Molecular Immunology 101 (2018) 527–530

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Short communication

Molecular patterns in the isotype-specific antibody responses to the major cedar aeroallergen Jun a 1

Randall M. Goldblum[a], Rumali S. Madagoda-Desilva[a], Yueqing Zhang[a], Julius van Bavel[b], Terumi Midoro-Horiuti[a,*]

[a] Department of Pediatrics, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0366, USA
[b] Isis Clinical Research, LLC., 6836 Austin Center Blvd Ste 180, Austin, TX 78731, USA

ARTICLE INFO

Keywords:
Allergen
Cedar pollen hypersensitivity
Jun a 1
Epitopes
IgG
Immunoglobulin isotypes

ABSTRACT

Most studies of the immune responses in allergic rhinitis have focused on IgE antibodies to mixtures of allergenic proteins. Based on our previous studies of the major mountain cedar allergen Jun a 1, we sought to describe a broader assessment of the humoral immune responses to a single, dominant allergen, in three groups of allergic subjects, all of whom had similarly exposures to the whole cedar pollen. The major outcomes of this study was that, with the onset of allergic rhinitis symptoms, and after treatment with immunotherapy, serum IgE and IgG (but not IgA) antibodies to Jun a 1 increased. Interestingly, both IgE and IgG4 antibodies to the Jun a 1 allergen were strongly focused on its conformation epitopes. These IgG antibodies to conformationalstructures may be a useful marker of the therapeutic response to immunotherapy.

1. Introduction

Seasonal allergic rhinitis due to cedar pollens are a common problem in multiple areas in the world (Goldblum et al., 2016; Pichler et al., 2015; Midoro-Horiuti, 1992; Midoro-Horiuti et al., 1992). For instance, about 30–40% of the populations of central US, Southern Europe and Japan, suffer from cedar pollen hypersensitivities to highly cross-reactive allergens of the mountain cedar (*Juniperus ashei*, Cupressaceae), Italian cypress (*Cupressus sempervirens*, Cupressaceae) and Japanese cedar (*Cryptomeria japonica*, Taxodiaceae). We have previously isolated, cloned, sequenced and elucidated the crystal or model structure and IgE epitopes of two of these allergens, Jun a 1 and Jun a 3, from mountain cedar pollen (Midoro-Horiuti et al., 1999, 2000; Soman et al., 2000; Midoro-Horiuti et al., 2003; Liu et al., 2003; Czerwinski et al., 2005; Varshney et al., 2007). We also reported that the majority of human IgE antibodies from the sera of mountain cedar allergy sufferers are directed against several conformational epitopes on the surface of the dominant allergen, Jun a 1 (Goldblum et al., 2014). There are reports about the analyses of IgE and IgG epitopes on the other pollen allergens. The conformational IgE epitopes are reported on Bet v 1(Mirza et al., 2000; Gieras et al., 2011; Subbarayal et al., 2013), Amb a 4 (Pablos et al., 2018), Amb a 8 (Offermann et al., 2016), Cry j 1 (Aoki et al., 2009), Phl p 2 (Padavattan et al., 2009), Phl p 3 (Devanaboyina et al., 2014) and Phl p 5 (Focke-Tejkl et al., 2014). Also, conformational IgG epitopes are reported on Bet v 1 (Subbarayal et al., 2013).

We describe here a population-based study comparing three groups of atopic subjects, all of whom were naturally exposed yearly to mountain cedar pollen, in the same region. The first group did not display allergic symptoms or skin prick test (SPT) responses to mountain cedar extracts (SPT^-). The second group had seasonal, clinical manifestations of cedar pollinosis and positive SPT, but had not been treated with specific immunotherapy (SPT^+SIT^+). The third group had seasonal symptoms, was SPT^+ and had been treated with subcutaneous immunotherapy with mountain cedar pollen extracts (SPT^+SIT^+). Our goal for this epidemiologic study was to identify the similarities and differences in the serum antibody responses to Jun a 1, in these three clinical groups.

2. Methods

2.1. Patient selection and serum collection

Each subject provided serum samples under a University of Texas Medical Branch IRB Protocol (06–050) and had a repeat SPT with a

Abbreviations: Jun a 1, Juniperus *ashei* allergen 1; SIP, specific immunotherapy; SPT, skin prick test

* Corresponding author.
E-mail addresses: rmgoldbl@utmb.edu (R.M. Goldblum), medagoda@allergyclinicoftulsa.com (R.S. Madagoda-Desilva), yuezhang@utmb.edu (Y. Zhang), jvanbavel@aol.com (J. van Bavel), tmidoro@utmb.edu (T. Midoro-Horiuti).

https://doi.org/10.1016/j.molimm.2018.08.007
Received 5 December 2017; Received in revised form 31 July 2018; Accepted 5 August 2018

commercial extract of mountain cedar pollen (Hollister-Stier, Spokane, WA), if they had not been tested in the previous year. Sera from the three groups of atopic adults, all of whom were seasonally exposed to mountain cedar pollen in the same region and were recruited from Dr. van Bavel's clinic at Austin, Texas. We distributed these subjects into three clinical groups. The first group did not display allergic symptoms or SPT responses to mountain cedar extract (SPT^-, n = 10). The second group had seasonal clinical manifestations of cedar pollinosis and positive skin prick tests, but were not treated with specific immunotherapy (SPT^+ SIT^-, n-12). The third group had seasonal symptoms, positive SPT and were treated with allergen specific immunotherapy (SPT^+SIT^+, n = 12).

2.2. Purification of Jun a 1

Jun a 1 was purified from mountain cedar pollen, as we previously described (Midoro-Horiuti et al., 1999).

2.3. Enzyme linked immuno assay (ELISA) assays

To quantify the IgE, IgA, IgG and IgG4 serum antibodies to Jun a 1, 96 well microtiter plates were coated by incubating with native, purified Jun a 1 (3 μg/mL) at 37 °C overnight (Midoro-Horiuti et al., 1999). Then various dilutions of the sera (1:10 to $1:10^9$ in 0.01% Tween 20-Tris buffered saline, TTBS) from each subject were incubated in duplicate wells for 4 h. After washing the microtiter plates, the quantity of IgE bound to the wells was detected, using biotinylated goat anti-human IgE (Vector, Burlingame, CA), followed by horseradish peroxidase (HRP)-streptavidin conjugates. The binding of IgA, IgG and IgG4 were detected by incubation with anti-human IgA, IgG or IgG4 enzyme conjugates (Zymed, San Francisco, CA). After washing the microtiter plates, the patient's immunoglobulins of each isotype, that bound to the wells was quantified using 3,3′,5,5′-Tetramethylbenzidine (TMB, Sigma). The results from the colorimetric enzyme assays were then plotted, to establish the highest dilution that had color significantly above the buffer control. The number of dilutions was considered the titer for that isotype.

2.4. ELISAs to distinguish between antibodies that bind to native and denatured Jun a 1

To determine the extent to which individual patient's IgE, IgA, IgG and IgG4 antibodies react to conformational and linear epitopes of Jun a 1, we used similar ELISA assays, which we described previously (Varshney et al., 2007; Goldblum et al., 2014). Some of these coated wells were incubated with 6 mol/L guanidine-HCl for 30 min, to denature the Jun a 1. The sera from the members of each of the three clinical groups were then serially diluted and incubated in either the guanidine-treated and untreated wells. The binding of each isotype, to native and denatured Jun a 1, was detected and the results plotted as described above.

3. Results and discussion

3.1. Patient demographics

There were no significant differences in the gender or age distribution between these three groups. The mean male: female ratios were (3:7, 7:5 and 4:6), and ages ± SD were 31.9 ± 11.7, 37.9 ± 12.1 and 44.2 ± 8.9) for SPT^-, SPT^+SIT^- and SPT^+SIT^+, respectively. (Table 1)

3.2. Titer of Jun a 1 specific immunoglobulins

We first assessed each subject's titers of serum IgE, IgA, IgG and IgG4 antibodies to Jun a 1, using ELISA assays, as described above (Fig. 1) (Goldblum et al., 2014). We could not distinguish any differences in the concentration of serum IgA anti-Jun a 1 antibodies between these three groups (Fig. 1). However, we found a significantly higher concentration of IgG anti-Jun a 1 antibodies in the sera of the SPT^+SIT^-, than in the other two groups. The IgG4 anti-Jun a 1 concentrations were too low to be accurately quantified in most of the 1:10 diluted sera. The lines above the graph indicate the significant differences between the groups for IgG and IgE isotypes.

Table 1
Patient demographics.

#	sex	age	*J. ashei*	positive SPT to other allergens
SPT^+SIT^- group				
1	M	26	+	tree, ragweed, dust mite, Alternaria
2	F	43	+	no other
3	M	27	+	ragweed, Alternaria
4	M	26	+	tree, ragweed, Alternaria
5	F	39	+	tree, ragweed, dust mite
6	M	30	+	tree, ragweed, dust mite, Alternaria, penicillium
7	M	55	+	tree, grass, ragweed, cat, Alternaria
8	M	45	+	tree, grass, ragweed, dust mite, cat
9	F	42	+	tree, grass, ragweed, dust mite, cat, Alternaria
10	F	56	+	tree, ragweed, cat
11	F	47	+	tree, grass, ragweed
12	M	19	+	grass, dust mite, ragweed
mean		37.9	12.1	
SPT^+SIT^+ group				
1	M	43	+	tree, grass,ragweed, dust mite, dog, cat
2	M	35	+	tree, grass, ragweed
3	F	42	+	tree, grass, ragweed, dog, cat
4	M	44	+	tree, grass, ragweed, dust mite
5	M	47	+	grass, dust mite
6	F	65	+	ragweed
7	F	34	+	tree, ragweed, penicillium, dog
8	F	50	+	tree, grass, ragweed, dust mite, dog, cat, cockroach
9	F	44	+	tree, dust mite, cat
10	F	38	+	tree, grass, ragweed, dust mite, Alternaria, dog, cat, cockroach
mean		44.2 ± 8.9		
SPT^- group				
1	F	39	–	tree, grass, dog, cat, Alternaria
2	F	31	–	tree, ragweed, dog, dust mite, Alternaria
3	M	20	–	tree, grass, dog, dust mite
4	F	11	–	tree, grass, ragweed, dust mite, cat, Alternaria
5	F	47	–	tree, dust mite, dog
6	F	32	–	tree, Alternaria, dog, cat, Alternaria
7	M	24	–	tree, cat, Alternaria
8	F	30	–	tree, grass, dust mite, Alternaria
9	F	36	–	tree, grass, dust mite, dog, Alternaria
10	M	49	–	dust mite, Alternaria
mean		31.9 ± 11.7		

SPT: skin pick test, SIT: specific immunotherapy.

3.3. Recognition of conformational vs. linear epitopes of Jun a 1 by each antibody isotype

We next examined the types of molecular structures on Jun a 1 that were recognized by each isotype, by testing the same sera in ELISA wells, in which the conformational (discontinuous) epitopes were disrupted by pretreating Jun a 1 coated wells with guanidine (Fig. 2), as we previously described. The results of these assays showed that IgE antibody are strongly directed toward conformational epitopes, while IgA and total IgG are less selective for these structures. This is indicated in the SPT^+SIT^- group (Fig. 2B), by the loss (80–100%) of their IgE reactivity when assayed on the denatured Jun a 1 (mean ± SD = 95 ± 3). However, binding of their IgA, IgG and IgG4 was less affected by this denaturation (43 ± 24, 27 ± 22 and 58 ± 27%, $p < 0.00005$, 0.0005 and 0.06), respectively. These findings strongly suggest that pathologic IgE antibodies to Jun a 1 predominantly bind to conformational epitopes, while the IgA and total IgG antibodies recognize both