IgE and IgG4 Repertoire in Asymptomatic HDM-Sensitized and HDM-Induced Allergic Rhinitis Patients

Qingxiu Xu Qing Jiang Lin Yang Wenjing Li Nan Huang Yaqi Yang
Dongxia Ma Shuchen Zhang Yin Wang Rongfei Zhu

Department of Allergy, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST), Wuhan, China

Keywords

House dust mite · Asymptomatic sensitization · Allergic rhinitis · Basophil activation · House dust mite component

Abstract

Introduction: Asymptomatic sensitization is defined as the presence of positive skin prick test (SPT) and/or positive serum allergen-specific IgE in the absence of clinical allergic symptoms. Currently, there is no convincing explanation why some people with positive allergen tests do not show symptoms. We aimed to investigate the house dust mite (HDM)-specific IgE and IgG4 repertoire in asymptomatic HDM-sensitized subjects and HDM-induced allergic rhinitis (AR) patients. **Methods:** A total of 48 subjects sensitized to HDM were included in this study: 27 had AR with/without asthma (symptomatic group), and 21 had no allergic symptoms (asymptomatic group). Six healthy individuals served as control group. Peripheral blood samples were collected for serum IgE and IgG4 assay and basophil activation tests (BATs). IgE and IgG4 assay included antibodies to *Der p1*, 2, 7, 10, 21, 23, and *Der f1*, 2. **Results:** AR patients had a larger wheal diameter of SPT (7.0 vs. 3.0 mm, $p < 0.0001$) and a higher specific IgE to *Der p* (15.50 vs. 0.70

KU/L, $p < 0.0001$) than asymptomatic subjects. They also showed more frequent sensitization to *Der p1* and *Der p2* (both $p < 0.05$). However, the total IgE and specific IgG4 did not differ significantly between the 2 groups. The basophil activation response after being stimulated with HDM was observed to be higher in AR patients (all $p < 0.05$). **Conclusions:** There are differences in SPT, serum-specific IgE to *Der p*, component allergen *Der p1* and *Der p2* level and BAT between AR patients and asymptomatic subjects sensitized to HDM. IgG4 alone cannot differentiate asymptomatic individuals from AR patients.

© 2021 The Author(s).

Published by S. Karger AG, Basel

Introduction

Allergic rhinitis (AR) is a common chronic respiratory disease affecting up to 40% of the population worldwide [1]. The typical symptoms of AR are sneezing, rhinorrhea, and itchy and blocked nose after one's exposure to certain allergens. Although the exact mechanism

Edited by: O. Palomares, Madrid.

of AR still needs to be clarified, AR has been regarded as an IgE-mediated disease, and the production of specific IgE antibodies against allergens is a major contributing factor for the development of allergic symptoms [2]. The term *sensitization* has been used to describe positive skin prick test (SPT) and/or IgE to one or more allergens in individuals [3]. Presently, it is still unclear why some people sensitized to a given allergen develop allergic symptoms while others don't (asymptomatic). Some studies suggest sensitization can (but not always) result in the development of clinical symptoms [4–6]. Then, what accounts for such difference between asymptomatic individuals and AR patients? Many factors had been reported to be responsible for the difference, including total IgE level, specific IgE or IgG profiles, T regulatory cells, and the high-affinity receptor for IgE (FcεRI) [7]. These factors work in a complex manner either to translate sensitization into clinical allergy or prevent that from happening. IgE has been identified to play a key role in this process. The allergen-specific IgE and their binding capacity to FcεRI partially explain the differences between the asymptomatic and clinical patients [8–10]. Several studies showed the allergen-specific IgE and major allergen component IgE levels were higher in the allergic patients than asymptomatic sensitized individuals [11–13]. Some studies also provided the *cutoff* value of serum IgE level to distinguish asymptomatic from allergic patients, although these *cutoff* values often failed to be validated in different study population [14–16]. Meanwhile, some studies reported that allergen-specific IgG4 could protect individuals against allergic symptoms after their exposure to relevant allergen [17–19]. However, one study found no differences of specific IgE and IgG4 levels among the 2 groups after investigating the immunological features of asymptomatic and AR patients [20]. Currently available tests, including quantitative IgE and IgG4 antibodies to distinguish between asymptomatic sensitization and clinical allergy, still need validation.

House dust mite (HDM) is a major allergen worldwide. Our previous study had shown that more than 90% of AR patients in central China were induced by HDM [21]. HDM has been identified to possess up to 37 allergenic proteins which play distinct roles in sensitization and symptom elicitation [22]. In our study, we investigated the specific IgE and IgG4 antibodies repertoire to HDM and HDM components and also basophil activation tests (BATs) among the asymptomatic HDM-sensitized and HDM-induced AR patients.

Methods

Study Subjects

This study was conducted in the Department of Allergy, Tongji Hospital. The symptomatic group included subjects who were diagnosed with HDM-induced AR with/without asthma according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines and Global Initiative for Asthma (GINA) guidelines and without a history of allergen immunotherapy [23, 24]. Asymptomatic sensitization subjects we enrolled were from a previous allergy epidemiological study cohort, and all these asymptomatic subjects had a negative clinical history of any allergic symptoms but with a positive SPT for HDM and/or positive level of serum HDM-specific IgE. To reconfirm these subjects were asymptomatic, we required them to record any nasal symptoms for 30 days before the enrollment. Healthy control subjects were enrolled from individuals who had no allergic disease and had a negative SPT for HDM and negative level of serum HDM-specific IgE. Demographics and characteristics of the participants such as sex, age, atopic family history, and smoking history were collected. This study had been approved by the Independent Ethics Committee of Tongji Hospital and all the participants had given their written informed consent.

Skin Prick Test

SPT was performed according to EAACI recommendations. The wheal diameter larger than 3 mm was considered positive [25]. The SPT panel included 19 common inhalant allergens in our area [21].

Serum-Specific IgE and Total IgE Measurements

The levels of serum-specific IgE to HDM allergens and total IgE were detected with the ImmunoCAP (Thermo Fisher, Uppsala, Sweden). The sIgE level ≥ 0.35 KU/L was considered positive. Moreover, sIgE to Der p/total IgE ratio was calculated.

HDM Component-Specific IgE and IgG4 Antibodies Test

HDM component-specific IgE and IgG4 antibodies were tested based on protein chip assay according to the manufacturer's instructions. HDM component-specific IgE and IgG4 antibodies test kits (Hangzhou Zheda Dixun Biological Gene Engineering Co., Ltd.) were applied to detect a series of HDM components, including Der p1, Der f1, Der p2, Der f2, Der p7, Der p10, Der p21, and Der p23. These allergen components were coated in the nitrocellulose strips. Sera from symptomatic and asymptomatic subjects as well as healthy individuals were added to the membranes, followed by anti-human IgE or IgG4 antibodies, enzymes, and corresponding substrates, which included incubating and washing 4 times in total. The testing process was performed automatically by the immunoblotting instrument (DX-Blot 45 II, Hangzhou Zheda Dixun Biological Gene Engineering Co., Ltd.). The plate detectors were dried and scanned (EPSON Perfection U370 Photo). Finally, the results were interpreted by automated immunoblotting software (Hangzhou Zheda Dixun Biological Gene Engineering Co., Ltd.). The test was positive if the IgE level was 0.35 IU/mL or greater and the IgG4 level 250 U/mL or greater, respectively.

Basophil Activation Test

The Flow CAST was used to quantify the in vitro basophil activation by flow cytometry according to the manufacturer's instructions. The BAT was performed by flow cytometer to measure

Table 1. Demographic, clinical data, and immunological parameters in AR patients, asymptomatic subjects, and healthy individuals

	Symptomatic group (n = 27)	Asymptomatic group (n = 21)	Healthy group (n = 6)	p value
Sex (male/female)	16/11	8/13	3/3	
Age, years*	21.6±12.8	24.3±5.2	24.7±1.0	0.19
Atopic family history (yes/no)	12/15	5/16	0/6	0.22
Smoking history (yes/no)	1/26	0/21	0/6	1.00
Monosensitized/polysensitized	15/12	17/4	–	0.075
AR duration, months	60.8	–	–	–
Mild/moderate-to-severe AR	13/14	–	–	–
AR symptom score	5.8	–	–	–
SPT, mm**				
<i>Der p</i>	7.0 (5.0–8.9)	3.0 (2.0–4.0)	0	<0.0001
<i>Der f</i>	8.0 (5.5–10.5)	3.0 (1.1–3.4)	0	<0.0001
sIgE, KU/L**				
<i>Der p</i>	15.50 (3.04–31.90)	0.70 (0.37–2.15)	0.05 (0.04–0.06)	<0.0001
<i>Der f</i>	24.92 (2.94–54.30)	0.71 (0.33–1.61)	0.01 (0.01–0.01)	<0.0001
tIgE, KU/L**	188.00 (73.70–802.00)	106.40 (60.18–325.25)	13.39 (5.38–27.38)	0.26
sIgE to <i>Der p</i> /tIgE**	0.08 (0.02–0.15)	0.01 (0.00–0.02)	0.005 (0.00–0.01)	0.0002
Number of positive IgE to HDM components***	4 (0–8)	0 (0–7)	0 (0–0)	0.0012

AR, allergic rhinitis; SPT, skin prick test; *Der p*, *Dermatophagoides pteronyssinus*; *Der f*, *Dermatophagoides farinae*; HDM, house dust mite; SD, standard deviation; IQR, interquartile range. Monosensitized: subjects who sensitized to HDM alone. Polysensitized: subjects with several allergens sensitization except for HDM. The *p* value was presented to describe the difference between symptomatic group and asymptomatic group. A two-sample *t* test was used for the age; Fisher's exact test was used for the atopic family history, smoking history, and mono- or polysensitized; and the Mann-Whitney U test was used for SPT, sIgE, tIgE, sIgE/tIgE, and the number of positive IgE to HDM components. * The result is presented as mean±SD. ** The result is presented as median and 25–75% IQR. *** The result is presented as median and range.

the expression of activation markers CD63 on the surface of basophils after stimulation. For each subject, allergen test tubes were prepared with 50 µL *Der p* allergen at the final concentration of 333, 111, and 33.3 ng/mL (*Der p* 0000537973, DW, Material 1035921). N-formyl-methionyl-leucyl-phenylalanine (fMLP; BUHLMANN Laboratories AG, CH-4124 Schönenbuch, Switzerland) and highly specific monoclonal antibody binding to the high-affinity IgE receptor (anti-FcεRI mAb; BUHLMANN Laboratories AG, CH-4124 Schönenbuch, Switzerland) were used as positive controls, while 50 µL stimulation buffer (containing 3 ng/mL IL-3; BUHLMANN Laboratories AG, CH-4124 Schönenbuch, Switzerland) was used as negative control. The basophil activation rate was calculated based on the percentage of CD63 cells compared with the CCR3 identified basophils. The test was considered positive if the CD63 response was 15% or more [26].

Statistical Analysis

The results were presented as means and standard deviations for normally distributed continuous data, frequencies for categorical data, medians and 25–75% interquartile ranges (IQRs) for abnormal distribution data. Either the two-sample *t* test or the Mann-Whitney U test was applied to evaluate the continuous variable, while Fisher's exact test was used to compare the categorical variable. Receiver-operating characteristic curve (ROC) was performed to analyze the optimal *cutoff* values for the discrimination between symptomatic and asymptomatic subjects. The correlations between different parameters were estimated with Spear-

man's rank correlation analysis and presented as Spearman's rank correlation coefficient (*r*). *p* < 0.05 was considered statistically significant. All statistical analyses were performed with Stata software, version 16.0 (StataCorp, College Station, TX 77845 USA) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

Results

Participant Characteristics

A total of 54 subjects participated in this study (27 males; mean age 23.0 years). Twenty-seven subjects were included in the AR group (16 males, 11 females; mean age 21.6 years), while 21 subjects were included in the asymptomatic group (8 males, 13 females; mean age 24.3 years) and 6 subjects were included in the healthy group (3 males, 3 females; mean age 24.7 years). Among the 27 AR patients, 6 individuals were diagnosed as AR with asthma. The AR duration ranged from 1 month to 240 months, and the mean duration was 60.8 months. Thirteen were classified as mild persistent AR, while 14 were moderate-to-severe persistent AR, and the mean symptom score

Fig. 1. Skin reaction, levels of serum-specific IgE to HDM allergens and total IgE in AR group, asymptomatic group and healthy group. AR, allergic rhinitis; Dp, *Dermatophagoides pteronyssinus*; Df, *Dermatophagoides farinae*.

was 5.8 (ranging from “0” to “10,” obtained by visual analog scale, “0” for “no symptom,” “10” for “very severe symptom”). In both groups, some subjects were polysensitized: 12 of 27 AR patients (3 to cockroaches, 3 to animal dander, 11 to pollen, and 2 to molds) and 4 of 21 asymptomatic subjects (4 to cockroaches). All the AR patients had positive SPTs and specific IgE to HDM. In the asymptomatic group, 11 subjects had positive SPTs and IgEs to HDM, 4 subjects had positive SPTs, and 6 subjects had positive IgE. The participant characteristics are presented in Table 1.

Skin Reaction

The AR group had greater skin reaction than the asymptomatic group (Table 1; Fig. 1). The wheal diameter of *Der p* was 7.0 mm in the AR group and 3.0 mm in the asymptomatic group. Additionally, the wheal diameter of *Der f* was 8.0 mm in the AR group and 3.0 mm in the asymptomatic group. These differences were statistically significant ($p < 0.0001$) (Table 1; Fig. 1).

Fig. 2. **a** In vitro basophil activation rate in the AR group, asymptomatic group, and healthy group. **b** Threshold for in vitro basophil activation in the AR group, asymptomatic group, and healthy group. *** $p < 0.05$. AR, allergic rhinitis; BAT, basophil activation test; Dp, *Dermatophagoides pteronyssinus*.

Table 2. Basophil activation rate after stimulation with different concentrations of *Der p*

	Symptomatic group ($n = 27$)	Asymptomatic group ($n = 21$)	Healthy group ($n = 6$)	p value
Basal CD63, %	0.65 (0.27–0.90)	0.68 (0.38–2.11)	1.33 (0.50–7.08)	0.228
fMLP CD63, %	5.52 (3.07–12.01)	12.93 (3.01–17.99)	15.54 (5.95–45.97)	0.218
anti-Fc ϵ RI mAb CD63, %	65.63 (50.47–74.50)	46.21 (11.29–70.19)	51.34 (40.01–63.75)	0.078
333 ng/mL CD63, %	34.50 (4.45–68.76)	1.94 (0.44–9.47)	2.11 (0.27–10.12)	0.0016
111 ng/mL CD63, %	16.10 (0.84–39.38)	0.99 (0.39–4.72)	0.97 (0.26–5.83)	0.019
33.3 ng/mL CD63, %	6.05 (0.51–10.19)	0.99 (0.36–3.14)	1.27 (0.38–7.55)	0.037

IQR, interquartile range. The results are presented as the medians and 25–75% IQRs. The p value was presented to describe the difference between the symptomatic group and asymptomatic group. The Mann-Whitney U test was used to compare the basophil activation rate between the 2 groups.

Levels of sIgE to HDM and tIgE

AR patients, in contrast with asymptomatic subjects sensitized to HDM, had higher levels of sIgE to *Der p* (15.50 vs. 0.70 KU/L, $p < 0.0001$) and to *Der f* (24.92 vs. 0.71 KU/L, $p < 0.0001$) (Table 1; Fig. 1, 4c). Nevertheless, no significant difference was observed between the 2 groups in terms of the levels of total IgE ($p = 0.26$) (Table 1; Fig. 1, 4c). In addition, there was a significant difference in the sIgE to *Der p*/total IgE ratio between the AR and asymptomatic groups (0.08 vs. 0.01, $p = 0.0002$) (Table 1; Fig. 1).

Basophil Activation Rate

The basal basophil activation rate was comparable between the 2 groups ($p = 0.228$) (Table 2). The basophil activation rate at *Der p* concentration of 333 ng/mL, 111 ng/mL, and 33.3 ng/mL was significantly higher in AR patients than in asymptomatic subjects (all $p < 0.05$) (Table 2; Fig. 2a). Nonetheless, no significant difference was observed regarding the CD63 response to anti-Fc ϵ RI mAb in the AR group compared with the asymptomatic group ($p = 0.078$) (Table 2; Fig. 2a). Additionally, the threshold for basophil activation was significantly lower in the AR group in contrast with the asymptomatic group

Fig. 3. **a** Three-dimensional map for SPT to Dp, sIgE to Dp, and BAT at 333 ng/mL Dp between groups. **b** Three-dimensional map for sIgE to Dp, sIgE/tIgE ratio, and BAT at 333 ng/mL Dp between groups. AR, allergic rhinitis; SPT, skin prick test; BAT, basophil activation test; Dp, *Dermatophagoides pteronyssinus*.

Table 3. HDM component-specific IgE and IgG4 in AR patients, asymptomatic subjects, and healthy individuals

	Symptomatic group (<i>n</i> = 27)	Asymptomatic group (<i>n</i> = 21)	Healthy group (<i>n</i> = 6)	<i>p</i> value
HDM component sIgE, IU/mL				
<i>Der p</i> 1	6.13 (0.05–27.20)	0.04 (0.01–0.21)	0.07 (0.05–0.09)	0.0007
<i>Der f</i> 1	4.20 (0.04–18.34)	0.03 (0.02–0.42)	0.09 (0.06–0.12)	0.0003
<i>Der p</i> 2	5.53 (0.62–28.10)	0.04 (0.03–0.22)	0.09 (0.07–0.11)	0.0024
<i>Der f</i> 2	31.53 (0.03–104.60)	0.09 (0.04–2.26)	0.11 (0.09–0.18)	0.0066
<i>Der p</i> 7	0.10 (0.04–0.47)	0.06 (0.03–0.12)	0.12 (0.09–0.18)	0.14
<i>Der p</i> 10	0.03 (0.02–0.07)	0.04 (0.03–0.12)	0.08 (0.05–0.16)	0.24
<i>Der p</i> 21	0.05 (0.03–0.96)	0.07 (0.03–0.16)	0.11 (0.07–0.12)	0.81
<i>Der p</i> 23	0.07 (0.04–0.15)	0.06 (0.04–0.30)	0.10 (0.07–0.11)	0.79
HDM component sIgG4, U/mL				
<i>Der p</i> 1	32.2 (16.1–41.6)	25.1 (15.7–36.4)	23.9 (20.0–28.6)	0.80
<i>Der f</i> 1	23.6 (14.2–34.3)	25.0 (16.5–36.8)	11.0 (0.0–26.6)	0.56
<i>Der p</i> 2	140.5 (103.7–185.9)	111.5 (76.3–167.0)	74.9 (32.5–132.5)	0.20
<i>Der f</i> 2	116.3 (77.1–142.5)	109.4 (53.8–130.1)	59.9 (25.6–140.3)	0.36
<i>Der p</i> 7	60.7 (36.0–90.2)	40.9 (30.0–84.4)	69.5 (34.2–241.4)	0.23
<i>Der p</i> 10	23.2 (18.7–49.4)	34.0 (15.2–102.2)	20.0 (14.9–32.4)	0.41
<i>Der p</i> 21	59.0 (27.9–78.9)	38.2 (23.3–53.9)	24.5 (18.3–42.2)	0.17
<i>Der p</i> 23	27.9 (16.4–57.2)	39.5 (26.6–56.3)	15.0 (10.9–30.1)	0.15

HDM, house dust mite; *Der p*, *Dermatophagoides pteronyssinus*; *Der f*, *Dermatophagoides farinae*; AR, allergic rhinitis; IQR, interquartile range. The results are presented as the medians and 25–75% IQRs. The *p* value was presented to describe the difference between the symptomatic group and asymptomatic group. The Mann-Whitney U test was used for the differences between the 2 groups.

Fig. 4. **a** HDM components sIgE level in the AR group, asymptomatic group, and healthy group. **b** HDM components sIgG4 level in the AR group, asymptomatic group, and healthy group. **c** Heatmap of IgE and IgG4 to HDM components between groups. *** $p < 0.01$. AR, allergic rhinitis; Der *p*, *Dermatophagoides pteronyssinus*; Der *f*, *Dermatophagoides farinae*.

($p < 0.01$) (Fig. 2b). Three-dimensional maps for BAT, sIgE, SPT, or sIgE/tIgE are shown in Figure 3.

HDM Allergens Components

Eight HDM components were detected in this study. There were significant differences between AR and asymptomatic group in the levels of HDM components sIgE to Der *p*1, Der *f*1, Der *p*2, and Der *f*2 (all $p < 0.05$) (Table 3, Fig. 4a, c). Additionally, AR patients were sensitized to more HDM components than asymptomatic subjects ($p = 0.0012$) (Table 1). However, no difference was found regarding the specific IgG4 to HDM components between the 2 groups (all $p > 0.05$) (Table 3; Fig. 4b, c).

ROC and Cutoff Values

By ROC analysis, we calculated the *cutoff* values for different parameters to differentiate AR patients from

asymptomatic subjects. The *cutoff* values and the area under the ROC (AUC) are shown in Table 4. SPT and serum sIgE to Der *p* showed good potency in distinguishing the AR patients from asymptomatic individuals. The optimal *cutoff* values for SPT to Der *p* was a wheal diameter of 4.5 mm (AUC 0.9240, 95% CI: 0.84856–0.99936) and for the level of sIgE to Der *p* was 2.29 KU/L (AUC 0.8677, 95% CI: 0.76494–0.97051) (Table 4).

Correlations between Immunological Parameters

We analyzed the correlation coefficients between SPT and sIgE to Der *p*, total IgE and BAT at 333 ng/mL to Der *p*, and sIgE to Der *p*1 and Der *p*2. The correlations are shown in Figure 5. The strongest correlations were observed between sIgE to Der *p* and sIgE to Der *p*2 ($r = 0.7623$, $p < 0.0001$) and between SPT to Der *p* and sIgE to Der *p* ($r = 0.6336$, $p < 0.0001$). In addition, strong correlations were found between sIgE to Der *p* and BAT at 333

Table 4. Cutoff values for parameters to distinguish AR patients from asymptomatic subjects

Parameters	AUC (95% CI)	Cutoff	Sensitivity, %	Specificity, %
SPT, mm				
Der p	0.9240 (0.84856–0.99936)	4.5	91.67	80.00
Der f	0.8958 (0.79858–0.99309)	5.5	83.33	90.00
sIgE, KU/L				
Der p	0.8677 (0.76494–0.97051)	2.29	85.19	76.19
Der f	0.8801 (0.78498–0.97516)	1.71	81.48	80.95
tIgE, KU/L	0.5963 (0.42924–0.76335)	116	70.37	55.00
sIgE to Der p/tIgE	0.8157 (0.69067–0.94082)	0.03	74.07	85.00
anti-FcεRI mAb CD63, %	0.7000 (0.48793–0.91207)	49.75	80.00	60.00
333 ng/mL CD63, %	0.7672 (0.62486–0.90953)	16.72	59.26	90.48
111 ng/mL CD63, %	0.6993 (0.54766–0.85093)	25.42	48.15	95.24
33.3 ng/mL CD63, %	0.6772 (0.52257–0.83193)	6.05	51.85	90.48
HDM component sIgE, IU/mL				
Der p1	0.7857 (0.65571–0.91572)	0.39	70.37	80.85
Der f1	0.8051 (0.68212–0.92810)	3.74	55.56	95.24
Der p2	0.7566 (0.61003–0.90320)	0.62	77.78	80.95
Der f2	0.7302 (0.57752–0.88280)	5.40	70.37	90.48
Der p7	0.6252 (0.46374–0.78670)	0.20	48.15	80.95
Der p23	0.5229 (0.35392–0.69194)	0.07	51.85	57.14
HDM component sIgG4, U/mL				
Der p1	0.5220 (0.35453–0.68957)	32.2	51.85	66.67
Der p2	0.6076 (0.44369–0.77147)	99.0	88.89	38.10
Der f2	0.5785 (0.41161–0.74536)	57.6	92.59	28.57
Der p7	0.6014 (0.43385–0.76897)	41.6	70.37	52.38
Der p21	0.6155 (0.44915–0.78189)	59.0	51.85	80.95
HDM component sIgE/sIgG4				
Der p1	0.6138 (0.44833–0.77918)	0.0001	92.59	33.33
Der f1	0.7637 (0.62920–0.89814)	0.0270	70.37	76.19
Der p2	0.7601 (0.61983–0.90045)	0.0152	59.26	90.48
Der f2	0.7795 (0.64327–0.91581)	0.0108	74.07	85.71
Der p7	0.7284 (0.57222–0.88457)	0.1298	66.67	95.24
Der p10	0.6420 (0.48271–0.80124)	0.0090	44.44	90.48

AUC, area under the ROC; CI, confidence interval; SPT, skin prick test; Der p, Dermatophagoides pteronyssinus; Der f, Dermatophagoides farinae; HDM, house dust mite; AR, allergic rhinitis.

ng/mL Der p ($r = 0.6246$, $p < 0.0001$), and also between sIgE to Der p and sIgE to Der p1 ($r = 0.6028$, $p < 0.0001$) (Fig. 5).

Discussion

Our understanding of allergy has always benefited from assessing the differences between asymptomatic and clinical allergic patients. Our study is a case in point. We compared the clinical and immunological parameters of HDM-sensitized asymptomatic and AR patients and investigated the correlations between these parameters which include SPTs, IgE and IgG4 profiles, and BATs. We

found that the asymptomatic individuals had a smaller area of wheal induced by HDM extract prick tests, and lower HDM IgE levels as well as serum Der p1 and Der p2 IgE levels than AR patients. They also showed a higher threshold for basophil activation than AR patients. Analysis of these differences proved the key role of IgE in the progression from sensitization to clinical allergy.

We first analyzed the differences between the 2 groups in SPT. SPT simulates the process of allergen entering the body, activating effector cells, and eliciting the allergic reaction. In the subject-enrolling stage, we found that the asymptomatic individuals had a significantly weaker skin reaction to the HDM SPT than AR patients. In other words, despite that the whole reaction chain elicited by

Fig. 5. Correlation networks in AR patients and asymptomatic subjects. Spearman's rank correlation analysis was performed for correlations between immunological parameters. All parameters were positively correlated. The width of the edge indicated the strength of association according to the Spearman's r . SPT, skin prick test; *Der p*, *Dermatophagoides pteronyssinus*; BAT, basophil activation test; AR, allergic rhinitis; r , rank correlation coefficient.

allergen was intact, the allergic reaction was attenuated in the asymptomatic individuals. Although the underlying mechanism is still unclear, IgE and IgG4 repertoire we investigated in our study helped explain the difference. We also found that a *cutoff* value of a wheal diameter of 4.5 mm induced by HDM SPT can with good sensitivity and specificity distinguish the asymptomatic from AR patients. This finding coincides with previous studies which show that it is possible to figure out a *cutoff* value of SPT wheal diameter to confirm allergen-induced symptoms and avoid nasal provocation test [15]. For example, a *cutoff* value of cat dander SPT equal to 6.5 mm can diagnose cat allergy with high negative predictive value [27]. Another finding of our study which accords with other studies [28, 29] is that the SPT reactions had no correlation with AR severity. In addition, SPT showed a weak-to-moderate correlation with serum-specific IgE and BAT. In short, the allergen entering route and amount, the interplay of allergen, IgE, and effector cells could all contribute to the discrepancy of SPT reaction, clinical outcome, and disease severity.

Also, in line with most reports, we found the AR patients had higher HDM-specific IgE level than the asymptomatic individuals [16, 30]. HDM, as we know, has more than 30 allergenic proteins, and *Der p1* and *Der p2* are the

major allergens which can be recognized by the IgE antibodies in more than 50% of the HDM-sensitized individuals [31, 32]. We found the IgE levels to *Der p1* and *Der p2* were significantly higher in the AR group, while other HDM component IgE including *Der p7*, *Der p10*, *Der p21*, and *Der p23* showed no difference in the 2 groups. The positive HDM component IgE number was also higher in the AR group. Although there may be discordance in serum IgE level and cell-bound IgE level in some individuals, in general, serum IgE correlates well with cell-bound IgE on FcεRI+ cells [33–35]. Thus, higher level of serum HDM and *Der p1/p2* IgE implies higher density of cell-bound IgE on the surface of mast cells and basophils, which facilitates the binding of HDM to IgE, crosslinks the IgE antibodies on the cell surface, and then triggers downstream mediator release. However, our findings differ from those of another similar IgE component study which showed that IgE to *Der p7* and *Der p23* but not *Der p1* and *Der p2* could distinguish asymptomatic from AR patients [20]. The difference, which may result from the relatively small population in the 2 studies, nevertheless shows that the allergen components are important for elicitation of allergic symptoms, since both studies found the numbers of positive allergen component IgE increased in the AR groups. In addition, we

found that only allergen-specific IgE but not total IgE was responsible for the allergic symptoms since the total IgE level did not see any difference between asymptomatic and AR patients. Moreover, it had no correlation with BAT and SPT. Seen from another perspective, specific IgG4, which has been regarded as an indicator of immune tolerance to certain allergen in allergic individuals [19, 36], may have a protective effect against allergic symptom. However, our study showed that sIgG4 alone could not account fully for this protective effect, for our data did not reveal any difference in all the HDM component sIgG4 levels between the asymptomatic and AR groups against allergic symptoms.

Apart from the IgE level differences in the 2 groups, the BAT results, useful for evaluation of food allergy and inhalant allergy [26], also showed a higher threshold of basophil reactivity to *Der p* allergen stimulation in the asymptomatic group than the AR group, which accorded with the study conducted by Zidarn et al. [20]. The number of basophils responding to a given amount of allergen represents the basophil reactivity conducted by the compound affinity of cell-bound allergen-specific IgE and free competing immunoglobulins (e.g., IgG and IgA). BAT has been reported to be a sensitive marker reflecting the clinical threshold for eliciting symptoms and discriminating between sensitization and allergic subjects [26, 37]. Our results were consistent with previous studies that show BAT can be a candidate biomarker to distinguish asymptomatic and AR patients. However, BAT did not show its advantages over SPT and *Der p*-specific IgE as the AUC of BAT was smaller than SPT and sIgE. In addition, BAT also showed a weak correlation with clinical severity, which is below our expectation as we thought BAT was an ideal model to simulate the allergen-induced reaction in vitro. However, we found that there were no differences in the basophil responses to positive controls anti-FcεRI mAb and fMLP between the asymptomatic group and the AR group. This result contrasts with another HDM study conducted by Zidarn et al. [20]. Our study suggested the ability of allergen-specific IgE binding to FcεRI may differ between AR patients and asymptomatic subjects. We reasoned that level of IgE and IgE affinity as well as allergen epitopes recognized by IgE were responsible for the absence of allergic symptoms in asymptomatic subjects sensitized to HDM. The BAT results combined with the IgE antibodies repertoire also implied a complex network functionally precluding the asymptomatic from developing clinical allergy.

Lastly, we compared the diagnostic potency of the clinical and immunological parameters in differentiating

asymptomatic from AR patients. In general, SPT, sIgE to *Der p* and *Der p2*, and BAT had higher potency than other parameters. Besides, there was also a strong correlation between these 3 parameters themselves. This correlation implied that the IgE-centralized chain played a more important role in the allergy process than other pathways [38]. We also provided the *cutoff* value of each parameter; however, it should be noticed that the *cutoff* value may not be in accordance with other studies considering the heterogeneity of populations [15, 16, 30]. Data from clinical trials had confirmed the clinical efficacy of anti-IgE therapy (omalizumab) in treating AR and asthma, which indicated the central role of IgE in allergic symptoms [39–41]. However, studies had confirmed that allergen skin test reactivity decreased after anti-IgE therapy, but the SPT result was not negative [42], which emphasized that the non-IgE-mediated pathway was partly responsible for the progression of sensitization into allergy. The underlying mechanism is still under investigation.

The analysis above does not mean that we are unaware of some limitations in our study. First, we did not perform nasal provocation test in the asymptomatic group. The enrollment of asymptomatic subjects was based on a negative history of any allergic diseases and no symptoms after HDM exposure. These individuals were confirmed to have been sensitized to HDM in another epidemiological study 3 years ago. To make sure they were really asymptomatic, we asked them to record any nasal symptoms for 4 weeks before the enrollment as some studies did. Thus, we believe these individuals were qualified for our study. In addition, to avoid the possible false-positive SPT in some asymptomatic individuals, we did a sensitivity analysis by removing the data of 4 subjects who had positive SPT but negative specific IgE to HDM (all in the asymptomatic group) and reanalyzed what was left of the data. We found the results were consistent with our original findings and the conclusions were the same. Second, the sample size for this study is relatively small, thus the findings in our study may need to be validated further in a larger population. Finally, the specific IgG and IgG4 and other antibodies against *Der p* were not obtained, and the whole antibodies repertoire is needed to elucidate the potential mechanisms of AR and asymptomatic subjects [43].

In conclusion, our study demonstrated that SPT, specific IgE to *Der p1* and *p2*, and BAT help distinguish asymptomatic from AR patients. IgG4 alone could not differentiate asymptomatic individuals from AR patients. Non-IgE-mediated pathways may also contribute to AR symptoms. Our findings help shed light on the underlying mechanism of HDM-induced allergic reaction.

Acknowledgement

We would like to thank Dr. Xiubo Zhu for her invaluable assistance throughout the preparation of the original manuscript.

Statement of Ethics

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki, and the study protocol was approved by the Independent Ethics Committee of Tongji Hospital (IRB ID: TJ-C20210501). All the participants had given their written informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was supported by Natural Science Foundation of Hubei Province, China (No. 2019CFB443).

Author Contributions

Q.X. and R.Z. conceived the study. Q.X. participated in the BAT and antibodies analysis and wrote the first draft. Q.J. and L.Y. participated in the BAT analysis. N.H., W.L., Y.Y., D.M., and S.Z. enrolled the patients and collected the clinical data; Y.W. collected the blood samples. R.Z. collected the clinical data, performed statistical analysis, and revised the manuscript.

References

- 1 Zhu R, Wang J, Wu Y, Yang Y, Huang N, Yang Y, et al. The allergic rhinitis control test questionnaire is valuable in guiding step-down pharmacotherapy treatment of allergic rhinitis. *J Allergy Clin Immunol Pract*. 2019; 7(1):272–8.
- 2 Bousquet J, Hellings PW, Agache I, Amat F, Annesi-Maesano I, Ansotegui IJ, et al. Mobile Airways Sentinel Network (MASK) Study Group. Allergic Rhinitis and its Impact on Asthma (ARIA) Phase 4 (2018): change management in allergic rhinitis and asthma multimorbidity using mobile technology. *J Allergy Clin Immunol*. 2019;143(3):864–79.
- 3 Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63(Suppl 86):8–160.
- 4 Dor-Wojnarowska A, Liebhart J, Miecielica J, Rabski M, Fal A, Samoliński B, et al. The impact of sex and age on the prevalence of clinically relevant sensitization and asymptomatic sensitization in the general population. *Arch Immunol Ther Exp (Warsz)*. 2017;65(3):253–61.
- 5 Su KW, Chiu CY, Tsai MH, Liao SL, Chen LC, Hua MC, et al. Asymptomatic toddlers with house dust mite sensitization at risk of asthma and abnormal lung functions at age 7 years. *World Allergy Organ J*. 2019;12(9):100056.
- 6 Bodtger U, Poulsen LK, Malling HJ. Asymptomatic skin sensitization to birch predicts later development of birch pollen allergy in adults: a 3-year follow-up study. *J Allergy Clin Immunol*. 2003;111(1):149–54.
- 7 Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Crameri R, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2LEN project. *Allergy*. 2006;61(6):671–80.
- 8 Niederberger V, Stübner P, Spitzauer S, Kraft D, Valenta R, Ehrenberger K, et al. Skin test results but not serology reflect immediate type respiratory sensitivity: a study performed with recombinant allergen molecules. *J Invest Dermatol*. 2001;117(4):848–51.
- 9 van Hage-Hamsten M, Johansson E, Kronqvist M, Loughry A, Cookson WO, Moffatt MF. Associations of Fc epsilon R1-beta polymorphisms with immunoglobulin E antibody responses to common inhalant allergens in a rural population. *Clin Exp Allergy*. 2002; 32(6):838–42.
- 10 Purohit A, Laffer S, Metz-Favre C, Verot A, Kricek F, Valenta R, et al. Poor association between allergen-specific serum immunoglobulin E levels, skin sensitivity and basophil degranulation: a study with recombinant birch pollen allergen Bet v 1 and an immunoglobulin E detection system measuring immunoglobulin E capable of binding to Fc epsilon RI. *Clin Exp Allergy*. 2005;35(2):186–92.
- 11 Hong X, Caruso D, Kumar R, Liu R, Liu X, Wang G, et al. IgE, but not IgG4, antibodies to Ara h 2 distinguish peanut allergy from asymptomatic peanut sensitization. *Allergy*. 2012;67(12):1538–46.
- 12 Gellrich D, Högerle C, Becker S, Gröger M. Is quantitative sIgE serology suitable for distinguishing between silent sensitization and allergic rhinitis to dermatophagoides pteronyssinus? *J Invest Allergol Clin Immunol*. 2019; 29(2):124–31.
- 13 Inoue Y, Sato S, Takahashi K, Yanagida N, Yamamoto H, Shimizu N, et al. Component-resolved diagnostics can be useful for identifying hazelnut allergy in Japanese children. *Allergol Int*. 2020;69(2):239–45.
- 14 Olivieri M, Heinrich J, Schlünssen V, Antó JM, Forsberg B, Janson C, et al. The risk of respiratory symptoms on allergen exposure increases with increasing specific IgE levels. *Allergy*. 2016;71(6):859–68.
- 15 Pastorello EA, Incorvaia C, Ortolani C, Bonini S, Canonica GW, Romagnani S, 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;96(5 Pt 1):580–7.
- 16 Comite P, Minale P, Ferrero F, Mussap M, Ciprandi G. Der p 1 IgE measurement for distinguishing between sensitization and allergy. *Immunol Lett*. 2015;166(2):145–6.
- 17 van Neerven RJ, Knol EF, Ejrnaes A, Würtzen PA. IgE-mediated allergen presentation and blocking antibodies: regulation of T-cell activation in allergy. *Int Arch Allergy Immunol*. 2006;141(2):119–29.
- 18 Savilahti EM, Rantanen V, Lin JS, Karinen S, Saarinen KM, Goldis M, et al. Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes. *J Allergy Clin Immunol*. 2010;125(6):1315–21.e9.
- 19 Tomićić S, Norrman G, Fälth-Magnusson K, Jenmalm MC, Devenney I, Böttcher MF. High levels of IgG4 antibodies to foods during infancy are associated with tolerance to corresponding foods later in life. *Pediatr Allergy Immunol*. 2009;20(1):35–41.

- 20 Zidarn M, Robič M, Krivec A, Šilar M, Resch-Marat Y, Vrtala S, et al. Clinical and immunological differences between asymptomatic HDM-sensitized and HDM-allergic rhinitis patients. *Clin Exp Allergy*. 2019;49(6):808–18.
- 21 Wang J, Wu Y, Li J, Huang X, Zhu R. Eight Aeroallergen Skin Extracts May Be the Optimal Panel for Allergic Rhinitis Patients in Central China. *Int Arch Allergy Immunol*. 2017;173(4):193–8.
- 22 Waldron R, McGowan J, Gordon N, McCarthy C, Mitchell EB, Fitzpatrick DA. Proteome and allergenome of the European house dust mite *Dermatophagoides pteronyssinus*. *PLoS One*. 2019;14(5):e0216171.
- 23 Brożek JL, Bousquet J, Agache I, Agarwal A, Bachert C, Bosnic-Anticevich S, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. *J Allergy Clin Immunol*. 2017;140(4):950–8.
- 24 Global Initiative for Asthma (GINA). *Pocket guide for asthma management and prevention (for adults and children older than 5 years)*; 2019. Available from: <https://ginasthma.org>.
- 25 Bousquet J, Heinzerling L, Bachert C, Papadopoulos NG, Bousquet PJ, Burney PG, et al. Global allergy and asthma European network; allergic rhinitis and its impact on asthma. Practical guide to skin prick tests in allergy to aeroallergens. *Allergy*. 2012;67(1):18–24.
- 26 Mehlich J, Fischer J, Hilger C, Swiontek K, Morisset M, Codreanu-Morel F, et al. The basophil activation test differentiates between patients with alpha-gal syndrome and asymptomatic alpha-gal sensitization. *J Allergy Clin Immunol*. 2019;143(1):182–9.
- 27 Al-Ahmad M, Jusufovic E, Arifhodzic N, Nurkic J, Hanoun AL. Sensitization to cat: when is nasal challenge needed? *Int Arch Allergy Immunol*. 2019;179(2):108–13.
- 28 Ibekwe PU, Ibekwe TS. Skin prick test analysis in allergic rhinitis patients: a preliminary study in Abuja, Nigeria. *J Allergy*. 2016;2016:3219104.
- 29 Srisuwatchari W, Kwanthong P, Bunnag C, Pacharn P, Jirapongsananuruk O, Visitsunthorn N. Association between skin prick test and serum specific immunoglobulin E to American cockroach allergens in allergic rhinitis patients. *Allergol Immunopathol*. 2020 Mar–Apr;48(2):170–4.
- 30 Alesina R, De Amici M, Ciprandi G. Serum IgE discriminates allergy from sensitisation better than skin testing. *Allergol Immunopathol*. 2014 Mar–Apr;42(2):171–3.
- 31 Kidon MI, Chiang WC, Chin CW, Liew WK, Kang LW, Ong TC, et al. Mite component-specific IgE repertoire and phenotypes of allergic disease in childhood: the tropical perspective. *Pediatr Allergy Immunol*. 2011;22(2):202–10.
- 32 Trombone AP, Tobias KR, Ferriani VP, Schuurman J, Aalberse RC, Smith AM, et al. Use of a chimeric ELISA to investigate immunoglobulin E antibody responses to Der p 1 and Der p 2 in mite-allergic patients with asthma, wheezing and/or rhinitis. *Clin Exp Allergy*. 2002;32(9):1323–8.
- 33 Conroy MC, Adkinson NF Jr, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol*. 1977;118(4):1317–21.
- 34 Dehlink E, Baker AH, Yen E, Nurko S, Fiebigler E. Relationships between levels of serum IgE, cell-bound IgE, and IgE-receptors on peripheral blood cells in a pediatric population. *PLoS One*. 2010;5(8):e12204.
- 35 Qiu C, Zhong L, Huang C, Long J, Ye X, Wu J, et al. Cell-bound IgE and plasma IgE as a combined clinical diagnostic indicator for allergic patients. *Sci Rep*. 2020;10(1):4700.
- 36 Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, 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):1249–56.
- 37 Santos AF, Douiri A, Bécaries N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J Allergy Clin Immunol*. 2014;134(3):645–52.
- 38 Poole JA, Rosenwasser LJ. The role of immunoglobulin E and immune inflammation: implications in allergic rhinitis. *Curr Allergy Asthma Rep*. 2005;5(3):252–8.
- 39 Vignola AM, Humbert M, Bousquet J, Boulet LP, Hedgecock S, Blogg M, et al. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with concomitant allergic asthma and persistent allergic rhinitis: SOLAR. *Allergy*. 2004;59(7):709–17.
- 40 Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med*. 2011;364(11):1005–15.
- 41 Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol*. 2005;115(3):459–65.
- 42 Corren J, Shapiro G, Reimann J, Deniz Y, Wong D, Adelman D, et al. Allergen skin tests and free IgE levels during reduction and cessation of omalizumab therapy. *J Allergy Clin Immunol*. 2008;121(2):506–11.
- 43 Aghayan-Ugurluoglu R, Ball T, Vrtala S, Schweiger C, Kraft D, Valenta R. Dissociation of allergen-specific IgE and IgA responses in sera and tears of pollen-allergic patients: a study performed with purified recombinant pollen allergens. *J Allergy Clin Immunol*. 2000;105(4):803–13.