Published in final edited form as:

Clin Exp Allergy. 2019 February 7; 49(6): 808–818. doi:10.1111/cea.13361.

Clinical and immunological differences between asymptomatic HDM-sensitized and HDM-allergic rhinitis patients

Mihaela Zidarn^{1,2,iD}, Maša Robi¹, Anja Krivec¹, Mira Šilar¹, Yvonne Resch-Marat³, Susanne Vrtala³, Peter Kopa¹, Nissera Bajrovi¹, Rudolf Valenta^{3,4,5}, and Peter Korošec^{1,iD}

¹University Clinic of Pulmonary and Allergic Diseases Golnik, Golnik, Slovenia

²Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

³Division of Immunopathology, Department of Pathophysiology and Allergy Research, Centre for Pathophysiology, Infectiology, and Immunology, Medical University of Vienna, Vienna, Austria

⁴NRC Institute of Immunology FMBA of Russia, Moscow, Russia

⁵Laboratory for Immunopathology, Department of Clinical Immunology and Allergy, Sechenov First Moscow State Medical University, Moscow, Russia

Summary

Background—Confirmation of the clinical relevance of sensitisation is important for the diagnosis of allergic rhinitis.

Objective—To investigate the usefulness of an in vitro basophil activation test and component-resolved diagnosis in distinguishing between symptomatic allergic rhinitis patients and asymptomatic sensitization to house dust mites (HDMs).

Methods—Thirty-six subjects with a positive skin prick test (SPT) for HDM were divided into a symptomatic (n = 17) and an asymptomatic (n = 19) group on the basis of their clinical history and a nasal provocation test. A basophil CD63 response to in vitro stimulation with *Dermatophagoides pteronyssinus* whole allergen extract and the IgE reactivity profiles for Der p 1, 2, 4, 5, 7, 10, 11, 14, 15, 18, 21, 23 were evaluated. Serum IgE and IgG specific to *D pteronyssinus* whole allergen extract and total IgE were measured.

Results—There were no statistically significant differences in the levels of IgE (IgE levels were higher in symptomatic patients with $P = 0.055$) and IgG specific to *D pteronyssinus* and total IgE.

iDORCID

Mihaela Zidarn <https://orcid.org/0000-0003-0515-5207>

Peter Korošec <https://orcid.org/0000-0002-0835-1599>

Correspondence: Mihaela Zidarn, University Clinic of Pulmonary and Allergic Diseases Golnik, Golnik, Slovenia. mihaela.zidarn@klinika-golnik.si.

Conflicts of Interest

RV has received research funding from Biomay AG, Vienna, Austria and Viravaxx, Vienna, Austria and serves as a consultant for both companies. Other authors declare no conflict of interest.

Author Contributions

MZ and P Korošec designed the study, analysed the data and wrote the manuscript. MR and AK collected the data and wrote the first draft of the manuscript. MŠ, YR, SV and RV contributed to data collection, interpretation of the results and manuscript revision. MZ, P Kopač and NB recruited patients. All authors read and approved the final version of the manuscript.

Symptomatic patients showed a lower threshold for in vitro basophil activation (3.33 ng/mL vs 33.3 ng/mL), a higher area under the curve (AUC) of basophil activation (171 vs 127) ($P = 0.017$), a higher response to positive control with anti-FcεRI stimulation (97% vs 79%) ($P < 0.001$), a recognition of more HDM allergens (4 vs 2) and more frequent sensitization to rDer p 7 ($P = 0.016$) and rDer p 23 compared to asymptomatic subjects ($P = 0.018$). There was a positive correlation ($r = 0.63$; $P < 0.001$) between the number of recognized allergens and the AUC of basophil activation.

Conclusion and Clinical Relevance—In the subjects studied, the differences in the basophil response to *D pteronyssinus* allergen extract, number of recognized HDM allergens and reactivity to rDer p 7 and rDer p 23 distinguish symptomatic from asymptomatic HDM sensitisation better than SPT or allergen extract-specific IgE. Information regarding the clinical relevance of sensitization is important for the prescription of allergen-specific immunotherapy.

Keywords

allergic rhinitis; basophil activation; CD63; component-resolved diagnosis; house dust mite

1 Introduction

The majority of patients with allergic rhinitis world-wide suffer from house dust mite (HDM) allergy.¹ In Europe, the mean prevalence of sensitization to *Dermatophagoides pteronyssinus* is 21.7%.² However, 45% of subjects with a positive skin prick test (SPT) and/or positive level of serum sIgE for *D pteronyssinus* in a community-based population study did not report any allergic rhinitis (AR) symptoms.³ The diagnosis of HDM-allergic rhinitis consists of a medical history confirmed with positive SPT and/or sIgE. Patients who are allergic to indoor allergens may predominantly suffer from chronic obstruction with a discharge that is primarily post-nasal.⁴ A similar medical history is also typical for non-allergic rhinitis.⁵ SPTs are performed with standardized batteries of allergen extracts that include HDM extracts. We are therefore often faced with positive SPT results of unknown clinical significance. Neither the size of SPT nor the concentration of sIgE antibodies provides direct information regarding the likelihood of rhinitis and/or asthma symptoms upon allergen exposure.^{6,7} Asymptomatic sensitization is a significant risk factor for the later development of allergic rhinitis, but many cases of asymptomatic sensitization remain unexplained.⁸ Confirming the clinical significance of sensitization is crucial because allergen immunotherapy is an effective treatment for chronic rhinitis and/or asthma only in patients with clinically relevant IgE sensitization.⁹ An objective evaluation of the impact of IgE sensitization would also be important to guide anti-IgE therapy in severe asthma cases.

Nasal provocation tests (NPTs) can be used as a diagnostic confirmation of HDM-allergic rhinitis when discrepancies arise between patient medical histories and the results of SPT and/or serological tests.¹⁰ However, a NPT is not always feasible in clinical practice because the test is time-consuming, demands patient cooperation and requires specially trained personnel and the presence of a physician. No universally accepted NPT method is available.¹¹ Basophil activation tests have proven useful for evaluating asymptomatic sensitization to grass pollen.¹² It is now also possible to determine the IgE antibody pattern to individual allergen components using component-resolved diagnosis (CRD)¹³ to study

molecular reactivity profiles.¹⁴ Moreover, it has been shown that measurement of allergen-specific IgE levels with recombinant allergens is superior to allergen extracts.¹⁵ In vitro experiments have also shown that the composition and complexity of the allergen-specific IgE repertoire determine the efficiency of basophil activation.¹⁶

The aim of our study was to establish whether novel approaches, including in vitro basophil activation with a whole dose-response curve to assess basophil sensitivity and IgE reactivity to a broad panel of natural and recombinant allergen components, may aid in the differentiation between symptomatic AR and asymptomatic HDM sensitization. We performed basophil activation tests (BATs) with *D pteronyssinus* whole allergen extract and evaluated IgE reactivity for a broad panel of purified *D pteronyssinus* allergens (nDer p 1, rDer p 2, rDer p 4, rDer p 5, rDer p 7, rDer p 10, rDer p 11, rDer p 14, rDer p 15, rDer p 18, rDer p 21 and rDer p 23). Total IgE and *D pteronyssinus*-specific IgE and IgG in the serum were measured. Subjects were included on the basis of a clinical history and positive SPT to *D pteronyssinus* and/or *Dermatophagoides farinae*. For all subjects, the history was supplemented with a questionnaire, and the clinical relevance of sensitization was defined using a NPT with a HDM allergen extract.

2 Methods

2.1 Study participants

Subjects who were skin prick tested with a standard panel of inhalant allergens at the University Clinic for Respiratory and Allergic Diseases were screened for this study. Two hundred and eighty-eight subjects who tested positive for *D pteronyssinus* and/or *D farinae* allergy were identified. One hundred and eighteen of the 288 subjects had a diagnosis of allergic rhinitis (AR), 53 had a diagnosis of asthma, 21 had a diagnosis of dermatitis, 17 had a diagnosis of urticaria, and for 79 subjects, no diagnosis of allergic disease was recorded. Subjects diagnosed with AR were considered for inclusion in the group of symptomatic patients, and subjects without allergic diseases were considered for inclusion in the asymptomatic group. The exclusion criteria for the study were dermatitis, urticaria, asthma without rhinitis, a history of allergen-specific immunotherapy, anatomic abnormalities of the nasal cavity, acute inflammation of nasal and paranasal cavities and pregnancy.¹⁷ Forty eligible subjects responded to an invitation to be included in the study. To exclude potential mistakes in SPT performance or other reasons for false positivity (such as dermographism), SPTs were repeated at the start of the study, and sensitization was confirmed in all enrolled subjects. Subjects were divided into symptomatic and asymptomatic groups on the basis of a past medical history of AR documented at the referral for allergy testing. To further confirm the group allocation, subjects responded to an allergic rhinitis (AR) questionnaire (according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines)¹⁸ and a NPT test was performed.

From 18 symptomatic patients who had a positive retrospective history for AR, AR was confirmed using an AR questionnaire for all patients. One patient was excluded because of a negative NPT, whereas the remaining patients had a positive NPT. Four symptomatic patients also had a history of asthma but had no history of other allergic disease. Among 22 asymptomatic subjects who had a negative retrospective history for AR and no other allergic

disease, two subjects were excluded because of a positive AR questionnaire (the remainder had a negative AR questionnaire), and one subject was excluded because of a positive NPT (the remainder had a negative NPT). Fifteen control subjects without rhinitis symptoms, no other allergic disease and with a negative SPT for *D pteronyssinus*, *D farinae* and negative HDM NPT were selected among students and medical personnel. Two subjects screened for the control group were excluded because of asymptomatic sensitizations to other allergens. The exact flow chart of the study subjects is presented in Figure 1. Demographical, serological and clinical data are presented in Table 1.

The study protocol was approved by the Slovenian National Medical Ethics Committee (158/10/11).

2.2 Skin prick testing

We performed SPT using a standard panel of inhalant allergens (Stallergenes, Antony, France) according to GA²LEN recommendations.¹⁹

2.3 Nasal provocation tests

Nasal provocation tests (NPTs) were performed as previously described.¹² Briefly, a nebulizer and a HDM allergen extract for NPT were used (Stallergene, Antony, France). NPTs were evaluated using a symptom score.²⁰ To evaluate non-specific reactivity, we sprayed two 50 µL puffs of saline into the nostril after deep inspiration. After 15 minutes, we assessed reactivity with a symptom score by rating sneezing, rhinorrhoea and stuffiness (each up to 3 points); itching of the nose and/or ears (up to 2 points); and itching or erythema of the eye (1 point). The maximum total score was 12 points.²⁰ The provocation with saline was considered positive if the sum of symptoms was 3 points or greater. If the symptom score sum was negative, we continued testing with increasing concentrations (0.1 IR, 1 IR, 10 IR and 100 IR) of HDM allergen. Two puffs (50 µL) of the lowest concentration were administered in full inspiration into the nostril. After 15 minutes, we determined the number of points obtained using the symptom score. The test was considered positive if the sum of the symptom score was 5 points or more.^{20,21} If the NPT was negative, we continued provocation with a higher concentration of allergen. Otherwise, the provocation was completed.

2.4 Basophil activation test

Basophil CD63 activation tests were performed as previously described in detail.²² Briefly, whole blood samples were stimulated with *D pteronyssinus* allergen extract containing a final concentration of 0.333, 3.33, 33.3 or 333 ng/mL of allergen (Buhlmann Laboratories, Schönenbuch, Switzerland); 0.55 µg/mL of anti-FcεRI mAb (Buhlmann Laboratories); or 2 µmol/L /mL L-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP; Sigma-Aldrich, St. Louis, MO, USA) or stimulation buffer alone (containing 3 ng/mL of IL-3; Buhlmann Laboratories). The differences in basophil allergen-specific response were evaluated by comparing basophil CD63 responses at different concentrations,²² and the cut-off value for positive results was set at 15%, which corresponded with previous results.²³ To evaluate allergen basophil sensitivity, the area under the curve (AUC) of the basophil response to stimulation with increasing concentrations of allergen was calculated.²⁴ In addition, we also

calculated and compared mean CD63 responses at three upper allergen concentrations (3.33, 33.3 and 333 ng/mL) as reduced for basal CD63 response and the percentage (%) of the allergen CD63 responses relative to the anti-FcεRI mAb responses.

2.5 Specific immunoglobulin E, total immunoglobulin E and specific immunoglobulin G measurements

The levels of sIgE to *D pteronyssinus* and total IgE and sIgG to *D pteronyssinus* were quantified using the CAP FEIA system (Phadia, Uppsala, Sweden). Specific IgE levels equal to or greater than 0.35 kU/L were considered positive. The amount of disease-relevant antibodies was defined and calculated as the ratio of sIgE to *D pteronyssinus* (kU/L) relative to total IgE (kU/L), as previously described.²⁵

2.6 Natural and recombinant HDM allergens

Natural Der p 1 was purified from spent mite medium by affinity chromatography using the monoclonal antibody 4C1.26 Recombinant Der p 2, Der p 4, Der p 11, Der p 14, Der p 15 and Der p 18 were expressed as hexa-histidine-tagged proteins in the inclusion body fraction of *Escherichia coli* extracts and purified by nickel affinity chromatography.²⁷ Recombinant Der p 5, Der p 7, Der p 10, Der p 21 and Der p 23 were expressed in *E coli* as soluble non-fusion proteins using the pET17 expression system and purified as previously described.^{28–31}

2.7 Non-denaturing IgE dot blot experiments

Sera were tested for the presence of HDM allergen-specific IgE antibodies in a radioallergosorbent test-based non-denaturing dot blot assay. Two-microlitre aliquots of natural Der p 1, rDer p 2, rDer p 4, rDer p 5, rDer p 7, rDer p 10, rDer p 11, rDer p 14, rDer p 15, rDer p 18, rDer p 21, rDer p 23 and BSA as a control (0.5 µg/dot) were dotted onto nitrocellulose strips (Schleicher & Schuell, Dassel, Germany). After blocking with buffer B [50 mol/L sodium phosphate pH 7.4, 0.5% (v/v) Tween-20, 0.5% (w/v) BSA and 0.05% (w/v) sodium azide], the membranes were incubated with sera from symptomatic and asymptomatic subjects as well as those from control subjects and, for control purposes, with serum from a non-allergic individual in a dilution of 1:10 overnight at 4°C. Bound IgE antibodies were detected with ¹²⁵I-labelled anti-human IgE antibodies (Demeditec Diagnostics, Kiel, Germany) and visualized by autoradiography.³²

2.8 Statistical analysis

The distribution of data was calculated using the D'Agostino-Pearson normality test. According to the distribution of the data, we used an unpaired *t* test or a Mann-Whitney *U* test. Fisher's exact test was used to assess the frequencies of natural and recombinant HDM allergens. Spearman's rank correlation coefficient (*r*) was used to calculate the correlation between recognized IgE allergens and the AUC. The performance of allergy tests was examined against the allergic status to HDM using receiver-operating characteristic (ROC) curve analyses. A probability value (*P*) of <0.05 was accepted as significant. The data are presented as the median and the ranges. Analyses were performed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA).

3 Results

3.1 Symptomatic patients and asymptomatic subjects do not differ significantly regarding skin prick test, specific IgE, total IgE and specific IgG

There was no significant difference between the extent of the skin reaction to *D pteronyssinus* and *D farinae* between symptomatic and asymptomatic subjects (Table 1). Two of 17 symptomatic patients and 4 of 19 asymptomatic subjects showed a positive SPT only to *D farinae*. One of 17 symptomatic patients and 2 of 19 asymptomatic subjects showed positive SPT to *D pteronyssinus* alone. The other subjects were SPT-positive to both. Based on the SPT results, some subjects were also sensitized to allergens other than HDM: 9 of 17 symptomatic patients (6 to animal epithelia, 3 to cockroaches, 2 to moulds and 7 to pollen) and 15 of 19 asymptomatic subjects (5 to animal epithelia, 4 to cockroaches, 4 to moulds and 10 to pollen).

The level of sIgE to *D pteronyssinus* was higher in symptomatic patients than in asymptomatic subjects. The significance test reached $P = 0.055$ for this comparison; thus, the difference was not significant. The sIgE *D pteronyssinus*/tIgE ratio revealed no significant differences between the symptomatic patients and asymptomatic subjects. Additionally, the analysis of sIgG to *D pteronyssinus* between these two groups also did not appear to be significant (Table 1).

3.2 Basophil activation tests discriminate symptomatic patients from asymptomatic subjects

We showed that the baseline CD63 response and the response to fMLP were highly comparable between the symptomatic patients, asymptomatic subjects and the controls (Table 2). Comparison of the anti-FcεRI mAb stimulus between the symptomatic patients and asymptomatic subjects revealed statistically significant differences ($P < 0.001$), with higher basophil activation among symptomatic patients (Table 2). There were two non-responders to anti-FcεRI mAb (<15% CD63 response) in the asymptomatic group; however, both had positive responses to fMLP. Allergen-induced responses for those two subjects were excluded from the analysis. All other study subjects had anti-FcεRI mAb responses above 15%. We found a significant difference in the proportion of activated basophils between the symptomatic patients and asymptomatic subjects at *D pteronyssinus* allergen extract concentrations of 3.33 ng/mL ($P = 0.010$), 33.3 ng/mL ($P = 0.021$) and 333 ng/mL ($P = 0.011$). The threshold for an in vitro basophilic activation of >15% was 3.33 ng/mL in symptomatic patients and 33.3 ng/mL in the asymptomatic group (Figure 2, Table 2). Importantly, we also detected that the symptomatic patients had significantly higher AUC compared to those in the asymptomatic group ($P = 0.017$) (Figure 2B, Table 2). There was a correlation between the ratio of specific/total IgE and AUC, $r = 0.617$, ($P < 0.001$). We also calculated and compared some additional parameters of basophil activation tests: the mean CD63 response at three of the higher allergen concentrations (3.33, 33.3 and 333 ng/mL) as reduced for basal CD63 response was significantly higher in symptomatic patients ($P = 0.017$); the percentage (%) of allergen CD63 relative to anti-FcεRI mAb response was higher in the symptomatic patients at 3.33 ng/mL (although this difference did not reach significance), and there were no differences at 33.3 and 333 ng/mL (Table 2).

3.3 Symptomatic patients recognize more HDM allergens than asymptomatic subjects

We found that 16 of the 17 (94.1%) symptomatic patients and 14 of the 19 (73.7%) asymptomatic subjects showed reactivity to at least one of the tested HDM allergens. Symptomatic patients were on an average sensitized to 4 of the 12 tested natural or recombinant *D pteronyssinus* allergens; in comparison, the asymptomatic subjects were on an average sensitized to 2 of the 12 tested allergens ($P = 0.02$) (Table 1). The frequency of sensitization to nDer p 1 and rDer p 2 was the same, although not all positive patients were sensitized to both allergens. A total of 82.3% of the symptomatic patients and 57.9% of the asymptomatic subjects were sensitized to nDer p 1 and rDer p 2, respectively. Sensitization to rDer p 5 was also highly prevalent in both groups (symptomatic 41.2% and asymptomatic 21.0%). Differences were observed in the frequency of sensitization to rDer p 7 and rDer p 23. A total of 41.2% of the symptomatic patients were sensitized to rDer p 7 compared to 5.3% of asymptomatic subjects ($P = 0.016$). A total of 70.6% of the symptomatic patients were sensitized to rDer p 23 compared to 26.3% of asymptomatic subjects ($P = 0.018$). The IgEs to rDer p 4, rDer p 10, rDer p 11, rDer p 14, rDer p 15, rDer p 18 and rDer p 21 were rarely present in the sera of the study subjects, with no significant difference between the symptomatic patients and asymptomatic subjects (Figure 3). None of the controls were sensitized to any of the natural or recombinant *D pteronyssinus* allergens.

3.4 Correlation between BAT and the number of recognized HDM allergens

The correlation between the number of positive HDM allergens and the basophil response was also evaluated. The subjects were divided into 5 groups according to the number of recognized HDM allergens. Six subjects showed negative results on CRD. Twelve subjects were positive for 1-2, 7 subjects were positive for 3-4, 6 subjects were positive for 5-6 and 2 subjects were positive for more than 7 HDM allergens. The basophil response was assessed based on the AUC. The median AUC in subjects who were negative for all tested HDM allergens was 7.9 (range 2.5-127.5). In the 1-2 group, the median AUC was 123.9 (range 9.9-175.7; $P = 0.01$) compared to the negative group, that in the 3-4 group was 142.8 (range 23.1-224.8; $P = 0.008$) compared to the negative group and that in the 5-6 group was 181.9 (range 8.9-255.6; $P = 0.002$) compared to the negative group (Figure 4A). Two subjects with more than 7 positive components had AUC values of 161.4 and 219.7, respectively. There was a significant positive correlation between the number of positive HDM allergens and the AUC value ($r = 0.63$, $P < 0.001$). (Figure 4B).

3.5 Receiver-operating characteristic curve

The ROC AUC for SPT for *D pteronyssinus* was 0.501 ($P = 0.986$), that for sIgE for *D pteronyssinus* was 0.687 ($P = 0.055$), that for BAT at 333 ng/mL was 0.749 ($P = 0.010$), that for BAT at 33.3 ng/mL was 0.727 ($P = 0.019$) and that for BAT at 3.33 ng/mL was 0.710 ($P = 0.031$); for the BAT response at all concentrations, the ROC AUC was 0.733 ($P = 0.016$), and for number of positive HMD allergens, the ROC AUC was 0.727 ($P = 0.019$).

4 Discussion

This study showed that basophil sensitivity measurements and component-resolved diagnostics (CRD) are useful diagnostic tools for differentiating between symptomatic

patients with HDM-allergic rhinitis and subjects with asymptomatic sensitization. Symptomatic patients have a lower threshold for basophil activation and a higher AUC of in vitro basophil stimulation with increasing allergen concentrations compared to asymptomatic subjects. There was also a higher basophil response to stimulation with anti-FcεRI in symptomatic patients. We demonstrated that symptomatic patients were sensitized to more HDM allergens and that sensitization to rDer p 7 and rDer p 23 was more prevalent in symptomatic patients than in asymptomatic subjects. Importantly, there was also a significant positive correlation between the number of recognized HDM allergens and the basophil AUC.

The factors responsible for differences between patients with allergic rhinitis and asymptomatic subjects have been studied previously.³⁴ In this study, we could not confirm a previous observation of reduced skin test reactivity in asymptomatic subjects³⁵ because the size of the skin test result was similar between symptomatic patients and asymptomatic subjects. Similarly, Ha et al³⁶ showed no correlation between retrospective nasal symptom severity and weal diameter; however, they showed a significant correlation between weal diameter and nasal provocation symptom score. We showed that symptomatic patients have a higher sIgE compared to asymptomatic subjects. However, the significance test reached $P = 0.055$ for this comparison; thus, the difference was not significant. The reason for this borderline P value might be the small number of study participants, especially as previous studies have shown significant differences in sIgE levels and provided cut-off values for different allergens.³⁷ However, no absolute cut-off value has been confirmed for HDM by other studies, similar to the result obtained in our study.³⁴ Kirerieri et al³⁸ showed that rhinomanometric response to nasal allergen provocation is not correlated with the diameter of the skin prick test result and the level of IgE. Recently, Wanjun et al³⁹ showed a correlation between nasal provocation with HDM, skin prick weal size and IgE; however, only nasal provocation was associated with the severity of nasal symptoms. Haxel et al⁴⁰ showed that the ratio between negative and positive NPT is decreased in patients with larger weal size and higher IgE classes, although some patients with a weal diameter of >5 mm and an IgE class 4 still had negative NPT. Interestingly, in the Pan-European standard prick test panel,⁴¹ polysensitization was less common in symptomatic patients than in asymptomatic subjects, in contrast to other studies that associated polysensitization with symptomatic allergic disease.³⁴ Specific IgG levels that can be considered as markers of exposure were similar in both groups as well as in control subjects without HDM sensitization. Studies in cat owners have shown that high levels of exposure can induce an allergen-specific IgG response⁴² that may act as a blocking antibody.⁴³ Total IgE titres were higher in asymptomatic subjects, but the difference was not significant. High titres of IgE have been supposed to saturate FcεRI and prevent the binding of allergen-specific IgE.⁴⁴ In this study, we could not confirm the importance of naturally occurring HDM IgG-blocking antibodies or the hypothesis regarding the saturation of IgE receptors with high total IgE as reasons for asymptomatic sensitization. An evaluation of the ratio of sIgE to *D pteronyssinus*/tIgE showed no significant differences between the symptomatic and asymptomatic groups; this result contrasts with our previous study, in which we showed that this ratio was significantly different between symptomatic patients and asymptomatic subjects who were sensitized to grass pollen.¹²

Basophil activation test is an in vitro flow cytometric-based test that quantifies the expression of CD63 or other markers of basophil activation after specific allergen stimulation.⁴⁵ Currently, BAT can be used to investigate IgE-mediated allergy caused by inhalant allergens, Hevea latex, food, drugs and hymenoptera venom.⁴⁶ It may also be used to diagnose non-IgE-mediated reactions (drug hypersensitivity and the detection of auto-antibodies in certain forms of chronic urticaria) and to predict venom immunotherapy side-effects.⁴⁷ In this study, the basophil response to positive control anti-FcεRI was higher in symptomatic patients than in asymptomatic subjects. In contrast, the response to another positive control, fMLP, was similar in both groups. Puan et al⁴⁸ also showed that basophil energy is associated with a reduced incidence of AR. Our previous study in grass pollen asymptomatic sensitization showed comparable responses to anti-FcεRI stimulation in symptomatic patients and asymptomatic subjects.²⁴ This difference could be attributed to the seasonal presence of grass pollen compared to the perennial presence of HDM, which may up-regulate the response of basophils to IgE-mediated stimulation. Plasma-free IgE levels influence FcεRI receptor density on basophils and the responsiveness of the cells to IgE-mediated stimulation,⁴⁹ but total IgE levels were similar between the groups. FcεRI receptor polymorphisms may be among the most relevant genetic factors involved in the differences between asymptomatic subjects and patients,³⁴ and this may serve as another possible explanation for the different reactions to the anti-FcεRI stimulus. Santos et al⁵⁰ showed that FcεRI non-responders were predominantly peanut tolerant and not peanut allergic. Similarly, in our study, we had two FcεRI non-responders, and both were in the asymptomatic group. Thus, we might speculate that in some cases, IgE signalling is important for allergen tolerance. Additional allergen/antiFcεRI mAb analysis also suggests the importance of FcεRI signalling. CD32b signalling, another important mechanism that modulates IgE-mediated response of basophils,⁵¹ might also be important. However, a substantially broader assessment and more studies are required to confirm these speculations.

With this study, we confirmed that basophil sensitivity was higher in symptomatic patients than in asymptomatic subjects, similar to grass pollen allergies. Basophil sensitivity testing provides information regarding how much allergen is needed to provoke basophils to react. In our study, basophil sensitivity was assessed using the threshold for basophil activation; this occurred at a 10-fold lower concentration of HDM allergen in symptomatic patients compared with asymptomatic subjects. Basophil allergen threshold sensitivity can also be assessed using CD-sens (data not shown)⁵²; however, this method is less useful for distinguishing symptomatic patients from asymptomatic subjects because some asymptomatic subjects have negative basophil activation test results. AUC represents both basophil sensitivity and basophil reactivity and is therefore more useful for comparing a wide array of different basophil responses that can be found especially in asymptomatic subjects (Figure 2). In this study, we showed that the AUC was 34% higher in symptomatic patients compared to asymptomatic subjects.

Component-resolved diagnosis is a promising diagnostic method that enables researchers to investigate the presence of antibodies to different HDM allergens. More than 30 HDM allergens have been described thus far,⁵³ and there is evidence that CRD can be useful for the selection of patients for HDM-specific allergen immunotherapy.⁵⁴ Furthermore,

recombinant allergen-based IgE serology seems to be superior to allergen extract-based testing¹⁵ and avoids contaminations of HDM allergen extracts with unrelated allergens.⁵⁴ With the availability of an extended set of HDM allergens, it is possible to study molecular reactivity profiles that may be associated with different manifestations of HDM sensitization.¹³ In our study, symptomatic patients were on an average sensitized to 4 of 12 tested HDM allergens; in comparison, asymptomatic subjects were on an average sensitized to 2 of 12 of the allergens. The AUC was correlated with the number of recognized HDM allergens. This was in accordance with previous observations that sensitization to more than one allergenic protein increases the likelihood of allergic disease.³⁴ In our study, sensitization to nDer p 1 and rDer p 2 proved to be the most prevalent both in symptomatic patients (nDer p 1 = 82.3%; rDer p 2 = 82.3%) and in asymptomatic subjects (nDer p 1 = 57.9%; rDer p 2 = 57.9%). This result is in accordance with other studies, which showed that nDer p 1 and rDer p 2 were the most prevalent HDM allergen components.⁵³ Sensitization to rDer p 5 was also highly prevalent in both groups (symptomatic 41.2% and asymptomatic 21.0%). A recent study of Weghofer et al identified rDer p 23 as a new major *D pteronyssinus* allergen with high allergenic activity. In that study, the prevalence among symptomatic patients was 74% (n = 347).³¹ Soh et al⁵⁵ showed that 54% of tested HDM-allergic patients displayed a Der p 23-specific IgE response. Becker et al⁵⁶ recently also showed that 31% of proven HDM-allergic patients who were negative for the commercially available allergens Der p 1, Der p 2 and Der p 10 tested positive for Der p 23 according to a MeDALL chip evaluation. Our results showed a higher prevalence of rDer p 23 sensitization in symptomatic patients (70.6%) than in asymptomatic subjects (26.3%). Similar results were also found for rDer p 7 sensitization, which was present in 41.2% of symptomatic patients but only in 5.3% of asymptomatic subjects. Lynch showed that patients with combined allergic rhinitis and asthma were more predisposed to react to Der p 2, 5 and 7 compared to those with either of these diseases alone, who react predominantly to Der p 2.⁵⁷ Other components were less prevalent, with no significant differences between groups. For food allergens, it is widely recognized that different sensitization patterns are connected with different phenotypes. It has also recently been shown that the molecular reactivity profile of asthmatic children differs from that of non-asthmatic children with HDM allergies.¹³ Our results showed that rDer p 23 and rDer p 7 are good indicators for distinguishing symptomatic patients from asymptomatic subjects.

The strength of this study is that we included clinically well-defined patients. Past patient histories were confirmed with questionnaires on rhinitis symptoms, and the clinical importance of sensitization was confirmed with NPT. A limitation of the study is the rather small number of included study participants. It would probably be better to use a mix of defined HDM allergen molecules for BAT instead of HDM allergen extract.

In summary, this study demonstrated that the use of CRD and BAT can help clinicians to distinguish between HDM-allergic rhinitis patients and asymptomatic subjects. Patients with allergic rhinitis recognize more HDM allergens and are more frequently sensitized to rDer p 23 and rDer p 7. Differences in allergen recognition are also reflected in a higher basophil response in allergic patients compared with that in asymptomatic subjects.

Funding information

This study was funded in part by the Austrian Science Fund (FWF) grants F4602 and F4605. Rudolf Valenta is recipient of a Megagrant of the Government of the Russian Federation, grant number 14.W03.31.0024.

References

1. Bousquet J. Allergic rhinitis and its impact on asthma (ARIA). *Clin Exp Allergy Rev.* 2003; 3(1): 43–45.
2. Bousquet PJ, Chinn S, Janson C, Kogevinas M, Burney P, Jarvis D. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I. *Allergy.* 2007; 62(3):301–309. [PubMed: 17298348]
3. Droste JH, Kerhof M, de Monchy JG, Schouten JP, Rijcken B. Association of skin test reactivity, specific IgE, total IgE, and eosinophils with nasal symptoms in a community-based population study. The Dutch ECRHS Group. *J Allergy Clin Immunol.* 1996; 97(4):922–932. [PubMed: 8655887]
4. Scadding G, Hellings P, Alobid I, et al. Diagnostic tools in rhinology EAACI position paper. *Clin Transl Allergy.* 2011; 1(1):2. [PubMed: 22410181]
5. Thomas M, Yawn BP, Price D, Lund V, Mullol J, Fokkens W. EPOS primary care guidelines: European position paper on the primary care diagnosis and management of rhinosinusitis and nasal polyps 2007 – a summary. *Prim Care Respir J.* 2008; 17(2):79–89. [PubMed: 18438594]
6. Haugaard L, Iversen M, Dahl R. Predictors of early- and late-phase reactions to bronchial allergen challenge. *Allergy.* 1997; 52(10):999–1004. [PubMed: 9360751]
7. van der Veen MJ, Lopuhaa CE, Aalberse RC, Jansen HM, van der Zee JS. Bronchial allergen challenge with isolated major allergens of *Dermatophagoides pteronyssinus*: the role of patient characteristics in the early asthmatic response. *J Allergy Clin Immunol.* 1998; 102(1):24–31. [PubMed: 9679844]
8. Bodtger U, Poulsen LK, Linneberg A. Rhinitis symptoms and IgE sensitization as risk factors for development of later allergic rhinitis in adults. *Allergy.* 2006; 61(6):712–716. [PubMed: 16677240]
9. Zuberbier T, Bachert C, Bousquet PJ, et al. GA²LEN/EAACI pocket guide for allergen-specific immunotherapy for allergic rhinitis and asthma. *Allergy.* 2010; 65(12):1525–1530. [PubMed: 21039596]
10. Chusakul S, Phannaso C, Sangsarsri S, Aemjaturapat S, Snidvongs K. House-dust mite nasal provocation: a diagnostic tool in perennial rhinitis. *Am J Rhinol Allergy.* 2013; 24(2):133–136.
11. Rondón C, Campo P, Herrera R, et al. Nasal allergen provocation test with multiple aeroallergens detects polysensitization in local allergic rhinitis. *J Allergy Clin Immunol.* 2011; 128(6):1192–1197. [PubMed: 21783237]
12. Zidarn M, Košnik M, Šilar M, Grahek A, Korošec P. Rhinitis symptoms caused by grass pollen are associated with elevated basophile allergen sensitivity and a larger grass-specific immunoglobulin E fraction. *Clin Exp Allergy.* 2012; 42(1):49–57. [PubMed: 22093044]
13. 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(4):1083–1091. [PubMed: 25956509]
14. Kraft D, Ferreira F, Ebner C, et al. Recombinant allergens: the future of the diagnosis and treatment of atopic allergy. *Allergy.* 1998; 53(45 Suppl):62–66.
15. Huang HJ, Resch-Marat Y, Rodriguez-Dominguez A, et al. Underestimation of house dust mite-specific IgE with extract-based ImmunoCAPs compared with molecular ImmunoCAPs. *J Allergy Clin Immunol.* 2018; 142(5):1656–1659.e9. [PubMed: 30059698]
16. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. *J Allergy Clin Immunol.* 2008; 122(2):298–304. [PubMed: 18572230]
17. Dordal MT, Lluch-Bernal M, Sánchez MC, et al. Allergen-specific nasal provocation testing: review by the Rhinoconjunctivitis Committee of the Spanish Society of Allergy and Clinical Immunology. *J Investig Allergol Clin Immunol.* 2011; 21(1):1–12.

18. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008*. *Allergy*. 2008; 63:8–160. [PubMed: 18331513]
19. Bousquet PJ, Burbach G, Heinzerling LM, et al. GA2LEN skin test study III: minimum battery of test inhalent allergens needed in epidemiological studies in patients. *Allergy*. 2009; 64(11):1656–1662. [PubMed: 19824887]
20. Lebel B, Bousquet J, Morel A, Chanal I, Godard P, Michel FB. Correlation between symptoms and the threshold for release of mediators in nasal secretions during nasal challenge with grass-pollen grains. *J Allergy Clin Immunol*. 1988; 82(5 Pt 1):869–877. [PubMed: 2461405]
21. Melillo G, Bonini S, Cocco G, et al. EAACI provocation tests with allergens. Report prepared by the European Academy of Allergology and Clinical Immunology Subcommittee on provocation tests with allergens. *Allergy*. 1997; 35(Suppl):1–35.
22. Lalek N, Košnik M, Šilar M, Korošec P. Immunoglobulin G-dependent changes in basophil allergen threshold sensitivity during birch pollen immunotherapy. *Clin Exp Allergy*. 2010; 40(8):1186–1193. [PubMed: 20528883]
23. Peternelj A, Šilar M, Eržen R, Košnik M, Korošec P. Basophil sensitivity in patients not responding to venom immunotherapy. *Int Arch Allergy Immunol*. 2008; 146(3):248–254. [PubMed: 18270492]
24. Zidarn M, Košnik M, Šilar M, Bajrovi N, Korošec P. Sustained effect of grass pollen subcutaneous immunotherapy on suppression of allergen-specific basophil response; a real-life, nonrandomized controlled study. *Allergy*. 2015; 70(5):547–555. [PubMed: 25627309]
25. Johansson SGO, Nopp A, Oman H, et al. The size of the disease relevant IgE antibody fraction in relation to “total-IgE” predicts the efficacy of anti-IgE (Xolair) treatment. *Allergy*. 2009; 64(10):1472–1477. [PubMed: 19393000]
26. Hales BJ, Shen HD, Thomas WR. Cross-reactivity of T-cell responses to *Dermatophagoides pteronyssinus* and *D. farinae*. Studies with group 1 and 7 allergens. *Clin Exp Allergy*. 2000; 30(7):927–933. [PubMed: 10848914]
27. Chen KW, Fuchs G, Sonneck K, et al. Reduction of the in vivo allergenicity of Der p 2, the major house-dust mite allergen, by genetic engineering. *Mol Immunol*. 2008; 45(9):2486–2498. [PubMed: 18295887]
28. Weghofer M, Grote M, Dall'Antonia Y, 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(2):101–109. [PubMed: 18520154]
29. Resch Y, Weghofer M, Seiberler S, et al. Molecular characterization of Der p 10: a diagnostic marker for broad sensitization in house dust mite allergy. *Clin Exp Allergy*. 2011; 41(10):1468–1477. [PubMed: 21711470]
30. Weghofer M, Dall'Antonia Y, Grote M, et al. Characterization of Der p 21, a new important allergen derived from the gut of house dust mites. *Allergy*. 2008; 63(6):758–767. [PubMed: 18445190]
31. Weghofer M, Grote M, Resch Y, 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(7):3059–3067. [PubMed: 23460742]
32. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med*. 1992; 175(2):377–385. [PubMed: 1370681]
33. Santos AF, James LK, Bahnson HT, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol*. 2015; 135(5):1249–1256. [PubMed: 25670011]
34. Bousquet J, Anto JM, Bachert C, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2LEN project. *Allergy*. 2006; 61(6):671–680. [PubMed: 16677235]
35. Backer V, Ulrik CS, Hansen KK, Laursen EM, Dirksen A, Bach-Mortensen N. Atopy and bronchial responsiveness in random population sample of 527 children and adolescents. *Ann Allergy*. 1992; 69(2):116–122. [PubMed: 1510285]

36. Ha EK, Na MS, Lee S, et al. Prevalence and clinical characteristics of local allergic rhinitis in children sensitized to house dust mites. *Int Arch Allergy Immunol.* 2017; 174(3–4):183–189. [PubMed: 29130983]
37. Pastorello EA, Incorvaia C, Ortolani C, et al. Studies on the relationship between the level of specific IgE antibodies and the clinical expression of allergy: I. Definition of levels distinguishing patients with symptomatic from patients with asymptomatic allergy to common aeroallergens. *J Allergy Clin Immunol.* 1995; 1:580–587.
38. Kırerler E, Güler N, Tamay Z, Ones U. Evaluation of the nasal provocation test for its necessity in the diagnosis of nasal allergy to house dust mite. *Asian Pac J Allergy Immunol.* 2006; 24(2–3): 117–121. [PubMed: 17136876]
39. Wanjun W, Qiurong H, Yanqing X, Mo X, Nili W, Jing L. Responsiveness of nasal provocation testing—but not skin test and specific immunoglobulin E blood level—correlates with severity of allergic rhinitis in *Dermatophagoides* species-sensitized patients. *Am J Rhinol Allergy.* 2018; 32(4):236–243. [PubMed: 29909644]
40. Haxel BR, Huppertz T, Boessert P, Bast F, Fruth K. Correlation of skin test results and specific immunoglobulin E blood levels with nasal provocation testing for house-dust mite allergies. *Am J Rhinol Allergy.* 2016; 30(1):60–64. [PubMed: 26867532]
41. Heinzerling L, Frew AJ, Bindslev-Jensen C, et al. Standard skin prick testing and sensitization to inhalant allergens across Europe—a survey from the GALEN network. *Allergy.* 2005; 60(10):1287–1300. [PubMed: 16134996]
42. Woodfolk JA, Platts-Mills TAE. The immune response to intrinsic and extrinsic allergens: determinants of allergic disease. *Int Arch Allergy Immunol.* 2002; 129(4):277–285. [PubMed: 12483032]
43. Flicker S, Valenta R. Renaissance of the blocking antibody concept in type I allergy. *Int Arch Allergy Immunol.* 2003; 132(1):13–24. [PubMed: 14555854]
44. Lynch NR, Hagel IA, Palenque ME, et al. Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. *J Allergy Clin Immunol.* 1998; 101(2 Pt 1):217–221. [PubMed: 9500755]
45. Ebo DG, Sainte-Laudy J, Bridts CH, et al. Flow-assisted allergy diagnosis: current applications and future perspectives. *Allergy.* 2006; 61(9):1028–1039. [PubMed: 16918504]
46. Hoffmann HJ, Santos AF, Mayorga C, et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. *Allergy.* 2015; 70(11):1393–1405. [PubMed: 26198455]
47. Korošec P, Žiberna K, Šilar M, et al. Immunological and clinical factors associated with adverse systemic reactions during the build-up phase of honeybee venom immunotherapy. *Clin Exp Allergy.* 2015; 45(10):1579–1589. [PubMed: 26046807]
48. Puan KJ, Andiappan AK, Lee B, et al. Systematic characterization of basophil anergy. *Allergy.* 2017; 72(3):373–384. [PubMed: 27271846]
49. MacGlashan DW, Bochner BS, Adelman DC, et al. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol.* 1997; 158(3):1438–1445. [PubMed: 9013989]
50. Santos AF, Douiri A, Bécares N, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J Allergy Clin Immunol.* 2014; 134(3):645–652. [PubMed: 25065721]
51. MacGlashan D, Moore G, Muchhal U. Regulation of IgE-mediated signalling in human basophils by CD32b and its role in Syk down-regulation. *Clin Exp Allergy.* 2014; 44(5):713–723. [PubMed: 24734927]
52. Glaumann S, Nopp A, Johansson SGO, Rudengren M, Borres MP, Nilsson C. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. *Allergy.* 2012; 67(2):242–247. [PubMed: 22126416]
53. Calderon MA, Linneberg A, Kleine-Tebbe J, et al. Respiratory allergy caused by house dust mites: what do we really know? *J Allergy Clin Immunol.* 2015; 136(1):38–48. [PubMed: 25457152]

54. Chen KW, Zieglmayer P, Zieglmayer R, et al. Selection of house dust mite allergic patients by molecular diagnosis may enhance success of specific immunotherapy. *J Allergy Clin Immunol*. 2019
55. Soh WT, Le Mignon M, Suratannon N, et al. The house dust mite major allergen Der p 23 displays o-glycan-independent IgE reactivities but no chitin-binding activity. *Int Arch Allergy Immunol*. 2016; 168(3):150–160.
56. Becker S, Schleder T, Kramer MF, et al. Real-life study for the diagnosis of house dust mite allergy – the value of recombinant allergen-based IgE serology. *Int Arch Allergy Immunol*. 2016; 170(2):132–137. [PubMed: 27505432]
57. Lynch NR, Puccio FA, Di Prisco MC, et al. Reactivity to recombinant house-dust-mite allergens in asthma and rhinitis in a tropical environment. *Allergy*. 1998; 53(8):808–811. [PubMed: 9722232]

Figure 1.

Flow chart of patient inclusion. AR Q, allergic rhinitis questionnaire; HDM, house dust mite; NPT, nasal provocation test; SPT, skin prick test

Figure 2.

A, Comparison of basophil CD63 response at different concentrations of *Dermatophagoides pteronyssinus* in symptomatic patients and asymptomatic subjects. B, Box plot showing the area under the curve of the CD63 basophil response after in vitro activation with increasing allergen concentrations

Figure 3.

IgE reactivity to individual HDM allergens in symptomatic patients with HDM-related allergic rhinitis and asymptomatic subjects with HDM sensitization. Fisher's exact test was used for assessing the frequencies of natural and recombinant HDM allergen

Figure 4.

A, Comparison of the AUC of basophil response to in vitro stimulation with *Dermatophagoides pteronyssinus* extract between groups of subjects with increasing numbers of recognized HDM allergens in all subjects with positive SPT for HDM allergen. B, Correlation between the number of recognized HDM allergens and the AUC. AR Q, allergic rhinitis questionnaire; HDM, house dust mite; NPT, nasal provocation test; SPT, skin prick test

Table 1
Test subject characteristics, including demographical and clinical data and humoral parameters

	Symptomatic patients (n = 17)	Asymptomatic subjects (n = 19)	Control subjects (n = 13)	<i>P</i> -value
Age (y)	27 (18-54)	26 (19-51)	25 (20-32)	
Sex (male/female)	8/9	9/10	3/10	
SPT				
<i>Dermatophagoides pteronyssinus</i> (mm)	4.5 (0-9)	5 (0-12)	0	0.827
<i>D. farinae</i> (mm)	5 (0-10)	4 (0-12)	0	0.678
Other standard panel allergens (pos./neg)	9/8	15/4	0/13	0.273
sIgE to <i>D. pteronyssinus</i> (kU/L)	12.4 (0-100)	2.17 (0-41.7)	0 (0-0)	0.055
tIgE (kU/L)	77.2 (17-1361)	99.4 (9.0-443)	25.7 (6.9-1165)	0.890
sIgE to <i>D. pteronyssinus</i> /tIgE	0.1 (0-0.5)	0.07 (0-0.2)	0 (0-0)	0.321
sIgG to <i>D. pteronyssinus</i> (mgA/L)	15.8 (4.22-30.0)	12.7 (4.1-64.3)	14.4 (5.1-43.8)	0.498
Number of positive HDM components	4 (0-10)	2 (0-8)	0 (0-0)	0.019

HDM, house dust mite; SPT, skin prick test.

The results are presented as the medians and ranges.

P-value in bold is statistically significant.

P-values were calculated for the difference between symptomatic patients and asymptomatic subjects. An unpaired *t* test was used for the SPT. The Mann-Whitney *U* test was used for other standard panel allergens, sIgE, tIgE, sIgE/tIgE, sIgG and the number of positive HDM components.

Table 2
Basophil CD63 response at the basal level (stimulation buffer alone), after in vitro stimulation with positive controls (fMLP, anti-FcεRI mAb), and after stimulation with increasing concentrations of *Dermatophagoides pteronyssinus* allergen extract

	Symptomatic patients (n = 17)	Asymptomatic subjects (n = 19)	Control subjects (n = 13)	P-value
Basal CD63 (%)	3.4 (2.0-4.4)	3.1 (1.2-4.2)	2.8 (1.7-3.7)	0.270
fMLP CD63 (%)	47.5 (17.1-82.6)	46.2 (15.4-88.6)	59.6 (20.8-76.4)	0.878
Anti-FcεRI mAb CD63 (%)	96.97 (70.3-99.6)	79.5 (9.0-99.5)	95.0 (21.9-98.0)	<0.001
Allergen CD63 (%)				
0.333 ng/mL	1.9 (0-15.0)	0.8 (0-5.5)	0.3 (0-4.2)	0.120
3.33 ng/mL	26.9 ^a (0.3-88.9)	2.3 (0.5-42.2)	1.1 (0.3-2.0)	0.010
33.3 ng/mL	93.7 (0.3-99.7)	77.2 ^a (0-94.9)	1.7 (0.4-4.1)	0.021
333 ng/mL	97.3 (5.0-99.5)	85.1 (2.6-98.7)	3.9 (1.3-10.9)	0.011
AUC	170.9 (7.6-255.6)	127.4 (2.4-181.9)	4.3 (2.1-12.7)	0.017
Mean allergen (3.33-333 ng/mL) minus basal CD63 (%)	69.7 (-0.09 to 92.5)	53.8 (-1.5 to 74.4)	-1.125 (-2.2 to 1.9)	0.017
Allergen CD63 (%) / anti-FcεRI mAb CD63 (%)				
3.33 ng/mL	27.5 (0.3-101.6)	13.2 (0.6-49.6)	1.3 (0.3-2.0)	0.093
33 ng/mL	98.6 (0.3-118.1)	92.0 (0-108.3)	1.7 (0.4-4.0)	0.254
333 ng/mL	100.2 (5.3-121.7)	92.0 (0-108.3)	3.8 (1.3-10.9)	0.526

AUC represents the area under the curve of the CD63 basophil response after in vitro activation with increasing allergen concentrations. We also compared mean allergen CD63 responses at 3.33-333 ng/mL as corrected by subtracting the basal response and the percentages (%) of the allergen CD63 responses relative to anti-FcεRI mAb responses.

The results are presented as the medians and ranges.

P-values in bold are statistically significant.

P-values were calculated for the difference between symptomatic patients and asymptomatic subjects using the Mann-Whitney *U* test.

^aThe threshold for in vitro basophilic activation.