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Effect of Japanese cedar–specific sublingual immunotherapy on allergen-specific T_H2 cell counts in blood

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ABSTRACT

Background: The contribution of antigen-specific T_H cells in peripheral blood to immunologic mechanisms underlying sublingual immunotherapy (SLIT) remains unclear, partly because of the lack of a standardized method for the analysis of this rare lymphocyte subset.

Objective: To clarify the role of antigen-specific T_H cells during SLIT using a sensitive method analyzing activation marker CD154-positive T_H cells with multicolor flow cytometry.

Methods: We assessed antigen-specific T_H cells using multicolor flow cytometry based on the expression of the activation marker CD154 and intracellular cytokines in patients with Japanese cedar pollinosis receiving SLIT at baseline and during the first pollen season after the initiation of SLIT.

Results: A total of 18 patients between 12 and 44 years of age were enrolled in the present study. Of these, 8 patients received SLIT (SLIT group) and 10 patients received symptomatic treatment only (control group). Although seasonal pollen exposure significantly increased the number of Japanese cedar–specific interleukin 5– and interleukin 4–producing T_H cells in the control group ($P < .01$ for both), SLIT ameliorated this increase in the SLIT group ($P = .64$ and $P = .84$, respectively).

Conclusion: The present study indicates that allergen-specific T_H2 cells in peripheral blood are involved in mechanisms underlying SLIT. The analysis of antigen-specific T_H cells using multicolor flow cytometry based on the expression of the activation marker CD154 represents a sensitive and relatively simple, standardized method for monitoring peripheral antigen-specific T_H cells during allergen-specific immunotherapy.

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Introduction

Japanese cedar pollinosis is one of the most common allergic disorders in Japan.¹ The prevalence of Japanese cedar pollinosis has increased in a couple of decades,² with one-third of Japanese adults estimated to have allergic nasal and ocular symptoms between February and April.³ Allergen-specific immunotherapy (SIT) is the only treatment capable of modifying the natural history of allergic diseases⁴; however, most patients are currently treated with symptomatic therapy, such as oral antihistamine or intranasal corticosteroid administration. Sublingual immunotherapy (SLIT) for allergic rhinitis^{5,6} was developed in Western countries as an

alternative method with lesser systemic adverse effects than conventional subcutaneous immunotherapy (SCIT).^{7,8} Recently, Japanese cedar–specific SLIT was found to have clinical efficacy in improving nasal and ocular symptoms and reducing the use of relief medication⁹ and has recently become clinically available for patients with Japanese cedar pollinosis.

Immunologic mechanisms underlying SLIT for allergic rhinitis are not completely understood.^{10,11} Transient early increases and subsequent reductions in serum specific IgE,⁷ sustained increases in serum specific IgG4,^{12,13} and suppression of the numbers of effector cells, such as neutrophils and eosinophils, in the local sites¹⁴ are observed during SLIT because these mechanisms are observed after SCIT.^{15–17} Increased numbers of regulatory T cells (Tregs) have been reported in local tissues¹⁸ and peripheral blood¹⁹ of patients who receive SLIT. The oral mucosa, which consists of Langerhans cells, epithelial cells, monocytes, and oral dendritic cells, appears to play a central role in the mechanisms underlying SLIT as a natural tolerogenic network.¹⁰

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Antigen-specific T_H cells in peripheral blood play an important role in the immunologic networks; however, the role of T_H cells in allergen-SIT mechanisms remain unclear because of the technical difficulty in analyzing antigen-specific T_H cells.²⁰ Although the functions of peripheral antigen-specific T_H cells were reported to remain unchanged during SCIT in a well-controlled study,²¹ recent technical advances in the use of major histocompatibility complex (MHC) class II tetramers²² and activation marker CD154²³ enabled the quantification of this rare lymphocyte subset (10^{-4} to 10^{-6} of total T_H cells) and eventually revealed the contribution of antigen-specific T_H2 cells to the effect of SCIT.^{24,25} Using the activation marker CD154 combined with multicolor flow cytometry, we previously found that Japanese cedar-specific SCIT attenuated seasonal increases in Japanese cedar-specific interleukin (IL) 5- and IL-4-producing T_H cells.²⁶ In contrast, Bonvalet et al²⁷ analyzed peripheral antigen-specific T_H cells from patients with SLIT using MHC class II tetramer technology and concluded that antigen-specific T_H2 cell numbers did not change during the early phase of SLIT.

In the present study, we found that antigen-specific T_H2 cell numbers in peripheral blood decrease during the early phase of SLIT. Furthermore, antigen-specific T_H cell quantification according to the expression of the activation marker CD154 using multicolor flow cytometry was found to have utility in monitoring SIT.

Methods

Patients and Study Design

Between November and December 2014, Japanese patients with Japanese cedar pollinosis, who were interested in our research, were consecutively invited to participate in the present study. The diagnosis of Japanese cedar pollinosis was made on the basis of typical seasonal allergic symptoms accompanied by Japanese cedar sensitization, as determined by the ImmunoCAP test (Phadia AB, Uppsala, Sweden). The SLIT protocol started with a 2-week dose escalation phase before increases in Japanese cedar pollen counts followed by a maintenance phase, wherein 1.0 mL of 2,000 Japanese allergy units (JAU) per milliliter of standardized Japanese cedar pollen extract (Torii Pharmaceutical, Tokyo, Japan) containing 1.46 to 4.2 $\mu\text{g/mL}$ of Cry j 1 was administered daily.⁹ During the pollen season, patients in both groups were allowed to use therapeutic treatments, such as oral antihistamine or nasal corticosteroid administration, with the use of these therapeutic treatments defined for statistical analyses as use for more than half a week.

Patients were voluntary assigned to 1 of 2 groups: patients who received SLIT after enrollment in the present study (SLIT group) and those who received standard symptomatic treatment only (control group) (Fig 1). Because the Japanese cedar pollen season is between February and April, study time points were defined as follows: preseason (before initiation of SLIT between early November and late December 2014) and postseason (after seasonal pollen exposure between late March and middle April 2015).

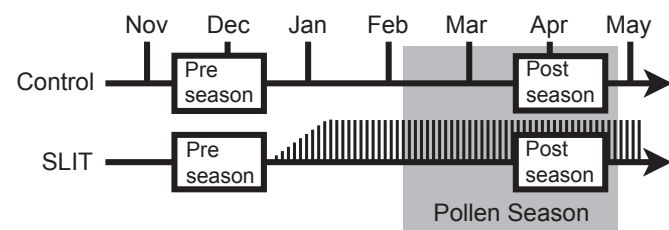


Figure 1. Study overview. Solid bars represent intakes of Japanese cedar extract for sublingual immunotherapy (SLIT).

The present study was conducted at Nagoya City University Hospital and approved by the research ethics committee of Nagoya City University. All participants provided written informed consent.

Serum Antigen-Specific Immunoglobulins

Serum Japanese cedar-specific IgE was measured using ImmunoCAP specific IgE (0.1–100 U_A/mL ; Phadia AB). For values over the detection limit ($>100 \text{ U}_A/\text{mL}$), samples were diluted to determine exact values. We used ImmunoCAP specific IgG4 (0.07–30 mg_A/L ; Phadia AB) to measure serum Japanese cedar-specific IgG4 levels. Values under the detection limit ($<0.07 \text{ mg}_A/\text{L}$) were considered 0.035 mg_A/L for statistical analysis.

Peripheral Blood Mononuclear Cell Preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from 10-mL samples of heparinized blood using Ficoll-Paque PLUS (GE Healthcare, Pittsburgh, Pennsylvania) and were frozen in RPMI 1640 that contained 12.5% human serum albumin (Gemini Bio-Products, Woodland, California) and 10% dimethyl sulfoxide (Sigma-Aldrich, St Louis, Missouri) according to the optimal cryopreservation protocol for intracellular cytokine staining.²⁸ To minimize experimental error, samples from the same individual were thawed on the same day. As a result, samples were stored in liquid nitrogen for up to 8 months. Viability was evaluated using Trypan blue solution (Sigma-Aldrich), with a mean viability at preseason and postseason before cryopreservation of 99.1% and 99.3% compared with 99.0% and 98.6% after thawing, respectively. Thawed PBMCs were resuspended at a concentration of $5 \times 10^6/\text{mL}$ in AIM-V medium (Life Technologies, Carlsbad, California) and cultured in an incubator overnight at 37°C under 5% carbon dioxide.

Antigen Stimulation and Intracellular Cytokine Staining

Antigen stimulation and intracellular cytokine staining were performed according to the intracellular cytokine staining protocol.²⁹ Briefly, 400 μL of thawed PBMCs supplemented with anti-CD28 (1 $\mu\text{g/mL}$; BD Biosciences, San Jose, California) was incubated with crude Japanese cedar antigen (100 $\mu\text{g/mL}$) for 6 hours. After a 2-hour incubation, Brefeldin A (10 $\mu\text{g/mL}$; Sigma-Aldrich) was added to the samples. After incubation for 6 hours, samples were stained with ethidium monoazide bromide (EMA; Life Technologies), fixed with IC Fixation Buffer (eBioscience, San Diego, California), permeabilized with IC Permeabilization Buffer (eBioscience), and stained with fluorescence-conjugated antibodies described below. The control medium was identical except for the omission of antigen. Crude Japanese cedar antigen was extracted from the Japanese cedar pollen (Hayashibara, Okayama, Japan) using ammonium bicarbonate. Briefly, Japanese cedar pollen was suspended in 125 mM ammonium bicarbonate solution overnight at 4°C , and the suspension was centrifuged at $1,500g$ for 10 minutes at 4°C . The supernatant was filtered through a $0.20\text{-}\mu\text{m}$ -pore membrane filter to obtain crude extract. The final endotoxin concentration in the extract used in culture, as determined by the limulus assay (endotoxin single test; Wako Pure Chemical Industries, Osaka, Japan), was under the detection limit ($<0.8 \text{ pg/mL}$).

Multicolor Flow Cytometric Analyses

The 8-color panel used for multicolor flow cytometric analyses in the present study consisted of the following: fluorescein isothiocyanate-conjugated anti-CD154 (clone TRAP1), allophycocyanin-conjugated anti-IL-10 (clone JES3-19F1), and Brilliant Violet 510-conjugated anti-CD4 (clone SK3; all from BD Biosciences); phycoerythrin-conjugated anti-IL-5 (clone JES1-39D10), phycoerythrin/Cy7-conjugated anti-IL-4 (clone MP4-25D2),

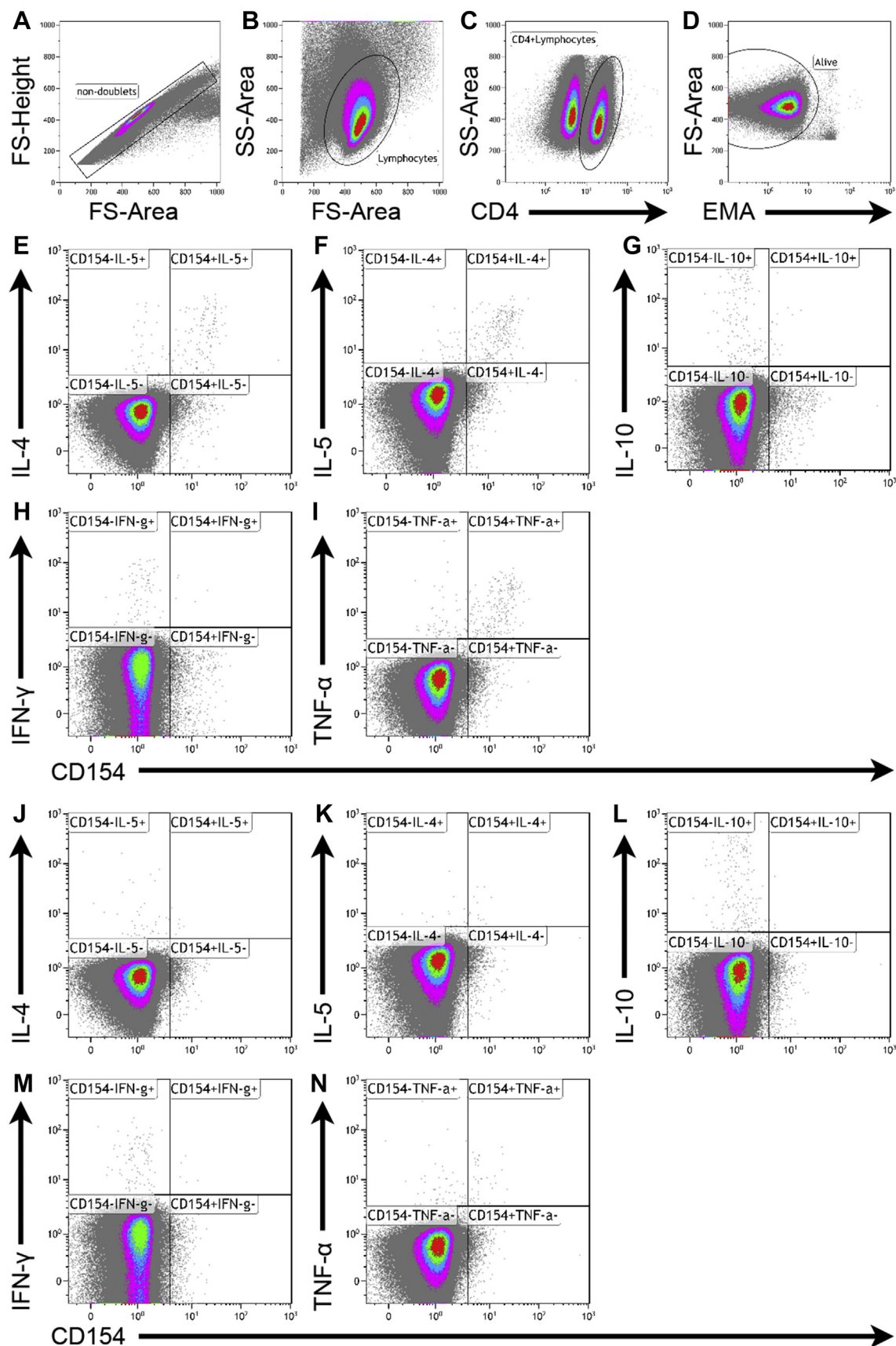


Figure 2. Detection of cytokine-producing T_H cells using multicolor flow cytometry after short-term (6 hours) stimulation with Japanese cedar antigen. (A–D), Gating of viable $CD4^+$ lymphocytes. (E–N), Five types of cytokine-producing T_H cells were simultaneously detected. Represents of a Japanese cedar–stimulated sample (E–I) and an unstimulated sample (J–N) are shown. The percentage of $CD154^+$ cytokine $^+$ T_H cells represents the proportion of viable $CD4^+$ lymphocytes. EMA indicates ethidium monoazide bromide; FS, forward scatter; IFN- γ , interferon γ ; IL, interleukin; SS, side scatter; and TNF- α , tumor necrosis factor α .

allophycocyanin/Cy7-conjugated anti-interferon γ (IFN- γ) (clone 4S.B3), Brilliant Violet 421-conjugated antitumor necrosis factor α (TNF- α) (clone MAb11; all from BioLegend, San Diego, California); and EMA. Stained samples were analyzed using a FACSCanto II multicolor flow cytometer (BD Biosciences) and Kaluza software (Beckman Coulter, Brea, California). Cell doublets were excluded using the forward scatter area vs forward scatter height parameter (Fig 2A). Viable CD4⁺ lymphocytes were identified by gating on forward scatter area vs side scatter area parameter (Fig 2B), CD4 vs side scatter parameter (Fig 2C), and then by negative EMA gating (Fig 2D). A total of 170,000 to 380,000 (median, 300,000) viable CD4⁺ lymphocytes were acquired in each sample. The proportion of CD154⁺cytokine⁺ cells in the viable CD4⁺ lymphocyte population was determined (Fig 2, E–I, Japanese cedar-stimulated sample, and Fig 2, J–N, unstimulated sample). The number of antigen-specific cytokine-producing T_H cells was determined by subtracting unstimulated CD154⁺cytokine⁺ cell numbers. Negative values were considered zero.

Statistical Analysis

Two independent groups of data were compared using the Fisher exact test or the Mann-Whitney *U* test. Paired data from the 2 groups were compared using the Wilcoxon matched-pairs signed-rank test. Correlations were assessed by the Spearman rank correlation coefficient. $P < .05$ was considered statistically significant. Statistical analyses were performed using Prism 6.0g software (GraphPad Software, San Diego, California).

Results

Eighteen patients between 12 and 44 years of age were enrolled in the present study. No significant differences in age, history of asthma, or Japanese cedar-specific IgE and total IgE levels were observed between the SLIT ($n = 8$) and control ($n = 10$) groups at baseline (Table 1). Some patients were sensitized to other allergens, such as grass, ragweed, birch, mite, or cat. No patients had a history of immunologic disorders. During the pollen season, the use of therapeutic treatments with oral antihistamine or nasal corticosteroid did not differ between the groups. All participants completed the study protocol with good adherence to SLIT and no systemic adverse effects. All samples

Table 1
Patient Characteristics^a

Characteristic	SLIT group ($n = 8$)	Control group ($n = 10$)	<i>P</i> value
Sex, female/male	3/5	4/6	>.99
Age, median (quartile range), y	24.5 (22.3–29.0)	36.0 (20.3–41.0)	.24
Physician-diagnosed asthma	1	0	.44
Polysensitization ^b	4	6	>.99
Japanese cedar-specific IgE, median (quartile range), IU/mL	45.1 (6.1–110.4)	15.0 (6.0–48.6)	.42
Total IgE, median (quartile range), IU/mL	303.0 (127.3–777.3)	172.0 (93.3–339.0)	.51
Oral antihistamine during the pollen season	4	4	>.99
Nasal corticosteroid during the pollen season	0	2	.48

Abbreviation: SLIT, sublingual immunotherapy.

^aData are presented as number of patients unless otherwise indicated. The SLIT group included patients who initiated SLIT after enrollment in the present study. The control group included individuals who did not receive SLIT.

^bPolysensitization indicates sensitization to other allergens (grass, ragweed, birch, mite, or cat).

obtained from patients were of sufficient quality for the quantification of immunoglobulin levels and antigen-specific T_H cell counts.

Effects of Japanese Cedar-Specific SLIT on Japanese Cedar-Specific Immunoglobulins

To access the effect of SLIT on humoral immunity, we measured serum Japanese cedar-specific IgE and IgG4 levels at preseason and postseason in both groups. Japanese cedar-specific IgE increased from preseason to postseason in the SLIT group ($P < .01$), with no increase observed in the control group ($P = .08$) (Fig 3A). Japanese cedar-specific IgG4 was increased in all patients of the SLIT group ($P < .01$) but not in the control group ($P > .99$) (Fig 3B).

Effects of Japanese Cedar-Specific SLIT on Japanese Cedar-Specific T_H Cells

The effect of seasonal Japanese cedar pollen exposure on antigen-specific T_H cells was investigated in samples obtained at preseason and postseason in the control group. The number of Japanese cedar-specific IL-5- and IL-4-producing T_H cells significantly increased in the control group ($P < .01$ for both) (Fig 4). Then, we analyzed the SLIT group, and no increases in the numbers of either IL-5- or IL-4-producing T_H cells were observed despite seasonal pollen exposure ($P = .64$ and $P = .84$, respectively) (Fig 4). On the other hand, the numbers of Japanese cedar-specific IL-10-, IFN- γ -, and TNF- α -producing T_H cells were not modified in the SLIT and control groups. The direct comparison of Japanese cedar-specific cytokine-producing T_H cell numbers at the preseason and postseason time points failed to reveal a difference between the SLIT and control groups (Fig 5).

Some supportive data regarding the antigen specificity of CD154⁺ T_H cells were provided in this study. The effects of SLIT on IL-5- and IL-4-producing T_H cells were also seen in total (CD154^{+/+}) T_H cells (eFig 1A) but not in the CD154⁻ only population

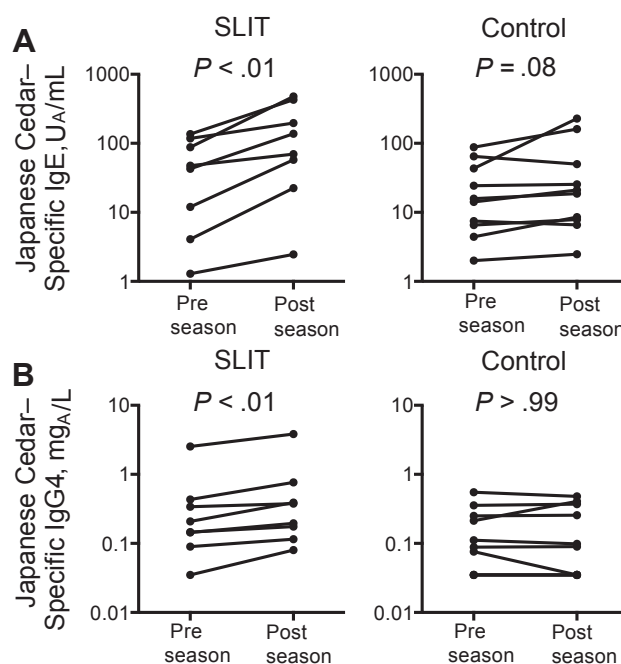


Figure 3. Effects of Japanese cedar-specific sublingual immunotherapy (SLIT) on serum Japanese cedar-specific IgE (A) and serum Japanese cedar-specific IgG4 levels (B) in the SLIT and control groups.

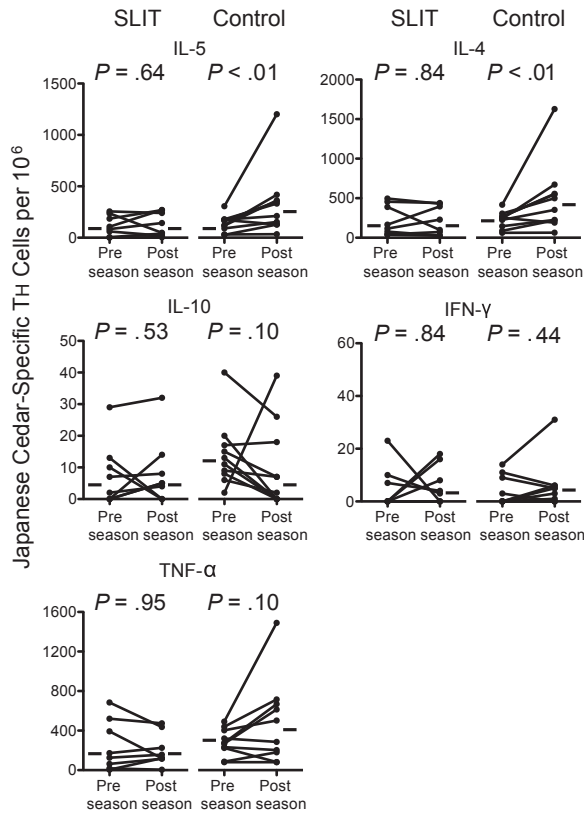


Figure 4. Effects of Japanese cedar-specific sublingual immunotherapy (SLIT) on Japanese cedar-specific cytokine-producing T_H cell numbers in the SLIT and control groups. Horizontal lines indicate median values. IFN- γ indicates interferon γ ; IL, interleukin; and TNF- α , tumor necrosis factor α .

(eFig 2). The background levels of Japanese cedar-activated cytokine-producing T_H cells in the $CD154^+$ population were relatively high in IL-10- and IFN- γ -producing T_H cells (Table 2 and eFig 3) but were lower than those in the $CD154^{+/-}$ T_H population (eTable 1 and eFig 1B). In addition, weak correlations were seen between Japanese cedar-specific IL-5- and IL-4-producing T_H cell numbers and Japanese cedar-specific IgEs, a conventional antigen-specific T_H2 activity marker, when we assessed all samples analyzed in the present study ($r_s = 0.36$, $P < .05$, and $r_s = 0.34$, $P < .05$, respectively) (eFig 4). These results indicate that seasonal increases in the number of Japanese cedar-specific IL-5- and IL-4-producing T_H cells were specifically ameliorated by Japanese cedar-specific SLIT.

Discussion

In the present study, we assessed the mechanisms underlying the effect of SLIT on antigen-specific T_H cell numbers in peripheral blood during the first pollen season after starting SLIT. Although seasonal Japanese cedar pollen exposure increased the number of Japanese cedar-specific IL-5- and IL-4-producing T_H cells, Japanese cedar-specific SLIT ameliorated the seasonal increase in the Japanese cedar-specific IL-5- and IL-4-producing T_H cell counts in peripheral blood. Seasonal pollen exposure did not affect Japanese cedar-specific IgE or Japanese cedar-specific IgG4 levels; however, SLIT increased both antigen-specific immunoglobulin levels.

The immunologic mechanisms underlying the effect of allergen-SIT are not fully understood, despite allergen-SIT being used for 100 years since first introduced by Leonard Noon in 1911.³⁰ The contribution of allergen-specific T_H cells in peripheral blood has remained controversial for several years because of the lack of a standardized method for analyzing this rare lymphocyte subset.²⁰ However, recent studies using sensitive methods involving MHC class II tetramers or the activation marker CD154 have reported a

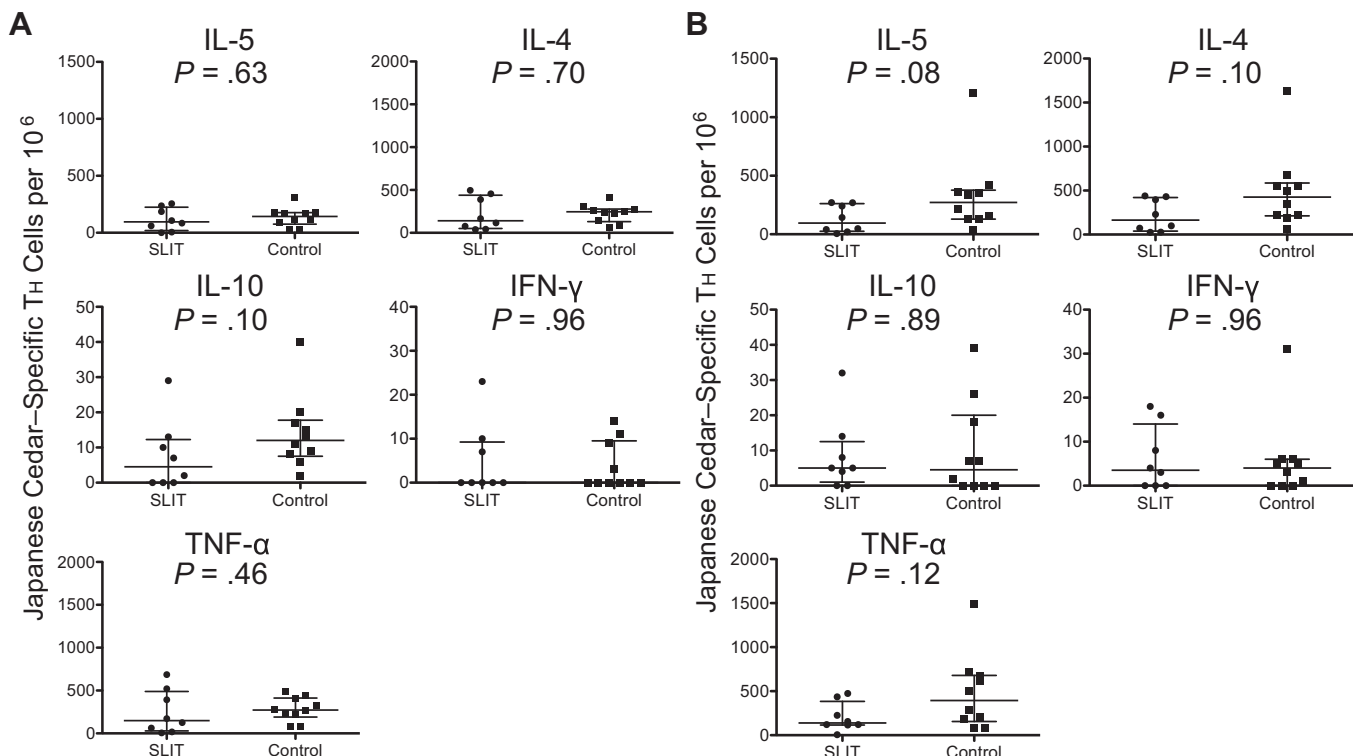


Figure 5. Direct comparison of Japanese cedar-specific cytokine-producing T_H cell numbers in the sublingual immunotherapy (SLIT) and control groups at the preseason (A) and postseason (B) time points. IFN- γ indicates interferon γ ; IL, interleukin; and TNF- α , tumor necrosis factor α .

Table 2Background Levels of Japanese Cedar–Specific (Japanese Cedar–Activated CD154⁺) Cytokine-Producing T_H cells (n = 36)^a

Component	Median (quartile range), ×10 ⁶	
	Japanese cedar–specific T _H cells	Unstimulated CD154 ⁺ T _H cells
IL-5	148.5 (51.3–252)	5.5 (0–12.3)
IL-4	234.5 (90.8–427)	9.5 (4.0–27.8)
IL-10	7.0 (0.5–14.8)	9.5 (3.3–18.0)
IFN- γ	2.0 (0–7.8)	8.0 (3.0–15.0)
TNF- α	229.5 (116–465)	89.5 (68.0–194)

Abbreviations: IFN- γ , interferon γ ; IL, interleukin; TNF- α , tumor necrosis factor α .^aThe number of Japanese cedar–specific cytokine-producing T_H cells was determined by subtracting unstimulated CD154⁺ cytokine-producing T_H cell counts from Japanese cedar–stimulated CD154⁺ cytokine-producing T_H cell counts.

contribution of allergen-specific T_H cells to the effects of SCIT.^{24–26} The detection of antigen-specific T_H cells by MHC class II tetramer is theoretically the gold standard method for the quantification of this rare lymphocyte subset; however, restriction of the MHC class II phenotype and variability of epitope peptides have limited the use of this technique as a standardized method in large-scale clinical studies. In contrast, the quantification of cells according to activation marker expression is relatively simple; however, whether all cells identified using this approach are genuine antigen-specific T_H cells remains controversial.^{23,31}

It is currently unknown whether the modification of allergen-specific T_H cell numbers in peripheral blood contributes to immunologic mechanisms underlying the effect of SLIT²⁷ because the clinical efficacy of SLIT is reportedly inferior to that of SCIT³² and local immunologic modification is thought to be important in SLIT.¹⁰ Conventional methods using messenger RNA expression analysis of the supernatants of antigen-stimulated PBMCs¹⁹ and intracellular cytokine staining without activation markers¹² have not been able to evaluate peripheral T_{H2} involvement with sufficient sensitivity. The results of the present study indicate an involvement of allergen-specific T_{H2} cells and that modification of peripheral antigen-specific T_H cell numbers may represent an immunologic mechanism underlying the effect of SLIT.

In addition to these results, we provide some informative data to support that CD154 is a specific activation marker in a clinical study. Although SLIT also suppressed seasonal increase of IL-5– and IL-4–producing T_H cell numbers in the CD154^{+/–} population, this modification was not seen in the CD154[–] population, indicating an importance of CD154⁺ population in the immunologic mechanisms of SLIT. Correlations between Japanese cedar–specific IL-5– and IL-4–producing T_H cell numbers and Japanese cedar–specific IgEs also imply the specificity of a Japanese cedar–specific T_{H2} response.

The immunologic effect of SLIT on antigen-specific immunoglobulin levels in the present study did not differ from our previous study of Japanese cedar–specific SLIT.⁹ Transient early increases and subsequent reductions in serum specific IgE cannot explain an early mechanism of SLIT because a clinical effectiveness is already seen when specific IgE is increasing.^{7,9} Serum specific IgG4 is considered to work as a blocking antibody in allergen SLIT.⁶ Sustained increases in serum specific IgG4 were accompanied by increases in serum inhibitory activity for the binding of allergen-IgE complexes to B cells (IgE-FAB), a validated surrogate of IgE-facilitated antigen presentation to T cells.³³ Our results support this humoral modification as a robust phenomenon underlying the effect of SLIT.

The limitations of the present study include a short study period and lack of data on the clinical efficacy for each individual, despite established clinical effectiveness of the therapy.⁹ Although an immunologic effect on T_{H2} cells was observed in this study period for allergen-SIT, whether this immunologic effect continued after

the study period or led to clinical effectiveness remains uncertain. In addition, this study was not randomized and included polysensitized patients because it was based on a clinical setting and many patients with Japanese cedar pollinosis sensitize other aeroallergens.³ Although immunologic assessments in this study were performed using antigen-specific methods, we need to carefully assess the effects of other allergens on the SLIT. Therefore, further long-term, controlled studies of the association between antigen-specific T_H cell counts and clinical efficacy are required for the development of strategies for monitoring allergen-SIT. An involvement of IL-10–producing T_H cells was not seen in the present study, same as that seen in our previous study for SCIT.²⁶ The time course of CD154 expression on IL-10–producing T_H cells may differ from that of effector T_H cells. Indeed, Litjens et al.³⁴ reported that the CD154 expression of FOXP⁺ natural Tregs was detectable 6 hours after stimulation, reaching maximal levels after 24 hours.

Despite these limitations, the present study yielded significant results. Although Bonvalet et al.²⁷ found that antigen-specific T_H cells were not involved in the early onset of clinical efficacy during grass pollen SLIT using MHC class II technology, Suárez-Fuey et al.³⁵ reported that the number of T_{H2} cells in peripheral blood changed during SLIT. This finding implied that the cells were modified by therapy; however, their study did not include a comparison between the changed number of treated cells and number of untreated controls cells.³⁵ There remains an urgent need for further studies assessing the involvement of peripheral T_{H2} cells in SLIT. To the best of our knowledge, the present study is the first to find an involvement of peripheral antigen-specific T_H cells during SLIT, including untreated controls.

In conclusion, the findings of the present study indicate that allergen-specific T_H cell counts in peripheral blood quantified according to CD154 expression measured by multicolor flow cytometry may play an important role in SLIT. This method of quantifying the numbers of this rare lymphocyte subset represents a sensitive and simple, standardized method for monitoring antigen-specific T_H cell counts during allergen-SIT.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2016.04.003>.

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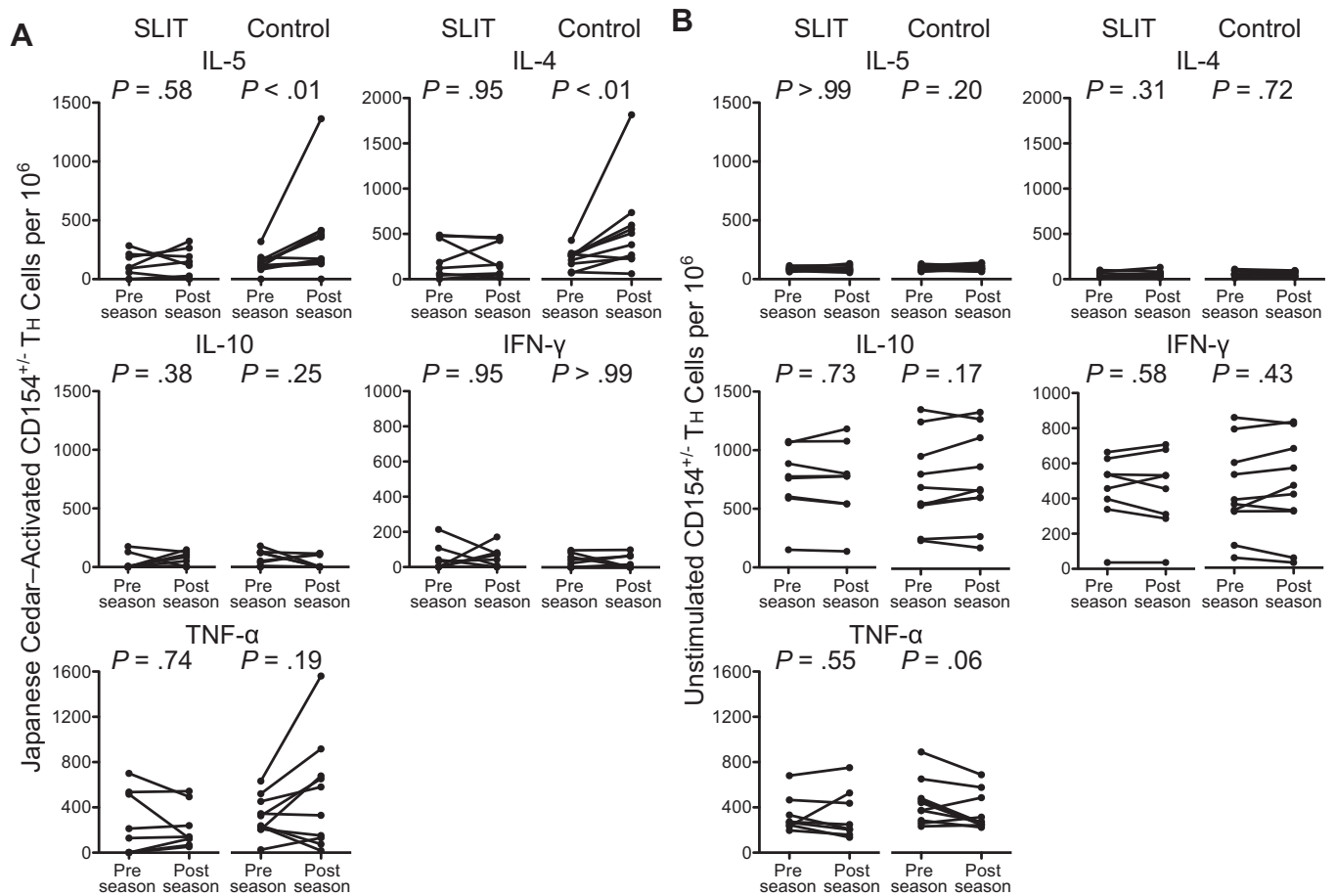


Figure 1. A, Effects of Japanese cedar–specific sublingual immunotherapy (SLIT) on Japanese cedar–activated CD154^{+/–} cytokine-producing T_H cell counts in the SLIT and control groups. B, Unstimulated CD154^{+/–} cytokine-producing T_H cells. IFN- γ indicates interferon γ ; IL, interleukin; TNF- α , tumor necrosis factor α .

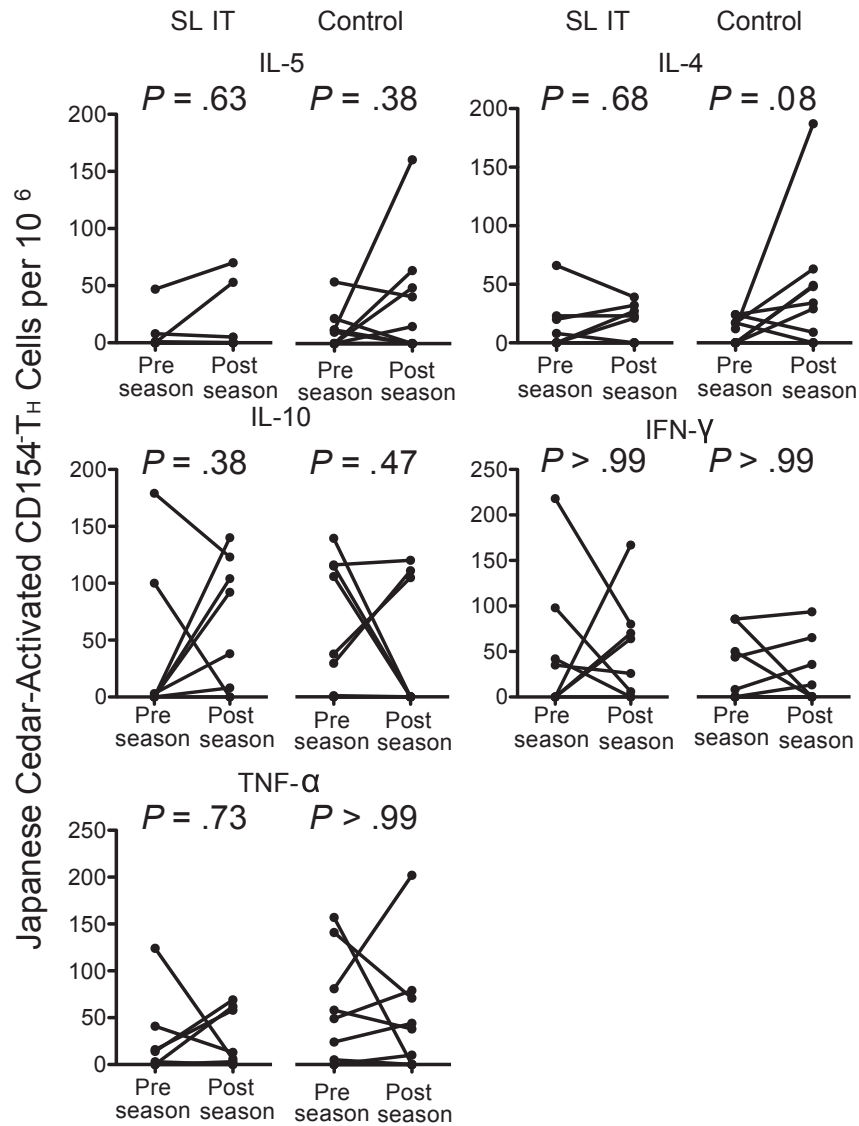
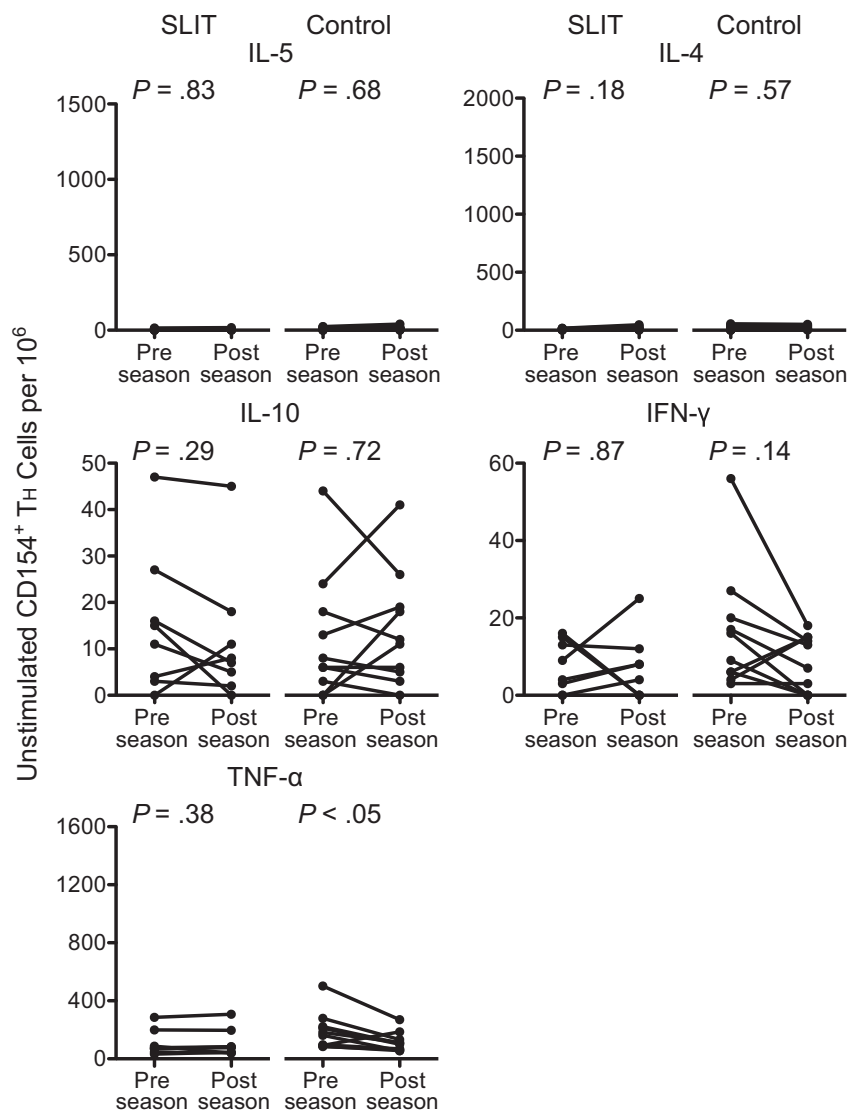


Figure 2. Effects of Japanese cedar-specific sublingual immunotherapy (SLIT) on Japanese cedar-activated CD154⁺ cytokine-producing T_H cell counts in the SLIT and control groups. IFN- γ indicates interferon γ ; IL, interleukin; TNF- α , tumor necrosis factor α .



eFigure 3. Unstimulated CD154⁺ cytokine-producing T_H cells. IFN-γ indicates interferon γ; IL, interleukin; TNF-α, tumor necrosis factor α.

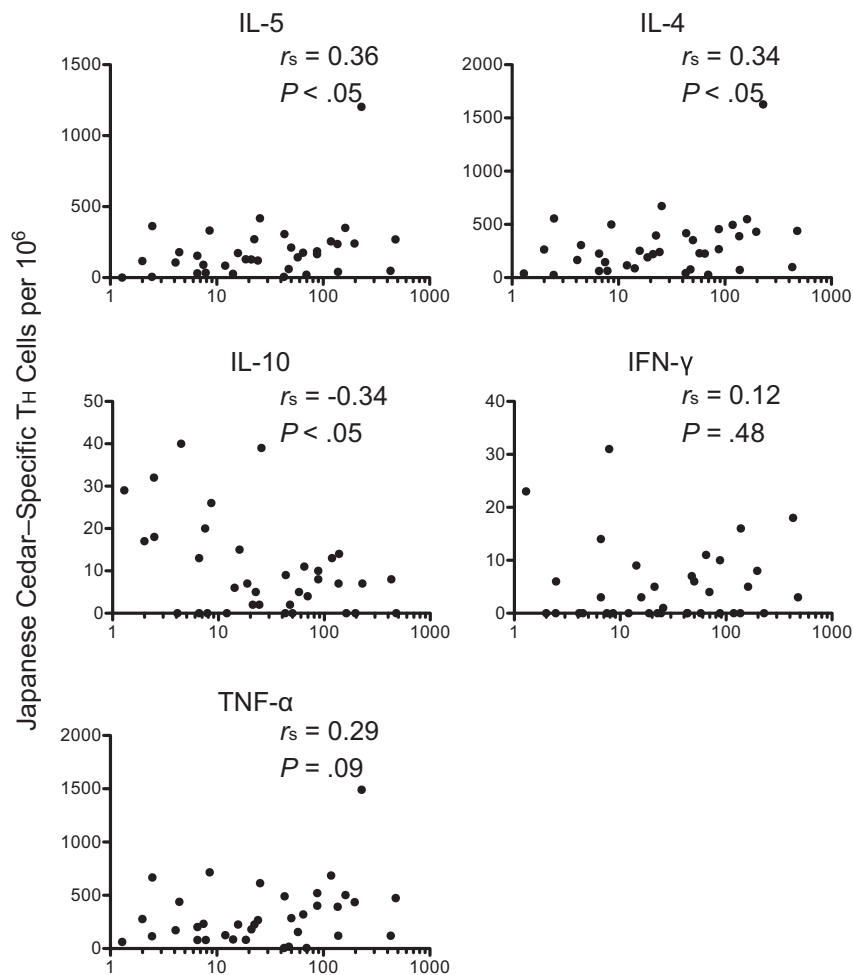


Figure 4. Correlation between Japanese cedar–specific cytokine-producing T_H cell numbers and Japanese cedar–specific IgEs of all samples analyzed in the present study ($n = 36$). IFN- γ indicates interferon γ ; IL, interleukin; TNF- α , tumor necrosis factor α .

Table 1

Background Levels of Japanese Cedar–Activated CD154^{+/−} Cytokine-Producing T_H Cells ($n = 36$)^a

Component	Median (quartile range), $\times 10^6$	
	Japanese cedar–activated CD154 ^{+/−} T_H cells	Unstimulated CD154 ^{+/−} T_H cells
IL-5	141.5 (83.8–252)	87.5 (75.3–109)
IL-4	256.5 (90.0–454)	55.0 (33.5–83.8)
IL-10	9.0 (0–115)	721.5 (541–1033)
IFN- γ	12.5 (0–69.0)	456.0 (316–618)
TNF- α	234.0 (124–531)	278.5 (244–476)

Abbreviations: IFN- γ , interferon γ ; IL, interleukin; TNF- α , tumor necrosis factor α .

^aThe number of Japanese cedar–activated CD154^{+/−} cytokine-producing T_H cells was determined by subtracting unstimulated CD154^{+/−} cytokine-producing T_H cell counts from Japanese cedar-stimulated CD154^{+/−} cytokine-producing T_H cell counts.