

Immunologic Modification in Mono- and Poly-sensitized Patients After Sublingual Immunotherapy

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Objectives/Hypothesis: To compare immunologic modification and treatment outcomes after 2 years of sublingual immunotherapy (SLIT) with house dust mite extracts (HDM) between monosensitized and polysensitized patients with allergic rhinitis.

Study Design: Retrospective cohort study.

Methods: Among the patients who were prospectively enrolled in the SLIT cohort study, patients with allergic rhinitis who were sensitized to HDM and treated with SLIT for at least 2 years were studied. All participants underwent serologic tests at baseline and after SLIT to evaluate changes in immunologic parameters. The total nasal symptom score (TNSS) was measured before and after SLIT, and effective and less effective responder groups were categorized depending on whether patients had a TNSS reduction of 50%, as compared with baseline.

Results: The increase in *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* specific immunoglobulin G4 levels was significantly higher in monosensitized patients than in polysensitized patients ($P = .020$ and $P = .005$, respectively). The TNSS significantly improved after SLIT in both the monosensitized and polysensitized groups ($P < .001$ in both groups). However, the difference in the changes in TNSS from baseline was not significant between the two groups ($P = .374$).

Conclusions: This study demonstrated different immunologic modifications after SLIT between monosensitized and polysensitized patients. However, patients in the polysensitized group who were treated with single-allergen SLIT experienced clinical improvement in TNSS that was comparable with that in the monosensitized group despite demonstrating different immunologic changes.

Key Words: Allergic rhinitis, immunologic modification, monosensitization, polysensitization, sublingual immunotherapy.

Level of Evidence: 2b.

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INTRODUCTION

Allergic rhinitis is the most common immunoglobulin E (IgE)-mediated disease, and its prevalence has gradually increased. Allergen-specific immunotherapy (AIT) has been performed for a century, as it can change the natural course of allergic rhinitis.¹ Subcutaneous immunotherapy has been the conventional form of immunotherapy. Recently, sublingual immunotherapy (SLIT) has been prescribed worldwide as a promising alternative tool to subcutaneous immunotherapy, and current meta-analyses have reported its efficacy.^{2–4} The effect of immunotherapy evokes specific immune responses, including the appearance of allergen-specific immunoglobulin G4 (IgG4) that inhibits IgE-mediated inflammatory

responses.^{5,6} Because of the role of IgG4 as a blocking antibody, increases in specific IgG4 may correlate with clinical improvement.^{7,8} It is well demonstrated that AIT with a single allergen is effective in monosensitized rhinitis; however, about 70% of patients with allergic rhinitis comprise those who are polysensitized.^{6,9–11} It has been debated whether AIT with a single allergen is effective for patients sensitized to multiple allergen families.¹² Nevertheless, recent studies demonstrated that single-allergen-extract SLIT is effective for patients who are polysensitized as much as it is for those who are monosensitized.^{13–15} Although SLIT with single-allergen extracts is efficacious for monosensitized and polysensitized allergic rhinitis in these studies, to our knowledge no study has compared the distinct immune responses after SLIT between monosensitized and polysensitized allergic rhinitis. This study aimed to compare the immunologic effects of SLIT with standardized house dust mite (HDM) extracts between monosensitized and polysensitized patients with allergic rhinitis.

MATERIALS AND METHODS

Study Design and Subjects

This study was based on the data of patients who were prospectively enrolled in the SLIT cohort study (number of

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institutional review board approval: 1111-081-387), which has been conducted since 2009 at a tertiary referral center. Between July 2009 and February 2015, a total of 502 patients with allergic rhinitis who were sensitized to HDM and treated with SLIT were enrolled initially; however, 210 patients maintained SLIT for at least 2 years. Skin-prick tests were performed initially on all participants, and a wheal diameter of ≥ 3 mm was considered positive. The monosensitized group was defined as subjects who were sensitized to *Dermatophagoides pteronyssinus* (Dp) and/or *Dermatophagoides farinae* (Df), and the polysensitized group was defined as subjects who were sensitized simultaneously to HDM and other allergens. Patients with asthma or atopic dermatitis who required regular medication therapy were excluded. Furthermore, pregnant or lactating women and subjects who had immunologic or hematologic disorders were also excluded. After screening, 80 eligible patients who completed serologic tests at baseline and after SLIT were selected and divided into two groups: a monosensitized group (n = 22) and a polysensitized group (n = 58).

Immunotherapy

A standardized HDM extract (50% Dp /50% Df; SLITone, ALK-Abelló, Milan, Italy) was used for immunotherapy. Each package of SLITone consists of 90 single-dose containers, and each single-dose container contains 0.2 mL of extractable volume, which equates to five drops of 1,000 STU/mL solution. A prepared single dose was taken daily in the morning or evening 30 minutes before a meal. The patients were told to keep drops of allergen under their tongue for 2 to 3 minutes before swallowing and not to consume any food or drink for 5 minutes after swallowing. After initiation of immunotherapy, patients required a monthly visit for the first 3 months, followed by a follow-up once every 3 months.

Immunologic Effects

A venous blood sample was obtained from all participants at baseline and after SLIT. After centrifuging (700 g for 10 minutes) the blood samples (5 mL), the serum was collected and stored in aliquots at -20°C until the serological assays. Total IgE (t-IgE), specific IgE (s-IgE) for Dp and Df, specific IgG4 (s-IgG4) for Dp and Df, and eosinophil cationic protein (ECP) were measured using ImmunoCAP (ThermoFisher Scientific,

Uppsala, Sweden). Peripheral blood eosinophils were counted using the XE-2100 analyzer (Sysmex, Kobe, Japan). In the laboratory study, the baseline t-IgE level was 4,685 (above the third quartile plus 10 interquartile range [IQR]) in one patient, and this patient's t-IgE level was excluded from the results; the range of baseline total IgE level in total patients was 7 to 1,480. To evaluate changes in these immunologic parameters, absolute changes from baseline were calculated (post–pre).

Clinical Effects

All participants were asked to complete questionnaires regarding their symptoms before and after treatment. Among the 80 subjects, 68 patients completed questionnaires on their symptoms at baseline and after 2 years of SLIT. The

TABLE II.
Comparison of Immunologic Parameters Between Monosensitized and Polysensitized Groups.

	Monosensitized Group, n = 22	Polysensitized Group, n = 58	P Value
Dp-specific IgE, kU/L, median (IQR)			
Baseline	13.3 (1.9 to 68.9)	28.9 (0.2 to 1 6.0)	.010*
Post	40.0 (6.1 to 98.8)	45.2 (0.2 to 365.0)	.442
Post – pre	22.0 (–14.6 to 81.7)	12.2 (–8.2 to 271.1)	.614
Df-specific IgE, kU/L, median (IQR)			
Baseline	31.6 (3.2 to 180.0)	53.1 (0.3 to 363.0)	.437
Post	71.2 (8.1 to 354)	68.8 (0.16 to 913.0)	.692
Post – pre	28.1 (–9.3 to 271.9)	8.5 (–29.9 to 550.0)	.095
Dp-specific IgG4, mg/L, median (IQR)			
Baseline	0.22 (0.04 to 0.80)	0.21 (0.02 to 0.80)	.510
Post	0.42 (0.10 to 1.78)	0.33 (0.04 to 1.20)	.140
Post – pre	0.22 (–0.24 to 1.41)	0.11 (–0.46 to 0.89)	.020*
Df-specific IgG4, mg/L, median (IQR)			
Baseline	0.23 (0.04 to 1.18)	0.26 (0.06 to 1.61)	.327
Post	0.43 (0.11 to 2.67)	0.36 (0.04 to 2.52)	.308
Post – pre	0.19 (–0.12 to 1.57)	0.07 (–0.80 to 2.13)	.005*
Total IgE, kU/L, median (IQR)			
Baseline	136 (24 to 1,289)	262 (7 to 1,480)	.015*
Post	254 (54 to 3,368)	367 (12 to 1,964)	.545
Post – pre	72 (–1,290 to 621)	25 (–1,078 to 1,169)	.549
Eosinophil count, / μL , median (IQR)			
Baseline	442 (121 to 1,248)	283 (52 to 1,428)	.192
Post	328 (70 to 923)	300 (22 to 972)	.360
Post – pre	–115 (–670 to 373)	–70 (–899 to 281)	.698
ECP, $\mu\text{g/L}$, median (IQR)			
Baseline	16.4 (2.3 to 53.1)	15.7 (1.0 to 112.0)	.584
Post	12.2 (2.4 to 78.9)	11.9 (1.0 to 58.4)	.718
Post – pre	–0.3 (–37.4 to 40.6)	–3.5 (–96.7 to 31.3)	.082

*Statistical significance ($P < .05$).

Df = *Dermatophagoides farinae*; Dp = *Dermatophagoides pteronyssinus*; ECP = eosinophil cationic protein; Ig = immunoglobulin; IQR = interquartile range.

TABLE I.
Demographics and Clinical Characteristics of the Study Patients.

	Monosensitized Group, n = 22	Polysensitized Group, n = 58	P Value
Age, yr, mean $\pm$ SD	10.6 $\pm$ 6.2	13.1 $\pm$ 8.8	.173*
Sex, M:F	20:2	39:19	.045†‡
Baseline TNSS, mean $\pm$ SD	8.6 $\pm$ 2.1	8.0 $\pm$ 2.9	.456*
Duration of symptoms, yr, median IQR	4.5 (1 to 13)	6.5 (0.5 to 30)	.300§
No. of sensitized allergen families, mean $\pm$ SD	1	4.4 $\pm$ 1.6	<.001*‡

*Student t test.

†Fisher exact test.

‡Statistical significance ($P < .05$).

§Mann–Whitney U test.

F = female; IQR = interquartile range; M = male; SD = standard deviation; TNSS = total nasal symptom score.

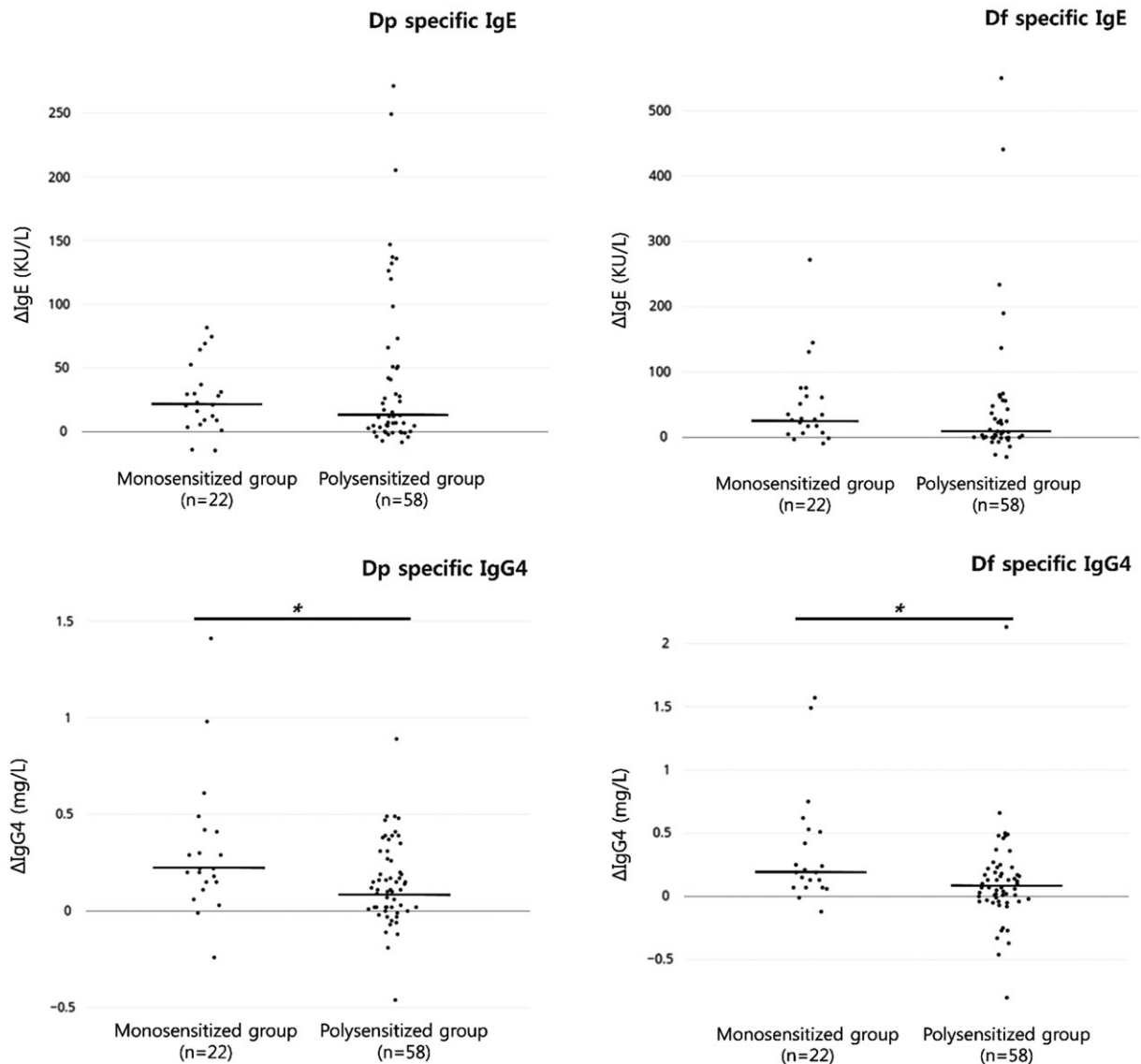


Fig. 1. The scatter plots show the post-pre Dp s-IgE, Df s-IgE, Dp s-IgG4, and Df s-IgG4 levels in the monosensitized and polysensitized groups. * $P < .05$. Df = *Dermatophagoides farinae*; Dp = *Dermatophagoides pteronyssinus*; s-Ig = specific immunoglobulin.

questionnaires included four nasal symptoms (rhinorrhea, sneezing, nasal obstruction, and nasal itching). Each symptom was graded from 0 to 3 points: 0 points for no symptoms, 1 point for mild symptoms, 2 points for moderate symptoms, and 3 points for severe symptoms. The total nasal symptom score (TNSS) was calculated using the sum of each symptom score. Effective and less effective responder groups were categorized depending on whether subjects had a reduction in the TNSS of 50% from their baseline level after 2 years of SLIT.

Statistical Analysis

Demographics and clinical characteristics are depicted as mean $\pm$ standard deviation or median (IQR), depending on their normality. Immunologic parameters at baseline and after SLIT are summarized as median (IQR). A Fisher exact test or χ^2 test was carried out to evaluate categorical variables such as demographic features and effectiveness of immunotherapy. Regarding the normality of continuous variables, nonparametric Mann-

Whitney U tests were applied to investigate immunologic parameters, whereas two-tailed Student t tests were used to evaluate clinical outcomes. All statistical analysis was conducted using SPSS version 19.0 for Windows (IBM Corp., Armonk, NY). A P value of $<.05$ was considered significant.

RESULTS

Eighty patients were enrolled in this study and divided into two groups: 22 monosensitized (27.5%) and 58 polysensitized (72.5%). The demographic features of the two groups are shown in Table I. There were no significant differences in age, baseline TNSS, or symptom duration between monosensitized and polysensitized patients. The male-female ratio for monosensitized patients was higher than that of polysensitized patients ($P = .045$).

Immunologic parameters at baseline and posttreatment, as well as absolute changes from baseline (post - pre),

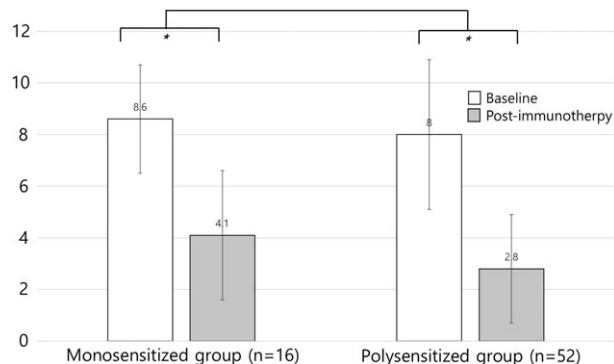


Fig. 2. A comparison of the changes in the TNSS between the monosensitized and polysensitized groups. * $P < .001$. TNSS = total nasal symptom score.

are provided in Table II. At baseline, the levels of Dp s-IgE and t-IgE were significantly higher in the polysensitized group than in the monosensitized group ($P = .010$ and $P = .015$, respectively). There were no significant differences in the post – pre Dp s-IgE or Df s-IgE concentration between the two groups (Fig. 1). However, the post – pre Dp s-IgG4 and Df s-IgG4 levels were significantly higher in the monosensitized patients than in the polysensitized patients ($P = .020$ and $P = .005$, respectively; Fig. 1).

TNSS was significantly decreased after SLIT in both the monosensitized and polysensitized groups (Fig. 2). However, changes in TNSS from baseline were not significantly different between the two groups ($P = .374$).

The effective responder group consisted of 50 patients, and the less effective responder group included 18 patients. There was no significant difference in immunologic parameters between the effective responder and less effective responder groups (Table III). Among the patients in the effective responder group, there were 10 monosensitized and 40 polysensitized patients (Table IV). In a subgroup analysis of the effective responder group, there was a significant difference in the baseline Dp s-IgE and t-IgE values and post – pre Dp s-IgG4 and Df s-IgG4 between the monosensitized and polysensitized groups that is consistent with the results in Table II. An additional subgroup analysis revealed that the less effective responder group consisted of six monosensitized and 12 polysensitized patients, indicating a relatively higher proportion of monosensitized patients, but there was no significant difference in the ratio of monosensitized and polysensitized patients between the effective responder and less effective responder groups ($P = .253$). In the less effective responder group, the post – pre Dp s-IgG4 and Df s-IgG4 levels were significantly higher in the monosensitized patients than in the polysensitized patients, as was demonstrated in the effective responder group (Table IV). In addition, a subgroup analysis of the less effective responder group showed that the posttreatment Dp s-IgG4 level was higher in the monosensitized group, and the baseline

eosinophil count was also higher in the monosensitized group.

DISCUSSION

Several double-blind, placebo-controlled studies demonstrated the clinical efficacy of SLIT that reduced nasal symptoms and medication usage in patients with allergic rhinitis.^{16–21} In addition, recent meta-analyses supported the significant effects of SLIT.^{2,22} Because SLIT is a treatment that specifically alters the immunologic response to a single allergen or an allergen component, proper selection of allergen extracts has been debated for polysensitized patients.⁶ Considering the mechanism of SLIT that modifies the immunologic response to specific allergen components, some clinicians prefer immunotherapy with multiple allergen extracts, which is common in the United States.²³ Clinicians in the United States commonly prescribe SLIT including all relevant allergens, and an average of eight allergens were included in aqueous SLIT.²⁴ However, the superiority of clinical efficiency of immunotherapy using multiple allergen extracts compared with that of monotherapy has been debated.⁶ In addition, the limited absorptive capacity of the sublingual mucosa, as well as economic factors for additional allergen extracts beyond the dominant allergen, should be considered if physicians select multiple allergen immunotherapy instead of single-allergen immunotherapy.^{6,13} Because of this controversy regarding multiple-allergen SLIT, in a previous study, we compared the efficacy of single-allergen (HDM) SLIT for 1 year in monosensitized and polysensitized patients to HDM; the clinical effects of SLIT were not different between the two groups.¹³ In the study by Ortiz et al.,²⁵ moreover, clinical effects were compared between three groups of patients who were sensitized to more than six allergens and treated with SLIT: patients treated with a single-allergen extract, three clinically most significant allergens, and all allergen extracts to which subjects were sensitized. Although significant decreases from baseline were found in Rhinoconjunctivitis Total Symptom Score and the mini-Rhinoconjunctivitis Quality of Life Questionnaire in all study groups after SLIT, there was no significant difference depending on the number of allergens used. Furthermore, current studies supported the idea that SLIT with a single-allergen extract is effective for patients who are polysensitized.^{14,15,26,27}

However, there is a lack of knowledge on the difference in immunologic modifications induced by single-allergen SLIT between monosensitized and polysensitized patients. In the present study, the immunologic modification observed in monosensitized and polysensitized patients after SLIT with HDM extracts was compared with serologic markers such as allergen-specific IgE and IgG4.

In this study, 22 monosensitized patients (27.5%) and 58 polysensitized patients (72.5%) were enrolled. It has been demonstrated in previous studies performed on various continents that polysensitization was more prevalent than monosensitization (IQR, 55%–80%).^{9,11,28–30} In the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) of the US general population, polysensitized patients accounted for 71.5% of patients who were sensitized to at least one allergen.¹¹ In

	Effective Responder Group, n = 50	Less Effective Responder Group, n = 18	P Value
Dp-specific IgE, kU/L, median IQR			
Baseline	21.0 (0.2 to 116.0)	20.7 (1.0 to 74.2)	.834
Post	39.3 (0.2 to 365.0)	51.1 (0.5 to 221.0)	.760
Post – pre	16.7 (–14.0 to 271.1)	26.0 (–0.7 to 146.8)	.412
Df-specific IgE, kU/L, median IQR			
Baseline	40.0 (0.3 to 363.0)	63.2 (1.3 to 180.0)	.408
Post	61.2 (0.2 to 913.0)	92.4 (0.5 to 354.0)	.328
Post – pre	9.2 (–29.9 to 550.0)	31.8 (–1.3 to 271.9)	.468
Dp-specific IgG4, mg/L, median IQR			
Baseline	0.20 (0.04 to 0.80)	0.28 (0.05 to 0.80)	.053
Post	0.33 (0.06 to 1.20)	0.61 (0.05 to 1.78)	.083
Post – pre	0.15 (–0.46 to 0.89)	0.28 (–0.12 to 1.41)	.226
Df-specific IgG4, mg/L, median IQR			
Baseline	0.23 (0.04 to 1.31)	0.32 (0.09 to 1.61)	.187
Post	0.36 (0.08 to 2.52)	0.51 (0.04 to 2.67)	.096
Post – pre	0.09 (–0.80 to 2.13)	0.12 (–0.05 to 1.57)	.158
Total IgE, kU/L, median IQR			
Baseline	204 (7.0 to 1,480)	364 (67 to 1,289)	.157
Post	340 (12.3 to 1,964)	518 (89 to 1,264)	.146
Post – pre	25 (–1,078 to 1,169)	53.4 (–126 to 668)	.411
Eosinophil count, / μ L, median IQR			
Baseline	291 (52 to 1,428)	384 (72 to 1,248)	.493
Post	310 (22 to 923)	332 (118 to 578)	.163
Post – pre	–52 (–899 to 373)	–48 (–670 to 281)	.955
ECP, μ g/L, median IQR			
Baseline	15.5 (1.0 to 112.0)	22.2 (5.3 to 72.6)	.066
Post	10.3 (1.0 to 78.9)	14.8 (2.9 to 39.8)	.276
Post – pre	–2.8 (–96.7 to 40.6)	–3.8 (–58.1 to 18.7)	.404

Df = *Dermatophagoides farinae*; Dp = *Dermatophagoides pteronyssinus*; ECP = eosinophil cationic protein; Ig = immunoglobulin; IQR = interquartile range.

addition, polysensitized patients constituted 55.9% and 74.3% in the first European Community Respiratory Health Survey²⁸ and the study of Ciprandi and Cirillo⁹ performed in European countries. Furthermore, in the study performed in Asia,²⁹ 67% of allergic rhinitis patients were polysensitized. These results showed rates of polysensitization comparable with the rate in this study (72.5%).

This study revealed that baseline Dp s-IgE and t-IgE levels were significantly higher in the polysensitized group than in the monosensitized group, in concordance with a study by Kim et al.³¹ in which the baseline Dp s-IgE and t-IgE concentrations were higher in polysensitized patients with asthma. Although there was no significant difference in the baseline and posttreatment IgG4 levels, post - pre

Dp and Df-specific IgG4 levels were significantly higher in the monosensitized group than in the polysensitized group. The increase in IgG4 levels is associated not only with blocking of IgE-mediated antigen presentation, but also with development of regulatory T-cells, which indicates favorable response to immunotherapy.^{32,33} Interleukin (IL)-10 is secreted by regulatory T-cells and has several potential antiallergic effects, such as suppression of mast cells, T-cells, and eosinophils, related to successful immunotherapy.⁷ Because IgG4 production is promoted by IL-10, the increase in specific IgG4 levels may reflect regulatory T-cells. However, in the present study, there was no significant difference in the changes in TNSS between monosensitized and polysensitized subjects.

First, immunologic characteristics of monosensitized and polysensitized allergic rhinitis might differ, leading to different post - pre specific IgG levels after immunotherapy. An increase in the number of sensitized allergens in the same allergic patients seems to be the typical natural course for allergies.³⁴ However, there are allergic patients who have persistent monosensitization over several years. In a study by Prigione et al.,³⁵ children who remained monosensitized had higher levels of IL-10 and interferon- γ than children who developed polysensitization. This result might suggest the existence of two different phenotypes for mono- and polysensitization that lead to different immunologic alterations after SLIT.^{9,35} Considering the allergen-specific response to immunotherapy, the resulting increase in Dp- and Df-specific IgG4 levels is a typical immunologic response and seems to be more prominent in monosensitized patients.

Moreover, there was a possibility that the changes in specific IgG levels had been influenced by cross-reactivity with HDM. The previous studies reported that the HDM allergen has high cross-reactivity with other allergens.^{15,36,37} It is reported that Der p 10, HDM tropomyosin, has a high cross-reactivity with other tropomyosin allergens such as Bla g 7 (cockroach) and Pen a 1 (shrimp).^{11,15} Moreover, Der p 4, Der p 5, and Der p 7 may play a role in HDM-snail cross-reactivity.³⁶ This cross-reactivity might play an important role in the progression of multiple sensitizations and could have an effect on immunologic modifications and treatment results that is a possible explanation for the low post–pre HDM specific IgG4 level with comparable therapeutic results demonstrated by polysensitized patients.¹⁵ Although SLIT induced only an allergen-specific immunologic response, immunologic modifications associated with other allergens, such as the induction of other allergen-specific IgG4, might occur in polysensitized patients because of cross-reactivity with HDM allergens, and these changes in the immunologic response to other allergens can influence clinical effects. Therefore, further investigation should be performed to measure total IgG4 or specific IgG4 for other allergens in polysensitized patients treated with single-allergen immunotherapy.

Lastly, another possibility is that the increase in IgG4 may not correlate with the treatment outcomes from immunotherapy. In contrast to previous studies^{7,8} that supported a relationship between the immunotherapy-induced increase in IgG4 and clinical improvement, some

TABLE IV.
Subgroup Analysis of Effective Responder and Less Effective Responder Groups.

	Effective Responder Group, n = 50			Less Effective Responder Group, n = 18		
	Monosensitized Patients, n = 10	Polysensitized Patients, n = 40	P Value	Monosensitized Patients, n = 6	Polysensitized Patients, n = 12	P Value
Dp-specific IgE, kU/L, median IQR						
Baseline	13.4 (1.9 to 68.9)	27.5 (0.2 to 116.0)	.028*	16.4 (10.0 to 34.8)	29.7 (0.95 to 74.2)	.448
Post	33.3 (6.1 to 98.8)	44.1 (0.2 to 365.0)	.489	47.3 (16.6 to 88.1)	62.1 (0.5 to 221.0)	.914
Post – pre	22.0 (–14.0 to 81.7)	12.2 (–8.2 to 271.1)	.613	29.7 (5.7 to 74.5)	18.4 (–0.7 to 146.8)	.664
Df-specific IgE, kU/L, median IQR						
Baseline	25.7 (3.2 to 85.3)	51.0 (0.3 to 363.0)	.177	72.6 (26.3 to 180.0)	51.6 (1.3 to 105.0)	.366
Post	59.2 (8.1 to 164.0)	67.5 (0.2 to 913.0)	.847	90.6 (30.9 to 354.0)	94.5 (0.5 to 289.0)	.519
Post – pre	27.0 (–9.3 to 130.9)	8.3 (–29.9 to 550.0)	.198	31.8 (–1.3 to 271.9)	20.3 (–1.0 to 233.8)	.699
Dp-specific IgG4, mg/L, median IQR						
Baseline	0.20 (0.04 to 0.30)	0.20 (0.10 to 0.80)	.110	0.32 (0.15 to 0.80)	0.26 (0.05 to 0.70)	.281
Post	0.34 (0.19 to 0.69)	0.32 (0.06 to 1.20)	.428	0.79 (0.32 to 1.78)	0.38 (0.05 to 1.19)	.019*
Post – pre	0.22 (0.11 to 0.49)	0.11 (–0.46 to 0.89)	.017*	0.51 (–0.01 to 1.41)	0.08 (–0.12 to 0.49)	.049*
Df-specific IgG4, mg/L, median IQR						
Baseline	0.22 (0.04 to 0.39)	0.23 (0.06 to 1.31)	.142	0.39 (0.19 to 1.18)	0.28 (0.09 to 1.61)	.223
Post	0.32 (0.17 to 0.97)	0.36 (0.08 to 2.52)	.802	0.95 (0.26 to 2.67)	0.38 (0.04 to 1.71)	.075
Post – pre	0.19 (–0.01 to 0.75)	0.06 (–0.80 to 2.13)	.026*	0.52 (0.07 to 1.57)	0.06 (–0.05 to 0.66)	.022*
Total IgE, kU/L, median IQR						
Baseline	105 (24 to 523)	250 (7.0 to 1,480)	.027*	295 (92 to 1,289)	264 (68 to 765)	.682
Post	216 (54 to 608)	354 (12 to 1,964)	.444	416 (114 to 1,264)	518 (89 to 1,249)	.964
Post – pre	85 (–38 to 396)	11 (–1,078 to 1,169)	.217	29 (–25 to 621)	73 (–126 to 668)	.682
Eosinophil count, / μ L, median IQR						
Baseline	328 (121 to 1,061)	262 (52 to 1,428)	.637	545 (337 to 1,248)	314 (72 to 746)	.025*
Post	329 (70 to 923)	250 (22 to 589)	.365	395 (260 to 578)	299 (118 to 531)	.223
Post – pre	–13 (–656 to 373)	–70 (–899 to 153)	.219	–198 (–670 to –27)	–2.0 (–255 to 281)	.092
ECP, μ g/L, median IQR						
Baseline	17.9 (2.8 to 53.1)	14.4 (1.0 to 112.0)	.495	13.1 (7.6 to 45.2)	29.8 (5.3 to 72.6)	.160
Post	15.5 (7.2 to 78.9)	9.6 (1.0 to 56.8)	.099	13.5 (2.9 to 27.8)	17.2 (3.2 to 39.8)	.574
Post – pre	3.4 (–37.4 to 40.6)	–2.9 (96.7 to 31.3)	.099	–2.6 (–21.3 to 7.4)	–4.5 (–58.1 to 18.7)	.426

*Statistical significance ($P < .05$).

Df = *Dermatophagoides farinae*; Dp = *Dermatophagoides pteronyssinus*; ECP = eosinophil cationic protein; Ig = immunoglobulin; IQR = interquartile range.

studies indicated a lack of correlation between the change in IgG4 and treatment outcomes.^{38,39} In the present study, the comparison between the effective responder and less effective responder groups revealed that there was no significant difference in post – pre IgG4 levels between the two groups. These results suggest that the quantitative increase in IgG4 does not always correlate with successful treatment. To explain the discordance between the change in specific IgG4 levels and clinical effects, the importance of the functional alteration of specific IgG4 was suggested.³³ In a recent study by Shamji et al.,⁴⁰ alteration of functional specific antibodies accounted for about 40% of the clinical outcomes, and quantitative changes in specific IgG4 levels accounted for only 13%, indicating the importance of functional changes in specific IgG4 on the clinical effect. In the present study, only quantitative changes in specific IgG4 after immunotherapy were compared between monosensitized and polysensitized patients. However, the functional alteration of specific IgG4

could have influenced the clinical effects of the immunotherapy but was not evaluated. Therefore, the difference in the functional alteration of specific IgG4 following immunotherapy between monosensitized and polysensitized patients should be assessed in further investigation.

In a subgroup analysis of the effective responder group that accounted for 73.5% of all patients, there was a significant difference in levels of baseline Dp s-IgE, t-IgE, post-pre Dp s-IgG4, and Df s-IgG4 between monosensitized and polysensitized patients that is consistent with the comparison between monosensitized and polysensitized groups for all patients. However, in a subgroup analysis of the less effective responder group, the posttreatment Dp s-IgG4 level was significantly higher in the monosensitized group, which was not demonstrated in a comparison of all patients. It seems that the greater increase in IgG in monosensitized patients led to the difference in Dp s-IgG4 levels between the two groups. In addition, the baseline eosinophil count was higher in monosensitized patients in a subgroup

analysis of the less effective responder group. In previous studies^{31,41} and a comparison of all patients, there was no significant difference in the eosinophil count between monosensitized and polysensitized patients. As the small number of patients in the less effective responder group might have led to this difference in eosinophil count, further investigation is necessitated with a larger number of patients.

Although the clinical efficacy of SLIT with a single-allergen extract was compared between monosensitized and polysensitized patients with allergic rhinitis in previous studies, to our knowledge this is the first study in which immunologic alterations involving monosensitized and polysensitized allergic rhinitis were compared after SLIT with HDM. In polysensitized patients with allergic rhinitis, 2 years of SLIT using an HDM extract showed an improvement in nasal symptoms comparable with that of monosensitized patients that was consistent with a previous comparison study after 1 year of SLIT.¹³ However, the present study had two limitations: no control group and a small number of monosensitized patients. To evaluate the immunologic effect of SLIT, natural changes in the immunologic parameters in monosensitized and polysensitized patients without any immunotherapy should be investigated as control groups. However, the objective of this study was to evaluate the immunologic parameters and clinical improvement of polysensitized subjects after immunotherapy, as compared with those of monosensitized subjects. Therefore, the monosensitized group could be considered a control group in the present study. In addition, there were fewer monosensitized patients than polysensitized patients. The reason for this limitation was that there were fewer patients with monosensitized allergic rhinitis in our study, because, according to the study by Ciprandi and Cirillo,⁹ 30% of patients with allergic rhinitis are monosensitized and 70% of patients with allergic rhinitis are polysensitized; therefore, fewer patients with allergic rhinitis are monosensitized in the allergic rhinitis patient population.

CONCLUSION

This study suggests an immunologically different response to SLIT with HDM extracts between monosensitized and polysensitized patients; the increase in Dp s-IgG4 and Df s-IgG4 levels was significantly higher in monosensitized patients after 2 years of SLIT. However, an important aspect of the present study is that comparable clinical improvement was demonstrated in both monosensitized and polysensitized patients after 2 years of SLIT, despite the difference in immunologic modifications. Based on these findings, although a proper selection of allergen extracts in polysensitized patients remains a great debate in current practice, SLIT with a single dominant allergen should be considered in polysensitized patients.

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