3$, and 1% wt/vol BSA in H_2O [pH 7.2]) for 1 hour at 37°C. The release of β-hexosaminidase in the medium from activated RBL cells was determined by a fluorometric assay by using 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide as a substrate (0.1 mM in 100 mM citrate [pH 4.5]). The reaction was stopped with 0.25 M glycine buffer after 60 minutes of incubation at room temperature. The plate was read on an Infinite M200 PRO series plate reader (TECAN Austria GmbH, Grödig, Austria) by using 380-nm excitation and 440-nm emission filters. All measurements were performed in triplicate, and the results were displayed as percentages of total release (means ± SDs).[43]

For measuring direct mediator release, cells were loaded with serum obtained from patients before immunotherapy at baseline (V0) or afterward (V4) and exposed to different concentrations of purified allergens (0.01 to 10 ng/mL). β-Hexosaminidase release was determined as described in the preceding paragraph.[43]

Statistics

The Mann-Whitney and Wilcoxon *U* tests were used to compare independent samples from different subjects to compare the IgE and IgG levels, as well as the IgG subclasses and allergen peptide ODs in different groups at the given different time points (GraphPad Prism software, version 7.00 for Windows, GraphPad Software, La Jolla, Calif). A *P* value less than .05 was considered statistically significant, as indicated in the figure legends.

RESULTS

Real-life study design and characterization of patients with HDM allergy

To study immune responses in patients with HDM allergy who were undergoing subcutaneous immunotherapy with HDM allergen extract–based Alutard SQ 510, an investigator-driven study was conducted at the Department of Otorhinolaryngology in Ghent University Hospital in Ghent, Belgium.[29] In this observational study patients with HDM allergy received subcutaneous immunotherapy with HDM allergen extract–based Alutard SQ 510 for 3 years (those in the HDMIT group); a control group without AIT (ie, those in the HDMA group) was also included. Serum samples from 24 of the 28 patients in the HDMIT group and all 9 patients in the HDMA group that had been obtained at baseline (ie, visit 0), after week 7 (ie, visit 1), after week 15 (ie, visit 2), after week 33 (ie, visit 3), and after week 52 (ie, visit 4) (Fig 1) were available for analysis. Table I provides an overview of the demographic and clinical characterization of the HDMIT group and HDMA group. Pseudonymized serum samples and nasal secretion samples were analyzed by an investigator who was blinded regarding the 2 groups.

Table II shows the molecular IgE recognition profiles of the patients in the HDMIT group and the patients in the HDMA group when tested with 13 microarrayed *D pteronyssinus* allergen molecules, as well as with Der f 1 and Der f 2 at the baseline visit. In the HDMIT group, Der p 2 (91.7%), Der p 1 (58.3%), and Der p 23 (45.8%) were the most frequently recognized allergen molecules, followed by Der p 7 (41.7%), Der p 4 (33.3%), Der p 21 (29.2%), Der p 10 (20.9%), Der p 5 and Der p 37 (both 12.5%), Der p 18 (8.3%), and Der p 15 (4.2%). In the HDMA group, frequencies of recognition of IgE to the individual allergen molecules were lower, which was in agreement with the lower levels of Der p– and Der f–specific IgE (Table I). Fewer members of the HDMA group had asthma than in the HDMIT group (ie, 11.1% vs 46.7%). A detailed demographic, clinical, and serologic characterization of each of those in the HDMIT group and those in the HDMA group can be found in Tables E2 and E3 (in this article's Online Repository at www.jacionline.org). Patients who were sensitized only to Der p 1 and/or Der p 2 are indicated in blue and those who are sensitized against at least 1 of the additional clinically relevant allergens (ie, Der p 5, Der p 7, Der p 21, and Der p 23) are highlighted in yellow in Tables E2 and E3.

Vaccination with Alutard SQ 510 induces specific IgG responses only to Der p 1, Der p 2, and Der p 23 and not to any of the other Der p allergen molecules

Fig 2 shows the development of allergen-specific IgG levels in the HDMIT group compared with the levels in the HDMA group during the observation period of 1 year. The patients in the HDMIT group showed a significant increase in Der p 1–, Der f 1–, Der p 2–, and Der f 2–specific IgG responses in serum samples obtained at visits 2, 3, and 4 compared with baseline. A much lower but significant increase in Der p 23–specific IgG levels was noted as well for the HDMIT group (Fig 2, *B*). However, no relevant induction of IgG antibodies specific for any of the other HDM allergens (Der p 5, Der p 7, or Der p 21 [Fig 2, *A* and *B*] and Der p 4, Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, or Der p 37 [see Fig E1, *A* and *B* in this article's Online Repository at www.jacionline.org and see also Table E3]) was found. No relevant HDM allergen–specific IgG increases were found in the untreated HDMA group (Fig 2, *A* and *B* [see Table E3]).

The analysis of allergen-specific IgE responses in the course of AIT showed that the patients in the HDMIT group also had increased levels of IgE to Der p 2, Der f 2, Der p 1, Der f 1, and Der p 23 beginning with visit 2, with the levels going down at visits 3 and 4 whereas levels of allergen-specific IgG continued to increase (Fig 2, *A* and *B*). These increases in allergen-specific IgE levels were observed only for those allergens against which IgG was induced but not for the other HDM allergen molecules. For certain patients in the HDMIT group (ie, patients 008, 030, 032, 034, 037, 039, and 040), we noted a *de novo* induction of allergen-specific IgE against allergens that were not recognized at baseline (see Table E4 in this article's Online Repository at www.jacionline.org). When basophils were loaded with sera obtained from the latter patients at baseline and after treatment we found that

TABLE II. Prevalence of IgE reactivity and mean IgE levels (ISU) to 15 HDM allergens

Group	HDMIT		HDMA	
No.	24		9	
	Prevalence (%)	Mean (SD)	Prevalence (%)	Mean (SD)
Der f 1	45.83	7.49 (12.15)	44.44	3.09 (4.45)
Der f 2	83.33	26.20 (37.43)	77.78	11.55 (23.64)
Der p 1	58.33	16.04 (27.63)	55.56	2.62 (4.79)
Der p 2	91.67	19.98 (31.83)	55.56	9.07 (18.07)
Der p 4	33.33	1.05 (0.68)	11.11	0.13 (0.22)
Der p 5	12.50	0.54 (0.16)	0.00	0.00 (0.00)
Der p 7	41.67	3.60 (3.96)	11.11	0.13 (0.34)
Der p 10	20.83	0.51 (0.17)	0.00	0.02 (0.03)
Der p 11	0.00	0.00 (0.00)	0.00	0.00 (0.00)
Der p 14	0.00	0.00 (0.00)	0.00	0.00 (0.01)
Der p 15	4.17	0.00 (0.00)	11.11	0.16 (0.47)
Der p 18	8.33	14.16 (19.37)	11.11	0.08 (0.23)
Der p 21	29.17	3.64 (5.52)	0.00	0.00 (0.00)
Der p 23	45.83	15.09 (34.66)	33.33	2.47 (7.07)
Der p 37	12.50	2.22 (2.58)	0.00	0.01 (0.03)

HDMA, Patients with HDM allergy who did not receive immunotherapy; *HDMIT*, patients with HCM allergy who received immunotherapy.

treatment-induced IgE led to an allergen-specific induction of mediator release in more than half of them (see Fig E2 in this article's Online Repository at www.jacionline.org). No increases in allergen-specific IgE levels were found for the untreated HDMA group (Fig 2, *A* and *B*).

Maintenance treatment continuously increases allergen-specific IgG_4 responses but only maintains IgG_1 and IgG_2 responses

Fig 3 presents the results obtained when allergen-specific IgG subclass responses were analyzed in the HDMIT group during the 1-year period of investigation. The IgG subclass analysis was performed only for those allergens against which increases in allergen-specific IgG responses were found (ie, Der p 1, Der p 2, and Der p 23) and for control purposes, for 1 allergen for which no IgG response was found (ie, Der p 5). Allergen-specific IgG subclass responses were observed mainly for IgG_1, IgG_4, and for IgG_2 but not for IgG_3, and they were limited to Der p 1, Der p 2, and Der p 23, whereas no Der p 5–specific IgG subclass responses were found (Fig 3). We noted interesting differences regarding the kinetics of the IgG subclass responses in the course of treatment: allergen-specific IgG_4 responses continued to increase during the course of treatment, as shown for Der p 1, Der p 2, and Der p 23. By contrast, allergen-specific IgG_1 and IgG_2 responses were lower and did not further increase during the maintenance treatment. Using a quantitative ELISA for measuring the concentrations of allergen-specific IgG_1 and IgG_4, we found that AIT-induced allergen-specific IgG_1 concentrations were in the range of 5 μg/mL, whereas allergen-specific IgG_4 concentrations went up to approximately 25 μg/mL (see Fig E3, *A* and *B* in this article's Online Repository at www.jacionline.org).

Allergen-specific IgG_1 and IgG_4 antibodies could be also detected in nasal secretions, but their levels were low and increases were noted only for specific IgG_4 antibodies (see Fig E4 in this article's Online Repository at www.jacionline.org).

In the untreated (HDMA) group, allergen-specific IgG subclass levels did not change during the year of investigation (data not shown).

IgG_4 antibodies are only partly directed against the IgE-binding regions of the allergens

IgG_4 levels specific for 8 overlapping peptides of Der p 1, 5 Der p 2 peptides, and 5 Der p 23 peptides, which covered the sequences of the allergens (see Table E1), were measured in sera of patients from the HDMIT group that had been obtained before immunotherapy (at V0), after 33 weeks (at V3), and after 1 year (at V4) of immunotherapy. Table E1 indicates the percentages in patients with allergy of inhibition of IgE binding to the corresponding allergens obtained with peptide-specific antibodies and thus define whether a peptide was derived from an IgE-binding region.[44,45] Statistically significant increases in IgG_4 levels were found for all but 1 of the Der p 1 peptides (ie, peptide 1), which were associated with the most significant increases in IgG_4 against peptides 6, 7, and 8 (Fig 4). Thus, no induction of IgG_4 responses against peptide 1, which is part of the Der p 1 IgE-binding region, was found. Der p 2–specific IgG_4 antibodies induced during immunotherapy were directed against peptides 2 and 3 and most significantly against peptides 4 and 5 (Fig 4). No development of IgG_4 responses against peptide 1 from Der p 2, which is part of the IgE epitope–containing region, was found. Der p 23–specific IgG_4 antibodies recognized mainly peptide 3, which represents an IgE-reactive part of the allergen[44] (Fig 4). We therefore found that AIT-induced IgG_4 was directed against some but not all IgE-binding regions of the allergens and also reacted with areas not relevant for IgE binding.

AIT-induced IgG antibodies inhibit mainly Der p 1–, Der p 2–, and Der p 23–induced basophil activation

Next, we investigated whether AIT-induced IgG antibodies can inhibit allergen-induced basophil activation (Fig 5 and see Fig E5 in this article's Online Repository at www.jacionline.org). Examples are shown for 9 patients from the treatment group (HDMIT group), which showed IgE reactivity not only to the major HDM allergens, Der p 1, Der p 2, and Der p 23, but also to 1 or more of the other HDM allergens (Fig 5 and see Fig E5). The detailed analysis showed that an inhibition of basophil activation by post-AIT sera was usually associated with the induction of allergen-specific IgG antibodies (Fig 5 and see Fig E5).

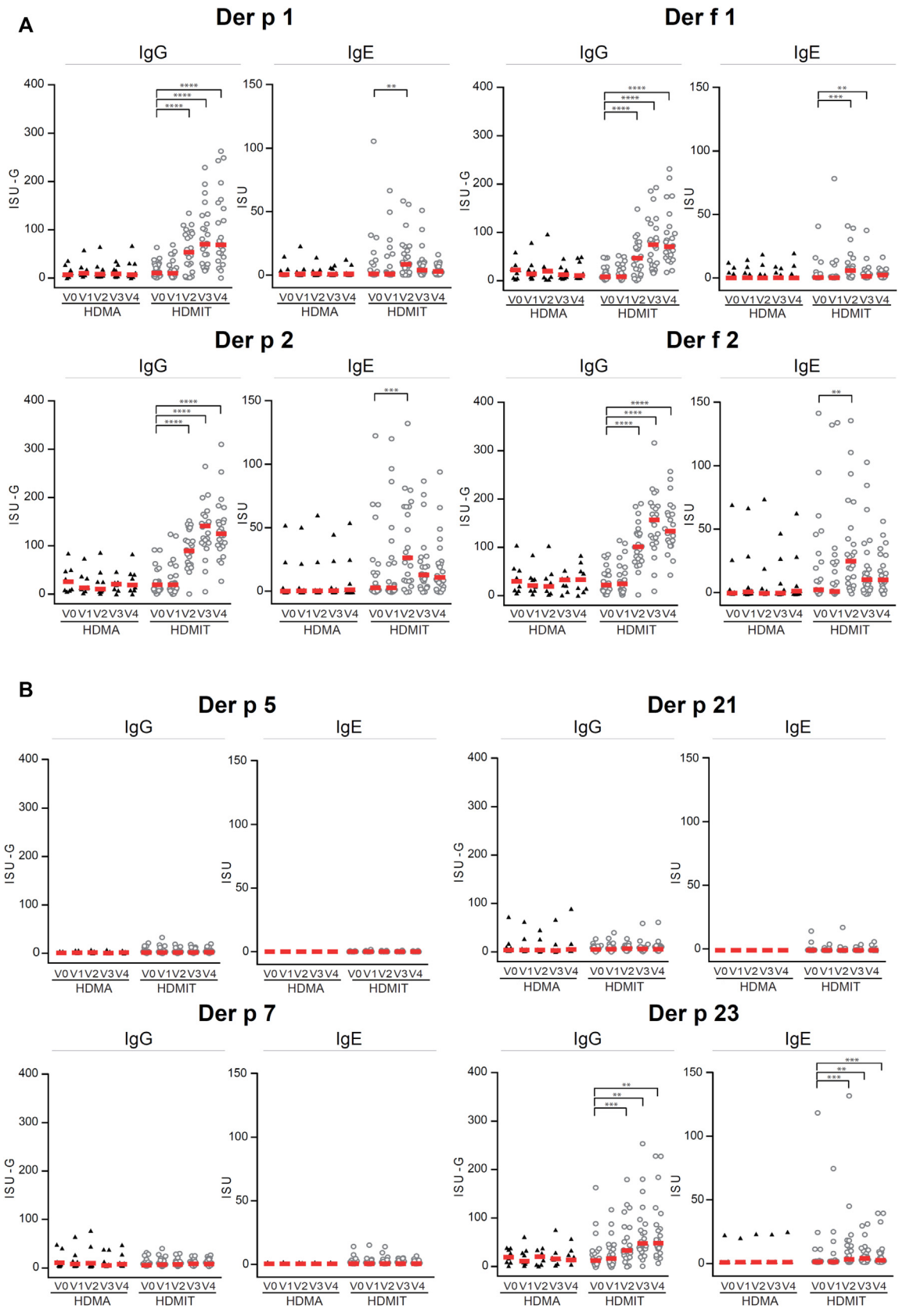


FIG 2. IgG and IgE reactivity to HDM allergen molecules before and during AIT. IgE and IgG levels (*y* axes show IgE in terms of standardized quality unit [ISU] and ISAC standardized quality unit for IgG antibodies [ISU-G]) to Der p 1, Der p 2, Der f 1, and Der f 2 (**A**) and Der p 5, Der p 7, Der p 21, and Der p 23 (**B**), as measured in sera of those patients in the HDMIT group (*gray dots* [n = 24]) and in the sera of those patients in the HDMA group (*black triangles* [n = 9]), as determined by ISAC at different visits (*x* axes show values at V0 [baseline], V1 [7 weeks], V2 [15 weeks], V3 [33 weeks], and V4 [1 year]). Red horizontal bars indicate median values. Statistically significant differences are indicated as follows: **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001.

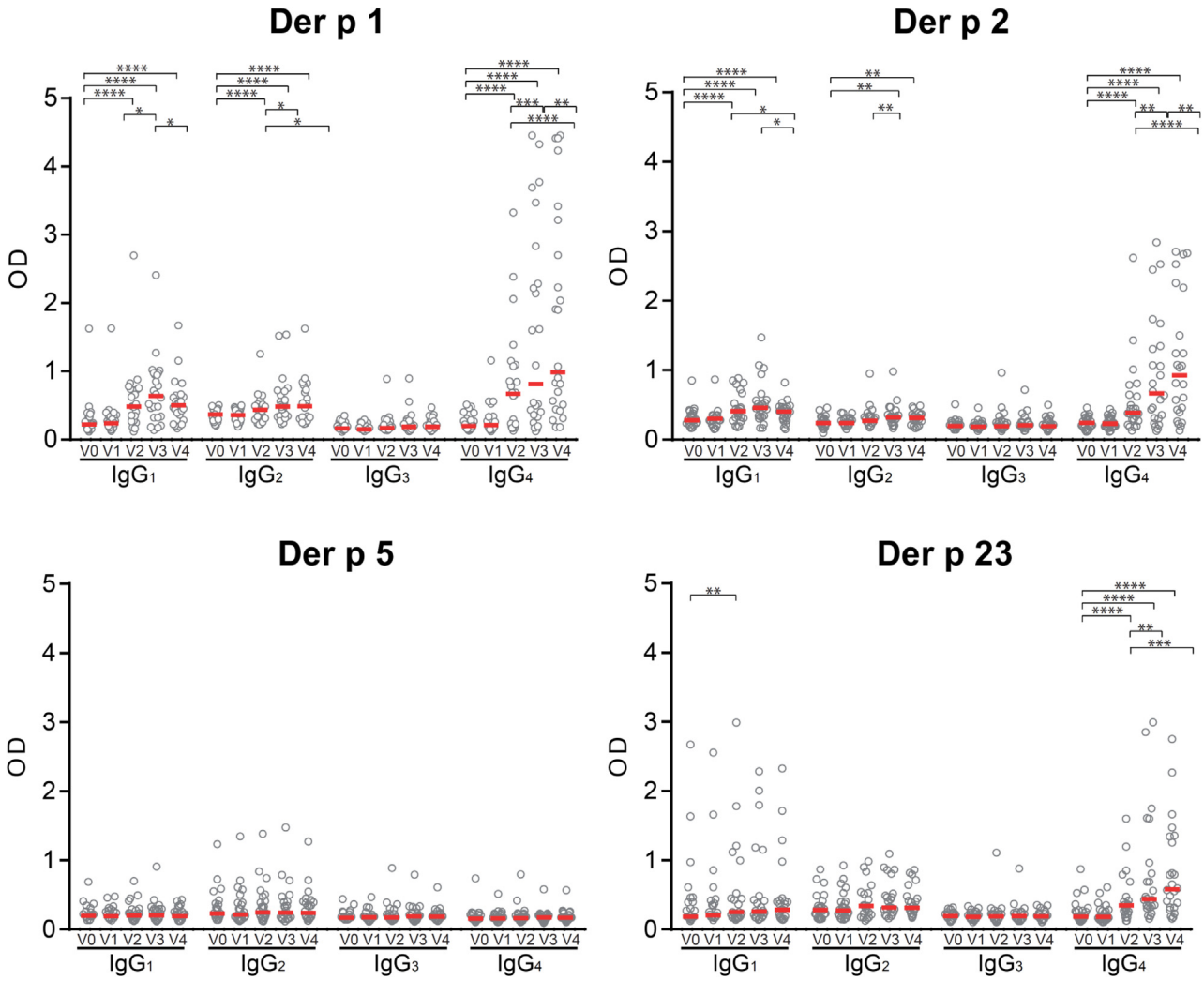


FIG 3. Development of allergen-specific IgG subclass responses in sera from patients in the HDMIT group during AIT. IgG_1, IgG_2, IgG_3, and IgG_4 levels to Der p 1, Der p 2, Der p 5, and Der p 23 (*y* axes show OD values) at different visits (*x* axes show values at V0 [baseline], V1 [7 weeks], V2 [15 weeks], V3 [33 weeks], and V4 [1 year]). Red horizontal bars indicate median values. Statistically significant differences are indicated as follows: $*P < .05$; $**P < .01$; $***P < .001$; $****P < .0001$.

Accordingly, the post-AIT sera inhibited mainly Der p 1– and Der p 2–induced basophil activation (Fig 5, *A* and *B* and see Fig E5, *A-G*). In the presence of AIT-induced IgG antibodies (V4) an approximately 10-fold concentration of allergen was necessary to induce basophil degranulation as compared with the concentration in serum obtained at baseline (at V0) (Fig 5 and see Fig E5, *A-G*). An inhibition of Der p 23–induced basophil activation was noted only for certain Der p 23–sensitized subjects (Fig 5, *B* and see Fig E5, *A*, *C*, and *E*) but not for others (Fig 5, *A* and see Fig E5, *B*, *D*, *F*, and *G*). Inhibition of basophil activation was observed for Der p 7 in only 2 subjects (see Fig E5, *A* and *B*) but not for the other allergens (ie, Der p 5 and Der p 21) and not for other subjects. Der p 4 showed no or only very low allergenic activity when basophil activation was tested in sensitized patients (patients 009, 019, 021, 027, 029, 033, 037, and 038 [see Table E2 and Fig E5, *H*]).

Evidence that AIT with Alutard SQ 510 is clinically less effective in patients with IgE reactivity to Der p 5, Der p 7, Der p 21, and/or Der p 23

CSMSs and VAS scores were assessed for each patient at each visit (V0-V4), and then the percentages of reduction of CSMSs and VAS scores between the baseline (V0) and the last visit (at 1 year [V4]) were calculated for each of the patients in the HDMIT group. Patients were then divided in groups with a reduction of more or less than 50% in their CSMS (Fig 6 [*upper part*]) or in groups with a reduction of more or less than 50% in their VAS score (Fig 6 [*lower part*]). We found that among patients with either a CSMS (n = 16) or a VAS score reduction less than 50% (n = 14) the frequencies of IgE binding to Der p 5, Der p 7, Der p 21, and Der p 23 were higher than in patients with a CSMS (n = 5) or VAS score reduction greater than 50% (n = 10), although no statistically

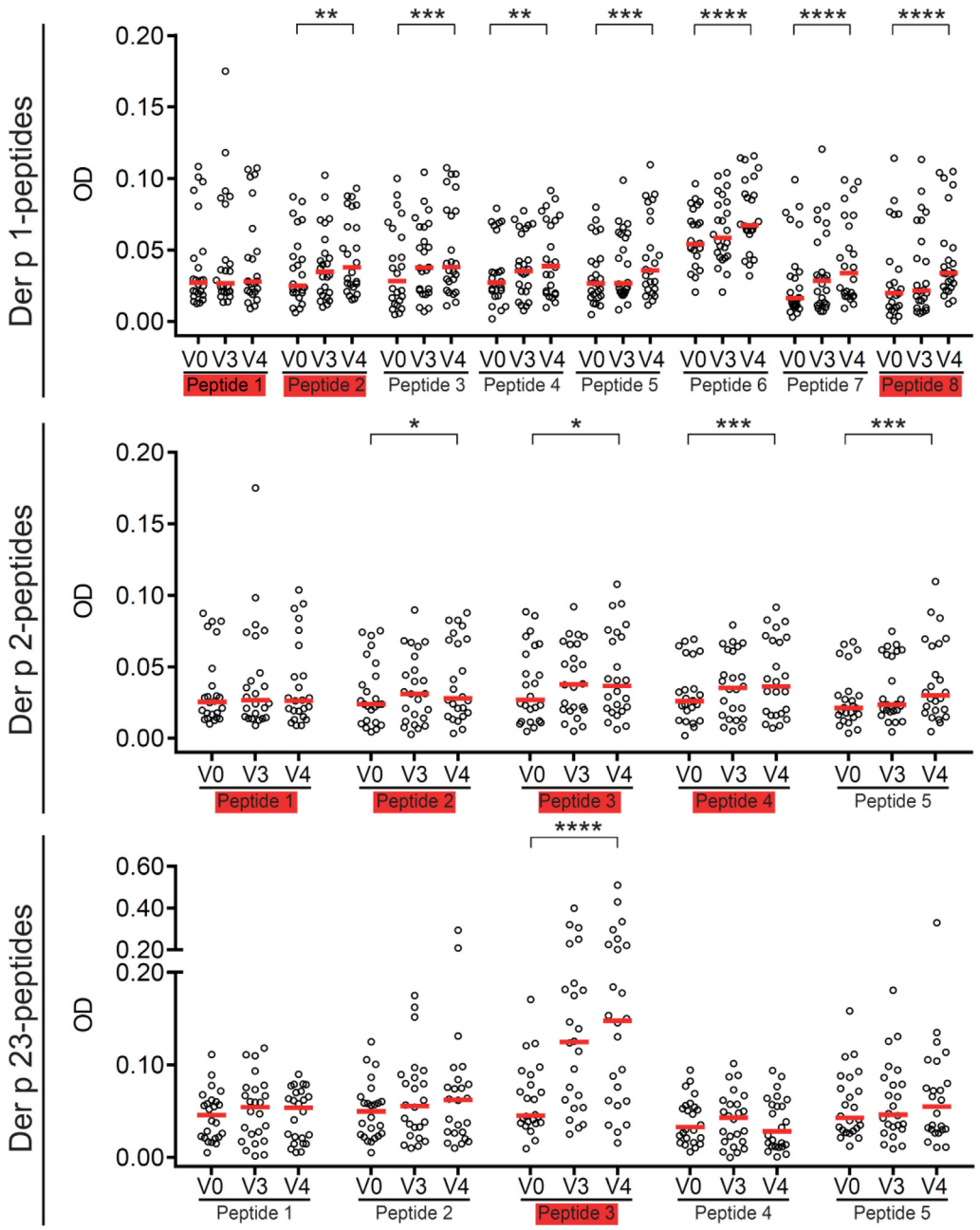
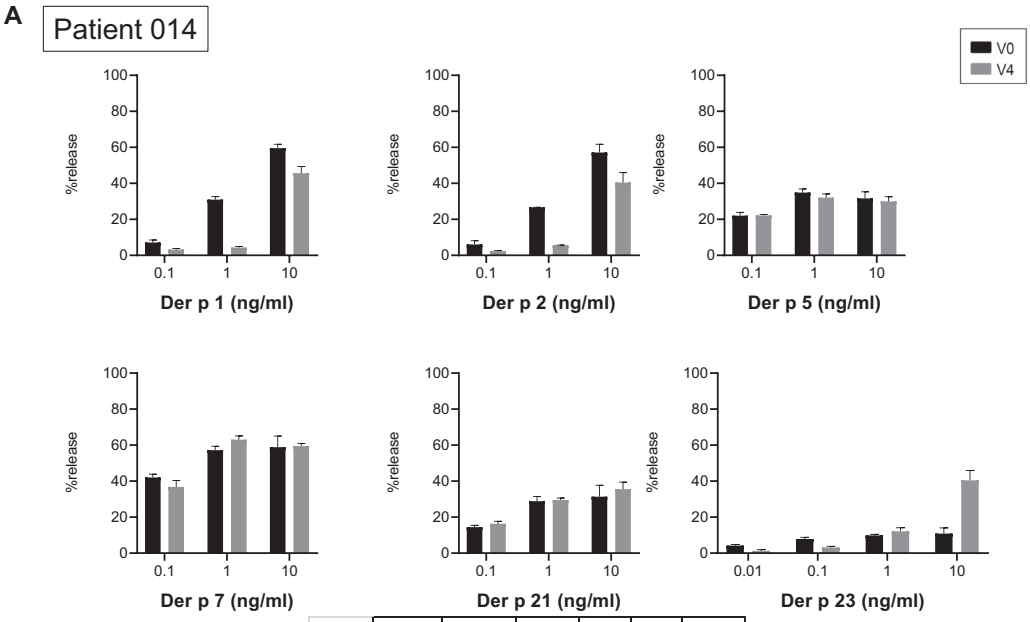


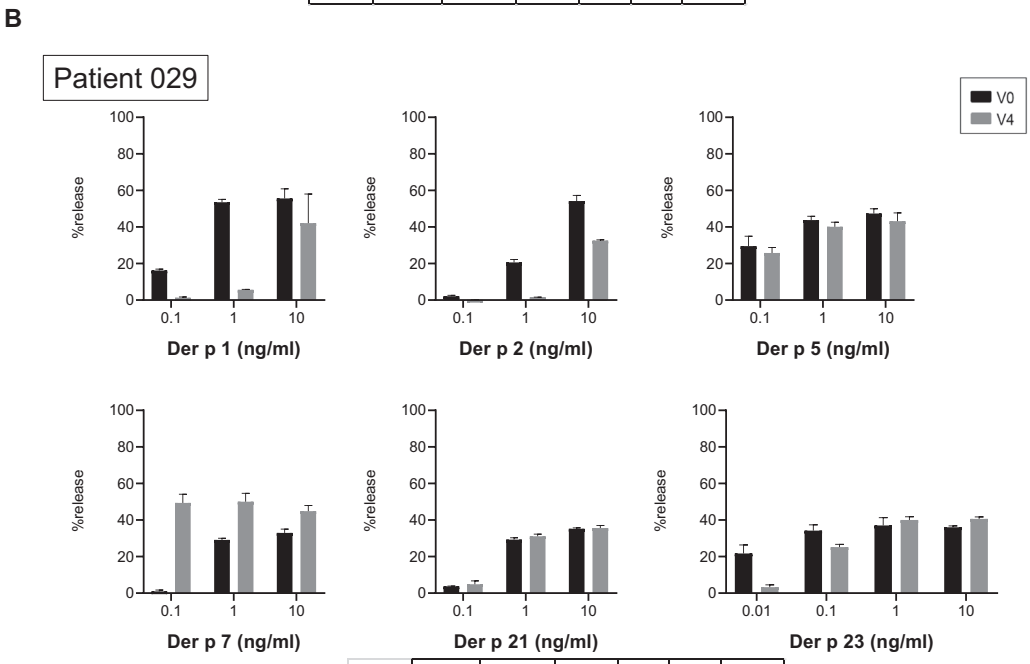
FIG 4. Development of IgG_4 responses to allergen-derived peptides in sera from patients in the HDMIT group during AIT. IgG_4 levels (*y* axes show OD levels) to Der p 1–, Der p 2–, and Der p 23–derived peptides (see Table E1) are displayed for different visits (*x* axes show values at V0 [baseline], V3 [33 weeks], and V4 [1 year]). Peptides that, on immunization of animals, have induced IgG antibodies blocking patients' IgE binding to an extent greater than 40%, as indicated in Table E1, are printed in red. Horizontal red bars indicate median values. Statistically significant differences are indicated as follows: $*P < .05$; $**P < .01$; $***P < .001$; $****P < .0001$.

significant differences were found (Fig 6). None of the patients with IgE reactivity to Der p 5 or Der p 21 had a CSMS reduction greater than 50%. Likewise, no VAS score reduction greater than 50% was found for patients with sensitization to Der p 5 or Der p 21 (Fig 6). Similarly, there was no relevant VAS score reduction for the 2 patients (ie, patients 008 and 024) who were negative for Der p 1 and Der p 2, and no relevant reduction in CSMS was found for 1 of the patients with available data (ie, patient 024) (see Table E2).

Patients who were sensitized exclusively to Der p 1 and/or Der p 2 (Fig E6, *A* in this article's Online Repository at www.jacionline.org) but not to any of the other important HDM allergens (ie, Der p 5, Der p 7, Der p 21, and Der p 23) showed a greater reduction in symptoms after 1 year of treatment (a median VAS score reduction of 59.33% [n = 10] and median CSMS reduction of 38.24% [n =9]) than did patients with additional sensitizations to Der p 5, Der p 7, Der p 21, and/or Der p 23 (a median VAS score reduction of 39.77% [n = 14] and median CSMS reduction of



ISU-G	Der p 1	Der p 2	Der p 5	Der p 7	Der p 21	Der p 23
V0	29.84	91.53	12.23	8.2	8.19	16.35
V4	43.51	153.96	11.02	6.25	5.86	44.00



ISU-G	Der p 1	Der p 2	Der p 5	Der p 7	Der p 21	Der p 23
V0	3.65	13.36	1.96	1.66	3.31	10.46
V4	51.07	122.22	2.22	1.87	4.33	65.18

FIG 5. Effects of treatment-induced IgG antibodies on allergen-induced basophil activation. Basophils were loaded with baseline serum IgE from patient 014 (**A**) and patient 029 (**B**) and then exposed to increasing concentrations of HDM allergen molecules (*x* axes show concentrations of 0.1 ng/mL, 1 ng/mL, and 10 ng/mL) that had been preincubated with serum obtained before AIT (V0 [*black bars*]) or after 1 year of AIT (V4 [*gray bars*]). Percentages of total β-hexosaminidase release are shown on the *y* axes (means ± SDs). Allergen-specific IgG levels measured at V0 and V4 are shown for each of the patients. *ISU-G*, ISAC standardized quality unit for IgG antibodies.

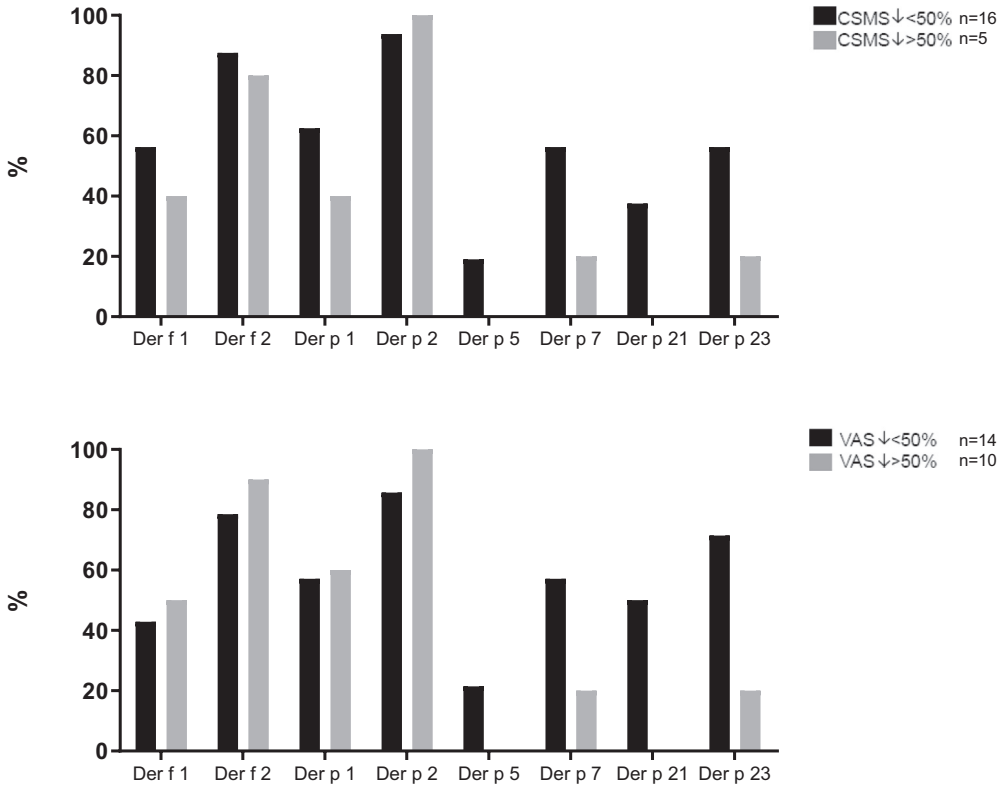


FIG 6. Percentages of patients with IgE reactivity (*y* axes) to the clinically relevant HDM allergen molecules (Der f 1, Der f 2, Der p 1, Der p 2, Der p 5, Der p 7, Der p 21, and Der p 23) (*x* axes) in the group with more (*gray bars*) or less (*black bars*) than a 50% reduction in CSMS and VAS scores between V0 (baseline) and V4 (1 year). The percentages of reduction were calculated as $\frac{(100 \times (V4 - V0)}{V0}$ and are shown for each patient in Table E2. Numbers of patients analyzed are indicated in the figure. Two patients without IgE sensitization to allergen molecules (patients 008 and 024) were not included.

17.89% [n = 12]) (Fig E6, *B*). However, these differences were also not significant. The VAS score and CSMS alterations of patients from the HDMA group who had not received AIT were different from the those of patients in the HDMIT group, with no advantage for patients sensitized only to Der p 1 and/or Der p 2 (see Table E3).

DISCUSSION

This is the first real-life study indicating that it may be possible to enhance the outcome HDM AIT by using a personalized approach based on the selection of patients according to their molecular sensitization profiles and by molecular monitoring of the development of AIT-induced allergen-specific IgG responses. We found that AIT with Alutard-SQ 510 induced allergen-specific IgG responses mainly against Der p 1 and Der p 2 and, to lower extent, to Der p 23 whereas no IgG responses to the other 3 important HDM allergens, Der p 5, Der p 7, and Der p 21, were detectable even after 1 year of treatment. The manufacturer of Alutard-SQ 510 does not provide information regarding the contents of the individual HDM allergens in the vaccine. However, Casset et al have shown that HDM SPT extracts from the company that produced Alutard-SQ 510 contained Der p 1 and/or Der p 2 and little Der p 7 but lacked Der p 5 and Der p 21.[15] Similar findings were made for natural HDM extracts produced by other companies,[15] and the analysis of an HDM AIT study also showed that there was no induction of IgG antibodies against Der p 5, Der p 7, and Der p 21.[28] It is thus conceivable that natural HDM allergen extracts contain much less Der p 5, Der p 7, Der p 21, and Der p 23 than Der p 1 and Der p 2.

The SCIT-induced allergen-specific IgG response consisted of a specific IgG_1 and IgG_2 response that was maintained during treatment and a continuously rising IgG_4 response similar to that observed for pollen AIT and AIT with the recombinant grass pollen allergy vaccine BM32.[46] Support for a beneficial role of the SCIT-induced IgG response comes from our finding that the specific IgG that developed during SCIT reduced allergen-specific basophil activation. Because our results were obtained with basophils, which do not express inhibitory Fcγ receptors, the mechanism behind the reduction of allergen-specific basophil activation is most likely competition of IgG with IgE for allergen binding. Furthermore, suppression of allergen-induced basophil activation was associated with a reduction of allergen-specific IgE binding during testing with low amounts of microarrayed allergens, an *in vitro* assay that has been described to visualize the competition of blocking IgG antibodies with specific IgE.[47,48] According to recent studies, it is also possible that SCIT-induced IgG antibodies may reduce effector cell activation by crosslinking the allergen/IgE/IgE receptor complex with the IgG-inhibitory receptor FcγRIIb,[49-51] although this mechanism was found to not be of relevance for Alutard-based AIT vaccines.[52]

Previous studies have shown that adult patients with allergy do not develop new IgE sensitizations to respiratory allergens in the natural course of disease.[13,53] Interestingly, we found that SCIT

with Alutard-SQ 510 also induced a *de novo* development of allergen-specific IgE in patients who were not sensitized against these allergens. We found that these *de novo*–induced allergen-specific IgE antibodies could lead to specific basophil activation when loaded onto basophils, but we have no evidence that they may be harmful considering that at the same time much higher levels of allergen-specific IgG antibodies appeared in the blood.

However, the most important finding from our real-life study was that SCIT with Alutard-SQ 510 induced only a partially protective IgG antibody response. SCIT-induced IgG responses were directed mainly against Der p 1 and Der p 2, and to a lesser extent to Der p 23, but not to the other 3 important HDM allergens, Der p 5, Der p 7, and Der p 21. Der p 5, Der p 7, Der p 21, and Der p 23, but not Der p 4, must be considered clinically relevant allergens for our patients because they induced strong basophil degranulation even when specific IgE levels were low. In this context, it should be noted that several other factors besides specific IgE levels such as structural allergen determinants (eg, number and proximity of IgE epitopes), as well as avidities of IgE antibodies, are important for the allergenic activity of an allergen.[54,55] We even noted that not all of the Der p 1– and Der p 2–specific IgG antibodies induced by SCIT were directed against the major IgE binding sites of the allergens. Similar findings were recently made for patients who had received SCIT with a grass pollen allergy vaccine and a new recombinant grass pollen vaccine, BM32, which is based on carrier-bound peptides derived from the major IgE binding sites of the allergens.[56,57] The comparison of IgG responses obtained by using the extract-based vaccine with the responses induced by BM32 showed that BM32 focused IgG responses against the IgE binding sites whereas the allergen extract–based vaccine also induced IgG against less relevant epitopes.[57]

Alutard-SQ 510 induced protective IgG mainly to Der p 1 and/or Der p 2 and, to some extent, to Der p 23. The comparison of clinical outcomes according to CSMS and VAS score assessment indeed indicated that patients who are sensitized only to Der p 1 and/or Der p 2 benefited more from this treatment than patients who were also sensitized to other clinically relevant HDM allergens such as Der p 5, Der p 7, Der p 21, and Der p 23, which are not cross-reactive with Der p 1 and/or Der p 2.[58] Der p 4 was not considered a clinically relevant allergen because it did not induce relevant basophil degranulation in our patients. Other explanations for the differences in clinical outcomes are unlikely. First of all, the assessment of CSMS and VAS score was performed outside pollen seasons, and our patients did not have relevant sensitizations against other indoor allergens that played a role. Another possibility is that patients benefiting less from AIT had higher IgE levels against Der p 1 and/or Der p 2 and the therapy-induced IgG could block IgE binding in these patients less. However, this is not the case because after AIT basophil sensitivity decreased in all Der p 1– and Der p 2–sensitized patients approximately 10-fold regardless of Der p 1– and/or Der p 2–specific IgE levels. We therefore attribute the lower success rate of AIT in the patients sensitized to Der p 1 and/or Der p 2 and additional allergens to the lack of induction of protective IgG antibodies against the additional allergens.

It is a limitation of our study that only a limited number of patients could be studied. Nevertheless, the results obtained in this real-life study and those from an earlier HDM immunotherapy study[28] would suggest that one could increase the clinical improvement in HDM-specific SCIT by using a personalized medicine approach that is either based on the stratification of patients with HDM allergy by molecular allergy diagnosis in patients who are mainly sensitized to Der p 1 and/or Der p 2 and/or by monitoring of SCIT by molecular measurement of IgG responses. It might therefore be possible to improve the success rate of HDM AIT with Alutard-SQ 510 by selecting for treatment those patients who show IgE reactivity mainly to Der p 1 and/or Der p 2 but not to Der p 5, Der p 7, and/or Der p 21. In addition, one may consider continuing treatment with other AIT vaccines if it turns out that the AIT vaccine that had been selected as the first option did not induce IgG antibodies against all the relevant allergens needed. Our results may be extendable to other populations with HDM allergy because they show IgE reactivity profiles that are similar to those of the patients in our study.[59-61] Furthermore, our results may also be applicable to other AIT vaccines because similar results were obtained for patients in a study of HDM AIT.[28] Of course, larger clinical studies with more patients with HDM allergy will be needed to confirm our initial results, but our study provides the first real-life evidence for such a personalized concept for HDM-specific AIT practice.

Clinical implications: Molecular diagnosis and molecular monitoring may improve the success of HDM-specific AIT in real life through a personalized approach.

REFERENCES

1. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol 2006;6:761-71.
2. Shamji MH, Durham SR. Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. J Allergy Clin Immunol 2017;140:1485-98.
3. Cox L. Allergy immunotherapy in reducing healthcare cost. Curr Opin Otolaryngol Head Neck Surg 2015;23:247-54.
4. Incorvaia C, Gritti BL, Ridolo E. The economic advantage of allergen immunotherapy over drug treatment in respiratory allergy. J Med Econ 2018;21:553-5.
5. Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Host A, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. Allergy 2007;62:943-8.
6. Valenta R, Campana R, Focke-Tejkl M, Niederberger V. Vaccine development for allergen-specific immunotherapy based on recombinant allergens and synthetic allergen peptides: Lessons from the past and novel mechanisms of action for the future. J Allergy Clin Immunol 2016;137:351-7.
7. Kiel MA, Röder E, Gerth van Wijk R, Al MJ, Hop WCJ, Rutten-van Mölken MPMH. Real-life compliance and persistence among users of subcutaneous and sublingual allergen immunotherapy. J Allergy Clin Immunol 2013;132:353-60.e2.
8. Valenta R, Karaulov A, Niederberger V, Zhernov Y, Elisyutina O, Campana R, et al. Allergen extracts for in vivo diagnosis and treatment of allergy: is there a future? J Allergy Clin Immunol Pract 2018;6:1845-55.e2.
9. Thomas WR. House dust mite allergens: new discoveries and relevance to the allergic patient. Curr Allergy Asthma Rep 2016;16:69.
10. Fernandez-Caldas E, Puerta L, Caraballo L. Mites and allergy. Chem Immunol Allergy 2014;100:234-42.
11. Acevedo N, Zakzuk J, Caraballo L. House dust mite allergy under changing environments. Allergy Asthma Immunol Res 2019;11:450-69.
12. Chevigne A, Jacquet A. Emerging roles of the protease allergen Der p 1 in house dust mite-induced airway inflammation. J Allergy Clin Immunol 2018;142:398-400.
13. Posa D, Perna S, Resch Y, Lupinek C, Panetta V, Hofmaier S, et al. Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life. J Allergy Clin Immunol 2016;139(2):541-9.
14. Huang HJ, Resch-Marat Y, Rodriguez-Dominguez A, Chen KW, Kiss R, Zieglmayer P, et al. Underestimation of house dust mite-specific IgE with extract-based ImmunoCAPs compared with molecular ImmunoCAPs. J Allergy Clin Immunol 2018;142(5):1656-8.
15. Casset A, Mari A, Purohit A, Resch Y, Weghofer M, Ferrara R, et al. Varying allergen composition and content affects the in vivo allergenic activity of

commercial Dermatophagoides pteronyssinus extracts. Int Arch Allergy Immunol 2012;159:253-62.
16. Park KH, Lee SC, Son YW, Jeong KY, Shin YS, Shin JU, et al. Different responses in induction of allergen specific immunoglobulin G4 and IgE-blocking factors for three mite subcutaneous immunotherapy products. Yonsei Med J 2016;57:1427-34.
17. Rondón C, Campo P, Salas M, Aranda A, Molina A, González M, et al. Efficacy and safety of D. pteronyssinus immunotherapy in local allergic rhinitis: a double-blind placebo-controlled clinical trial. Allergy 2016;71:1057-61.
18. Pfaar O, Nell MJ, Boot JD, Versteeg SA, van Ree R, Roger A, et al. A randomized, 5-arm dose finding study with a mite allergoid SCIT in allergic rhinoconjunctivitis patients. Allergy 2016;71:967-76.
19. Bousquet J, Lockey R, Malling HJ, Alvarez-Cuesta E, Canonica GW, Chapman MD, et al. Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American Academy of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 1998;81:401-5.
20. Eifan AO, Calderon MA, Durham SR. Allergen immunotherapy for house dust mite: clinical efficacy and immunological mechanisms in allergic rhinitis and asthma. Expert Opin Biol Ther 2013;13:1543-56.
21. Varney VA, Tabbah K, Mavroleon G, Frew AJ. Usefulness of specific immunotherapy in patients with severe perennial allergic rhinitis induced by house dust mite: a double-blind, randomized, placebo-controlled trial. Clin Exp Allergy 2003;33:1076-82.
22. Frew AJ, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006;117(2):319-25.
23. Lai X, Li J, Xiao X, Liu E, Zhang C, Wang H, et al. Specific IgG4 production during house dust mite immunotherapy among age, gender and allergic disease populations. Int Arch Allergy Immunol 2013;160:37-46.
24. Zhao D, Lai X, Tian M, Jiang Y, Zheng Y, Gjesing B, et al. The functional IgE-blocking factor induced by allergen-specific immunotherapy correlates with igg4 antibodies and a decrease of symptoms in house dust mite-allergic children. Int Arch Allergy Immunol 2016;169:113-20.
25. Shamji MH, Kappen JH, Akdis M, Jensen-Jarolim E, Knol EF, Kleine-Tebbe J, et al. Biomarkers for monitoring clinical efficacy of allergen immunotherapy for allergic rhinoconjunctivitis and allergic asthma: an EAACI position paper. Allergy 2017;72(8):1156-73.
26. Shamji MH, Kappen J, Abubakar-Waziri H, Zhang J, Steveling E, Watchman S, et al. Nasal allergen-neutralizing IgG_4 antibodies block IgE-mediated responses: novel biomarker of subcutaneous grass pollen immunotherapy. J Allergy Clin Immunol 2019;143:1067-76.
27. Orengo JM, Radin AR, Kamat V, Badithe A, Ben LH, Bennett BL, et al. Treating cat allergy with monoclonal IgG antibodies that bind allergen and prevent IgE engagement. Nat Commun 2018;9:1421.
28. Chen KW, Zieglmayer P, Zieglmayer R, Lemell P, Horak F, Bunu CP, et al. Selection of house dust mite-allergic patients by molecular diagnosis may enhance success of specific immunotherapy. J Allergy Clin Immunol 2018;143(3):1248-52.
29. Berings M, Gevaert P, De Ruyck N, Derycke L, Holtappels G, Pilette C, et al. FcepsilonRI expression and IgE binding by dendritic cells and basophils in allergic rhinitis and upon allergen immunotherapy. Clin Exp Allergy 2018;48(8):970-80.
30. Berings M, Arasi S, De Ruyck N, Perna S, Resch Y, Lupinek C, et al. Reliable mite-specific IgE testing in nasal secretions by means of allergen microarray. J Allergy Clin Immunol 2017;140(1):301-3.
31. Lupinek C, Wollmann E, Baar A, Banerjee S, Breiteneder H, Broecker BM, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. Methods 2014;66:106-19.
32. Jarnicki AG, Thomas WR. Stimulatory and inhibitory epitopes in the T cell responses of mice to Der p 1. Clin Exp Allergy 2002;32:942-50.
33. Weghofer M, Grote M, Dall'Antonia Y, Fernandez-Caldas E, Krauth MT, van Hage M, et al. Characterization of folded recombinant Der p 5, a potential diagnostic marker allergen for house dust mite allergy. Int Arch Allergy Immunol 2008;147:101-9.
34. Weghofer M, Dall'Antonia Y, Grote M, Stocklinger A, Kneidinger M, Balic N, et al. Characterization of Der p 21, a new important allergen derived from the gut of house dust mites. Allergy 2008;63:758-67.
35. Weghofer M, Grote M, Resch Y, Casset A, Kneidinger M, Kopec J, et al. Identification of Der p 23, a peritrophin-like protein, as a new major Dermatophagoides pteronyssinus allergen associated with the peritrophic matrix of mite fecal pellets. J Immunol 2013;190:3059-67.
36. Focke M, Mahler V, Ball T, Sperr WR, Majlesi Y, Valent P, et al. Nonanaphylactic synthetic peptides derived from B cell epitopes of the major grass pollen allergen, Phl p 1, for allergy vaccination. FASEB J 2001;15:2042-4.
37. Huang HJ, Curin M, Banerjee S, Chen KW, Garmatiuk T, Resch-Marat Y, et al. A hypoallergenic peptide mix containing T cell epitopes of the clinically relevant house dust mite allergens. Allergy 2019; https://doi.org/10.1111/all.13956.
38. Pree I, Reisinger J, Focke M, Vrtala S, Pauli G, van Hage M, et al. Analysis of epitope-specific immune responses induced by vaccination with structurally folded and unfolded recombinant Bet v 1 allergen derivatives in man. J Immunol 2007;179:5309-16.
39. Reisinger J, Horak F, Pauli G, van Hage M, Cromwell O, Konig F, et al. Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. J Allergy Clin Immunol 2005;116:347-54.
40. Flicker S, Steinberger P, Norderhaug L, Sperr WR, Majlesi Y, Valent P, et al. Conversion of grass pollen allergen-specific human IgE into a protective IgG(1) antibody. Eur J Immunol 2002;32:2156-62.
41. Madritsch C, Flicker S, Scheiblhofer S, Zafred D, Pavkov-Keller T, Thalhamer J, et al. Recombinant monoclonal human immunoglobulin E to investigate the allergenic activity of major grass pollen allergen Phl p 5. Clin Exp Allergy 2011;41:270-80.
42. Nakamura R, Uchida Y, Higuchi M, Tsuge I, Urisu A, Teshima R. A convenient and sensitive allergy test: IgE crosslinking-induced luciferase expression in cultured mast cells. Allergy 2010;65:1266-73.
43. Gieras A, Focke-Tejkl M, Ball T, Verdino P, Hartl A, Thalhamer J, et al. Molecular determinants of allergen-induced effector cell degranulation. J Allergy Clin Immunol 2007;119:384-90.
44. Banerjee S, Weber M, Blatt K, Swoboda I, Focke-Tejkl M, Valent P, et al. Conversion of Der p 23, a new major house dust mite allergen, into a hypoallergenic vaccine. J Immunol 2014;192:4867-75.
45. Chen KW, Focke-Tejkl M, Blatt K, Kneidinger M, Gieras A, Dall'Antonia F, et al. Carrier-bound nonallergenic Der p 2 peptides induce IgG antibodies blocking allergen-induced basophil activation in allergic patients. Allergy 2012;67:609-21.
46. Eckl-Dorna J, Weber M, Stanek V, Linhart B, Ristl R, Waltl EE, et al. Two years of treatment with the recombinant grass pollen allergy vaccine BM32 induces a continuously increasing allergen-specific IgG4 response. EBioMedicine 2019;1-12.
47. Wollmann E, Lupinek C, Kundi M, Selb R, Niederberger V, Valenta R. Reduction in allergen-specific IgE binding as measured by microarray: a possible surrogate marker for effects of specific immunotherapy. J Allergy Clin Immunol 2015;136:806-9.e7.
48. Schmid JM, Würtzen PA, Dahl R, Hoffmann HJ. Pretreatment IgE sensitization patterns determine the molecular profile of the IgG4 response during updosing of subcutaneous immunotherapy with timothy grass pollen extract. J Allergy Clin Immunol 2016;137:562-70.
49. Burton OT, Logsdon SL, Zhou JS, Medina-Tamayo J, Abdel-Gadir A, Rivas MN, et al. Oral immunotherapy induces IgG antibodies that act via FcγRIIb to suppress IgE-mediated hypersensitivity. J Allergy Clin Immunol 2014;134:1310-7.e6.
50. Burton OT, Tamayo JM, Stranks AJ, Koleoglou KJ, Oettgen HC. Allergen-specific IgG antibody signaling through FcgammaRIIb promotes food tolerance. J Allergy Clin Immunol 2018;141:189-201. e3.
51. Zha L, Leoratti FMS, He L, Mohsen MO, Cragg M, Storni F, et al. An unexpected protective role of low-affinity allergen-specific IgG through the inhibitory receptor FcγRIIb. J Allergy Clin Immunol 2018;142:1529-36.e6.
52. Ejrnaes AM, Svenson M, Lund G, Larsen JN, Jacobi H. Inhibition of rBet v 1-induced basophil histamine release with specific immunotherapy -induced serum immunoglobulin G: no evidence that FcgammaRIIB signalling is important. Clin Exp Allergy 2006;36:273-82.
53. Lupinek C, Marth K, Niederberger V, Valenta R. Analysis of serum IgE reactivity profiles with microarrayed allergens indicates absence of de novo IgE sensitizations in adults. J Allergy Clin Immunol 2012;130:1418-20.e4.
54. Gieras A, Linhart B, Roux KH, Dutta M, Khodoun M, Zafred D, et al. IgE epitope proximity determines immune complex shape and effector cell activation capacity. J Allergy Clin Immunol 2016;137:1557-65.
55. Valenta R, Karaulov A, Niederberger V, Gattinger P, van Hage M, Flicker S, et al. Molecular aspects of allergens and allergy. Adv Immunol 2018;138:195-256.
56. Focke-Tejkl M, Weber M, Niespodziana K, Neubauer A, Huber H, Henning R, et al. Development and characterization of a recombinant, hypoallergenic, peptide-based vaccine for grass pollen. J Allergy Clin Immunol 2015;135, 1207-7.e11.
57. Rauber MM, Möbs C, Campana R, Henning R, Schulze-Dasbeck M, Greene B, et al. Allergen immunotherapy with the hypoallergenic B-cell epitope-based vaccine BM32 modifies IL-10- and IL-5-secreting T cells. Allergy 2019; https://doi.org/10.1111/all.13996.
58. Thomas WR. IgE and T-cell responses to house dust mite allergen components. Mol Immunol 2018;100:120-5.
59. Weghofer M, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, et al. Variability of IgE reactivity profiles among European mite allergic patients. Eur J Clin Invest 2008;38:959-65.
60. Siroux V, Lupinek C, Resch Y, Curin M, Just J, Keil T, et al. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: the EGEA study. J Allergy Clin Immunol 2016.
61. Resch Y, Michel S, Kabesch M, Lupinek C, Valenta R, Vrtala S. Different IgE recognition of mite allergen components in asthmatic and nonasthmatic children. J Allergy Clin Immunol 2015;136:1083-91.