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Formation of IgE-Allergen-CD23 Complex Changes in Children Treated with Subcutaneous Immunotherapy for Japanese Cedar Pollinosis

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Japanese cedar pollinosis · Subcutaneous immunotherapy · Immune response · Children · CD23

Abstract
Background: Subcutaneous immunotherapy (SCIT) is used to treat Japanese cedar (JC) pollinosis. The formation of IgE-allergen-CD23 complex after SCIT for JC pollinosis has not yet been fully elucidated. ***Objective:*** The objective of this study was to investigate the formation of IgE-allergen-CD23 complex after SCIT for JC pollinosis. ***Methods:*** Eleven patients were treated with 3-year SCIT for JC pollinosis at Sagamihara National Hospital from 2013 to 2014. Nasal and ocular symptoms (in terms of symptom scores) during the scattering of JC pollen and immunological changes were investigated. Levels of JC pollen-specific antibodies (IgE and IgG_4) were measured by ImmunoCAP assays. To detect the changes in allergen-presenting ability of B cells, the levels of IgE-allergen-CD23 complexes in serum were measured by a cell-free, enzyme-linked immunosorbent-facilitated antigen-binding assay. ***Results:*** The median (interquartile range) age of the subjects was 8 (6–10) years. Three patients (27%) had comorbid atopic dermatitis, and 5 patients (45%) had comorbid bronchial asthma. Before starting SCIT, the total IgE level was 373 (75–2,870) kU/L, and the level of JC pollen-specific IgE was 77.2 (15.4–528) kU_A/L. Symptom scores improved significantly from the year after treatment. JC pollen-specific IgE levels did not change after 3 years of treatment. JC pollen-specific IgG_4 levels increased significantly throughout the treatment period. The levels of IgE-allergen-CD23 complexes decreased significantly after 3 years of treatment. ***Conclusion:*** The ability of IgE-allergen complexes to bind to CD23 decreased after SCIT, suggesting that increasing levels of IgE-blocking antibodies, including IgG_4, may play an important role in the mechanism of SCIT.

Introduction

Japanese cedar (JC) pollinosis, defined as seasonal rhinoconjunctivitis, is caused by JC pollen and has a prevalence of 36.7% in the Japanese population [1]. Subcutaneous immunotherapy (SCIT) is used to treat JC pollinosis

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Table 1. Patient characteristics (n = 11)

Age, years	8 (6–10)
Male:female	7:4
Associated atopic disorders, *n* (%)	
Atopic dermatitis	3 (27)
Bronchial asthma	5 (45)
Eosinophils in peripheral blood, %	3.3 (2.9–5.8)
Total IgE, kU/L	373 (75–2,870)
JC pollen-specific IgE, kU_A/L	77.2 (15.4–528)
JC pollen-specific IgG_4, mgA/L	0.39 (0.24–0.59)
IgE-allergen-CD23 complex, %	98 (95–101)
Maintenance dose, *n* (%)	
400 JAU	8 (73)
200 JAU	1 (9)
60 JAU	2 (18)

Values are presented as medians (IQR) or *n* (%). JAU, Japanese allergy unit; JC, Japanese cedar.

[2]. Allergy immunotherapy is the only treatment that can alter the disease course in patients [3] and can be used to effectively treat IgE-mediated allergic respiratory diseases. However, the mechanism of allergen immunotherapy has not yet been clarified. The low-affinity IgE receptor CD23 plays an important role in controlling the activity of allergen-specific T cells through IgE-facilitated allergen presentation. Therefore, the ability of IgE-allergen complexes to bind to CD23 was investigated after SCIT patients with JC pollen allergy.

Materials and Methods

Materials

Patients treated with 3-year SCIT for JC pollen allergy at Sagamihara National Hospital (Sagamihara, Japan) between 2013 and 2014 were recruited, and the study was concluded during the period from 2016 to 2017. Four patients who did not complete 3-year SCIT were excluded; hence, 11 patients were recruited (Table 1). Standardized JC pollen extract was used (Torii Pharmaceutical, Tokyo, Japan), which contains 7.3–21 μg Cry j 1, a major allergen of JC pollen, in 10,000 Japanese Allergen Unit (JAU), for SCIT.

Methods

At the treatment initiation in autumn (outside the JC pollen season), all patients received SCIT. When rush SCIT was initiated, the patients were hospitalized for the first 5 days of therapy (see online suppl. Table 1; for all online supplementary material, see www.karger.com/doi/10.1159/000510640), after which maintenance doses were administered at 1-week intervals for 2 weeks, followed by administration at 2-week intervals for 4 weeks, and finally, 1-month intervals for 3 years. The maintenance dose administered was 400 JAU or the maximum dose tolerated by the patient.

Nasal and ocular symptoms (in terms of symptom scores) and immunological changes were investigated [4]. JC pollen-specific antibody (IgE and IgG_4) levels in each patient were measured before treatment and 1, 3, 6, 12, 18, 24, 30, and 36 months post-initiation of treatment using an ImmunoCAP (Thermo Fisher Scientific/Phadia, Uppsala, Sweden) assay.

To detect changes in the levels of IgE-blocking antibodies during treatment, complex formation between IgE, the allergen, and low-affinity IgE-receptor CD23 (IgE-allergen-CD23 complex) in serum was measured using a cell-free, enzyme-linked immunosorbent-facilitated antigen-binding (ELIFAB) assay, both before and after 1, 2, and 3 years of treatment [5]. Briefly, a 96-well microplate was coated with recombinant CD23 (sCD23, 123-FE-050; R&D Systems, Minneapolis, MN, USA). The plate was then washed and blocked with 1% bovine serum albumin/phosphate-buffered saline. Serum was incubated with JC pollen extract before transfer to CD23-coated microtiter plates for IgE-allergen-CD23 complex formation. Allergen-IgE-CD23 complexes were detected using biotin-conjugated anti-human IgE antibodies. The levels of allergen-IgE complexes bound to CD23 were expressed as absorbance at 450 nm.

Dunnett's multiple comparison test was performed to analyze changes of IgE-allergen-complexes bound to CD23. Other differences in continuous variables were tested using the Wilcoxon rank-sum rank test. Statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) software, and values of $p < 0.05$ were considered significant.

Results

Eight patients (73%) reached a maintenance dose of 400 JAU. The maintenance dose for 1 patient was limited to 200 JAU because upper arm swelling occurred when a dose of 400 JAU was administered. The maintenance dose for 2 patients was limited to 60 JAU due to systemic symptoms; in 1 of these patients, the dose was later increased to 400 JAU without the recurrence of systemic symptoms 30 months after starting SCIT.

Symptom scores significantly decreased throughout the treatment period (shown in Fig. 1a). Transient increases in specific IgE levels occurred during the JC pollen seasons; however, no overall increase, compared to pretreatment levels, was seen after 3 years of treatment (shown in Fig. 1b). Additionally, JC pollen-specific IgG_4 levels increased significantly throughout the treatment period (shown in Fig. 1c).

The pretreatment mean (95% confidence interval) level of IgE-allergen-CD23 complexes was 89.8% (69.6–109.9). Mean (95% confidence internal) levels of IgE-allergen-CD23 complexes were 55.5% (31.8–79.2), 48.4% (22.1–74.8), and 48.5% (22.3–74.8) for the first, second, and third years after treatment initiation, respectively (shown in Fig. 1d).

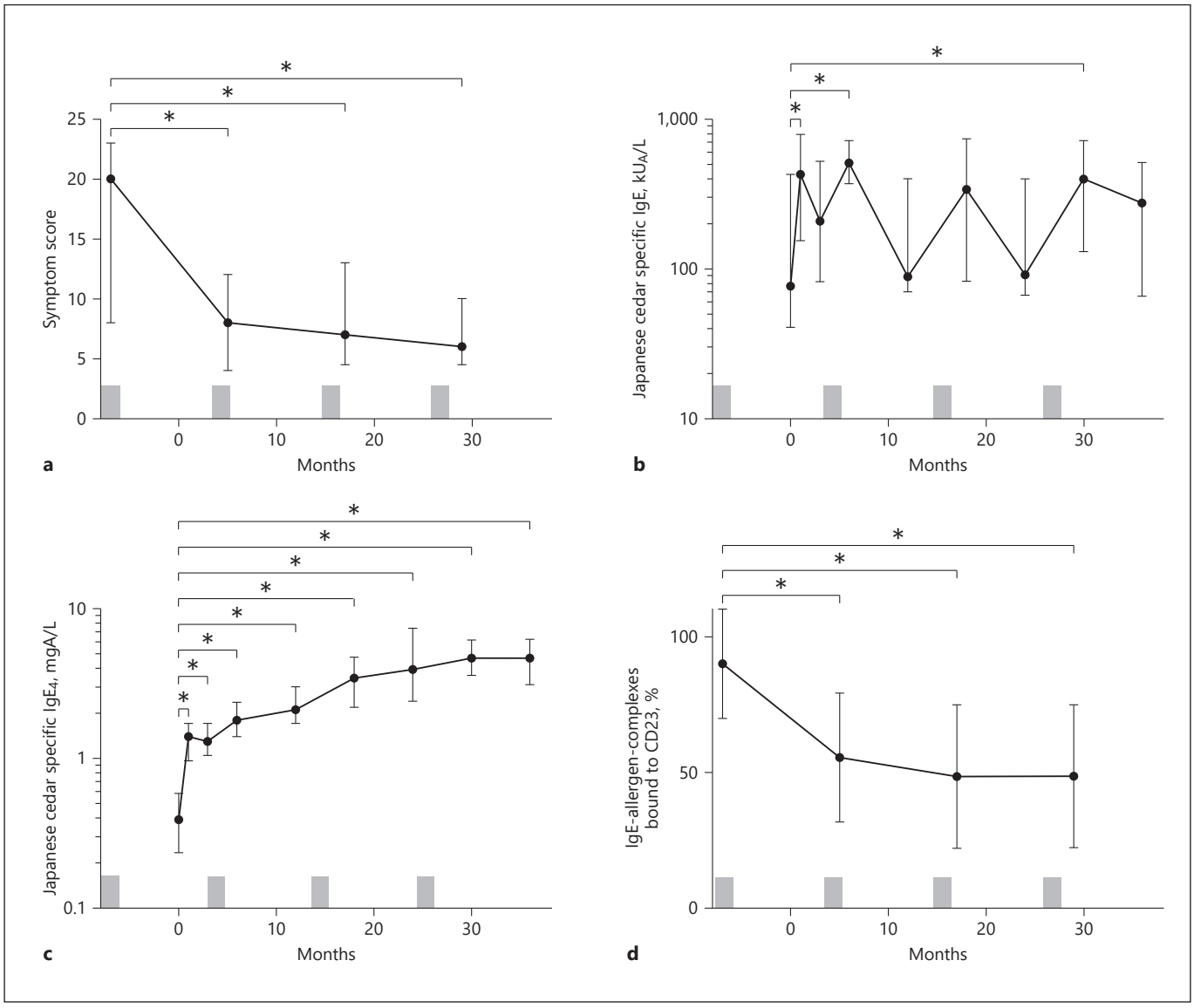

Fig. 1. Changes in symptom scores and immunological responses. **a** Symptom scores changed throughout the 3-year treatment period. **b** JC pollen-specific IgE levels did not change after 3 years of treatment. **c** JC pollen-specific IgG_4 levels increased significantly throughout the 3-year treatment period. **d** Levels of IgE-allergen-CD23 complexes decreased throughout the 3-year treatment period. The gray area indicates the dispersal period for JC pollen. Values are reported as medians (interquartile range), and data were assessed using the Wilcoxon rank-sum rank test, $*p < 0.05$ (**a**, **b**, **c**). Values are reported as means (confidence interval), and data were assessed using Dunnett's multiple comparisons test (**d**). JC, Japanese cedar.

Discussion

We administered SCIT to children with JC pollinosis and successfully reduced nasal and ocular allergic symptoms during the dispersal period for JC pollen. We found that the JC pollen-specific IgG_4 levels increased and allergen-IgE-CD23 complex formation decreased with SCIT. This is the first study that measured IgE-allergen-CD23 complex formation, before and after SCIT, in children with JC pollen allergy.

CD23 (FcεRII) is a low-affinity IgE receptor expressed in different cell types including B cells, macrophages, and eosinophils [6, 7]. When complexed with allergen-specific IgE, allergens can be taken up and presented to T cells through binding to CD23 located on the surface of B cells [7]. After the formation of IgE-allergen-CD23 complexes, these com-

plexes are internalized, and the allergen fragments are then presented to CD4-positive T cells via MHC class II molecules. The uptake of allergens through complex formation with IgE and CD23 allows efficient allergen presentation and activation of pro-inflammatory allergen-specific CD4 T cells at low allergen concentrations, a mechanism known as facilitated allergen binding [8]. Successful allergy immunotherapy is associated with the development of IgE-blocking antibodies and facilitated allergen binding inhibition, which inhibits allergen-specific T-cell activation [9].

Here, we showed that IgE-antigen-CD23 complex formation was inhibited after SCIT, possibly through the IgE-blocking effect of treatment-induced allergen-specific non-IgE antibodies (e.g., IgG_4). An ELIFAB assay has previously been used to assess IgE-blocking activity in serum, placebo, and 32 weeks post-initiation of grass pollen SCIT [10]. Similar to that in our study, ELIFAB data showed that grass pollen SCIT treatment reduced IgE-allergen-CD23 complex formation. The mechanism involving increased IgE-blocking activity as a result of SCIT is common for all allergens.

The rate of IgE-allergen-CD23 complex formation was determined to be 0% in 1 patient both before and after SCIT (Fig. 1d). At baseline, the JC pollen-specific IgE and IgG_4 levels in this patient were 822 kU_A/L and 0.87 mgA/L, respectively, and the total IgE level was 25,200 kU/L. Despite the high concentration of specific IgE in the serum from this patient, we could not detect IgE-allergen-CD23 complex formation using our system. The finding that the sera from some donors do not support IgE-allergen-CD23 complex formation is not uncommon [11]. The percentage of sera that do not support IgE-allergen-CD23 complex formation differs between extracts from different allergen sources. The reason for this is not clear, but it may be linked to the overall complexity of different allergen species with regard to the number of major and minor allergens and/or to the complexity of the IgE repertoire in individual serum samples [11].

In conclusion, we herein report the results of a successful JC pollen SCIT study with a rush up-dosing regimen in children. This is the first study that measured IgE-allergen-CD23 complex formation before and after SCIT in children with JC pollen allergy. The study showed a significant reduction in symptom scores over 3 consecutive JC pollen seasons. During the 3-year treatment period, a continuous increase in allergen-specific IgG_4 levels was seen. The ability of IgE-allergen complexes to bind to CD23 decreased after SCIT, suggesting that increasing levels of IgE-blocking antibodies, including IgG_4, may play an important role in the mechanism of SCIT.

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Statement of Ethics

This study was designed in accordance with the Declaration of Helsinki. Prior to analysis, all patient data were anonymized. Informed consent for the publication of data was obtained from all patients or their guardians. The study was approved by the Institutional Review Board of Sagamihara National Hospital (Approval No. 2013070912, UMIN000012117).

Conflict of Interest Statement

Hiroki Matsuhara, Satoko Kobayashi, Chiharu Fukano, and Katsuyo Ohashi-Doi are employees of Torii Pharmaceuticals Co., Ltd. Motohiro Ebisawa serves on the clinical medical advisory board of DBV Technologies. Sakura Sato and Motohiro Ebisawa had received speaker's honoraria from Mylan EPD. Tomoyuki Asaumi and Noriyuki Yanagida have no conflicts of interest to declare.

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Author Contributions

T.A., S.S., N.Y., K.O.D., and M.E. contributed to the conception and design of this study, analysis and interpretation of the data, and drafting and critical revision of the manuscript. H.M., S.K., and C.F. contributed to measure IgG_4 and IgE-allergen-CD23 complexes. All authors approved the submitted version of the manuscript.

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