

We thank all family members for their participation. We also thank Stanley Sing Hoi Cheuk for providing melanocytes, the Department of Clinical Genetics of the Karolinska University Hospital for help with MLPA, and the Clinical Genomics unit at SciLifeLab for whole-genome sequencing.

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This work was supported by a grant from the European Research Council (ERC) under the European Union's Seventh Framework Programme (grant no. FP/2007-2013; ERC grant agreement no. 311335), the Norwegian Research Council, the Swedish Foundation for Strategic Research, the Wallenberg Foundation, the Karolinska Institutet Center for Innovative Medicine (Y.T.B.), the Swedish Childhood Cancer Foundation (J.-I.H., Y.T.B., M.M.), as well as the Stockholm County Council, the Swedish Cancer Foundation, and the Swedish Research Council (J.-I.H., Y.T.B.), the Cancer and Allergy Foundation of Sweden (J.-I.H.), the German Ministry of Education and Research (grant no. BMBF 01EO1303), and the German Research Foundation (grant no. SFB1160, TP1; S.E.). B.T. is also supported by a doctoral student scholarship

from the Board of Postgraduate Studies at Karolinska Institutet. Computations were performed on resources provided by the Swedish National Infrastructure for Computing (SNIC) through the Uppsala Multidisciplinary Center for Advanced Computational Science under Project SNIC b2012204 and b2015280.

Disclosure of potential conflict of interest: S. Ehl reports a grant from Deutsche Forschungsgemeinschaft (grant no. SFB1160 TP4) and a grant from BMBF (grant no. BMBF 01EO1303) during the conduct of the study and grants from Union Chimique Belge outside the submitted work. J.-I. Henter reports grants from the Swedish Childhood Cancer Foundation, the Swedish Cancer Foundation, the Swedish Research Council, the Stockholm County Council, and the Cancer and Allergy Foundation of Sweden during the conduct of the study. M. Meeths reports grants from the Swedish Childhood Cancer Foundation during the conduct of the study. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online March 6, 2018.
<https://doi.org/10.1016/j.jaci.2018.02.031>

Correlation of allergen-specific T follicular helper cell counts with specific IgE levels and efficacy of allergen immunotherapy



To the Editor:

Allergic rhinitis (AR) is caused by an IgE-mediated hypersensitivity reaction to airborne allergens. T_H2 cells were long considered to regulate B-lymphocyte class-switch recombination to IgE by secreting the cytokines IL-4 or IL-13. However, recently, a novel CD4⁺ T-cell subset, follicular helper T (T_{FH}) cells, has been recognized as the central player in regulating B cells to support antibody response largely based on their localization in B-cell follicles and germinal centers.¹

Mouse studies indicate that T_{FH} cells, but not T_H2 cells, regulate IgE production in patients with allergic asthma.¹ We demonstrated that nasal IL-4⁺ T_{FH} cell counts correlate to local IgE production in patients with eosinophilic nasal polyps.² Kamekura et al³ reported a skewed T_{FH} phenotype toward type 2 polarization in peripheral blood of patients with AR. However, exactly how allergen-specific T_{FH} cells control the production of

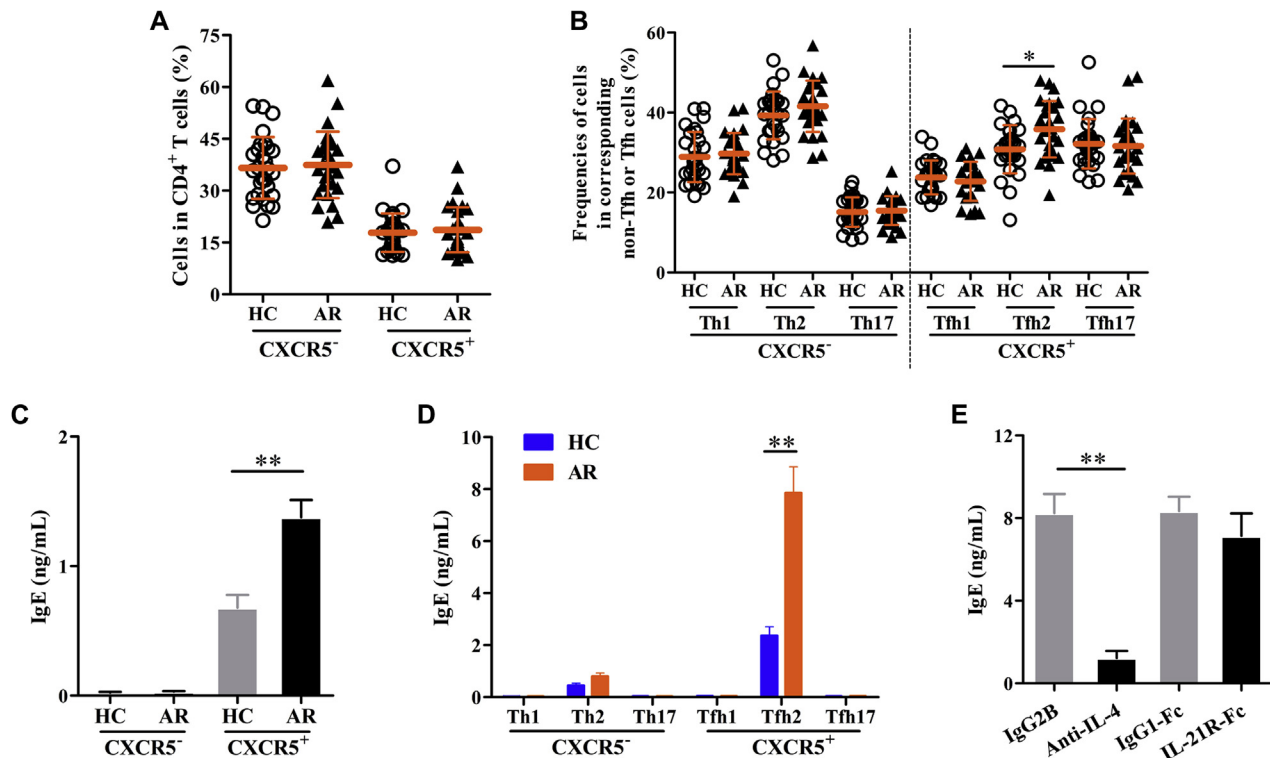


FIG 1. Increased numbers of circulating T_H2 cells in patients with AR. **A**, Percentages of circulating non-T_H and T_H cells among total CD4⁺ T cells in patients with AR (n = 27) and HC subjects (n = 28). **B**, Percentage of cell subsets among non-T_H and T_H cells in patients with AR (n = 27) and HC subjects (n = 28). **C**, T_H and non-T_H cells were cocultured with autologous naive B cells, and immunoglobulin levels were detected at day 8 (n = 8). **D**, Non-T_H and T_H cell subsets were cocultured with autologous naive B cells, and IgE levels were detected at day 8 (n = 6). **E**, T_H2 cells from patients with AR were cocultured with autologous naive B cells in the presence of blocking antibody or protein, and IgE levels were detected at day 8 (n = 6). In Fig 1, A and B, data are presented as means $\pm$ SDs. In Fig 1, C-E, data are presented as means $\pm$ SEMs. **P* < .05 and ***P* < .01.

allergen-specific IgE (sIgE) in human patients with allergic diseases remains to be defined.

In this study we investigated the frequency and phenotype of circulating T_H subsets, especially Der p 1-specific T_H cells, in patients with house dust mite-induced AR and examined the associations between Der p 1-specific T_H cell counts with sIgE production and their changes accompanying allergen immunotherapy (AIT).

PBMCs were isolated from 41 patients with AR and 42 healthy control (HC) subjects for cytometric and functional analysis of circulating T_H cell subsets (see Table E1 in this article's Online Repository at www.jacionline.org). Twenty-two patients with AR with subcutaneous AIT were recruited to explore the effect of AIT on T_H cells, with 20 patients with AR without such treatment as control subjects (see Table E2 in this article's Online Repository at www.jacionline.org). Combined symptom and medication score (CSMS), T_H cell subsets, and immunoglobulin levels were assessed before treatment (baseline) and at 3, 6, and 12 months after treatment. More information regarding study design is provided in the Methods section in this article's Online Repository at www.jacionline.org.

The gating strategy is shown in Fig E1, A, in this article's Online Repository at www.jacionline.org, as previously described (see Table E3 in this article's Online Repository at www.jacionline.org).⁴ Although no difference in percentages of circulating non-

T_H and T_H cells among total CD4⁺ T cells was found between patients with AR and HC subjects (Fig 1, A), we observed a significant increase in percentages of type 2 T_H (T_H2) cells among either T_H cells or total CD4⁺ T cells in patients with AR compared with control subjects (Fig 1, B, and see Fig E1, B). Notably, numbers of counterpart type 2 non-T_H cells (T_H2) remained comparable between patients with AR and HC subjects (Fig 1, B, and see Fig E1, B). Because there was no difference in all other examined T_H cell subsets between patients with AR and HC subjects (Fig 1, B, and see Fig E1, B), the difference of T_H2 cells suggests this T_H subset might play a prominent role in patients with AR.

Contrary to our findings, a recent study reported that numbers of circulating PD-1⁺CXCR5⁺CD4⁺ T_H cells were reduced in patients with AR.⁵ This discrepancy might be caused at least in part by different methods used to define T_H cells. Our study included staining for regulatory T cells and excluded this population in the analysis, and therefore we carefully avoided the interference of data interpretation by CXCR5⁺ regulatory T cells, which are considered regulatory follicular T cells. In addition, allergen exposure can affect blood cell dynamics and therefore possibly contributes to the difference in numbers of circulating T_H cells observed in different studies.⁶

Circulating T_H cells have been indicated as a peripheral counterpart of *bona fide* T_H cells in lymphoid follicles and share similar phenotypes and functions.⁴ When coculturing sorted

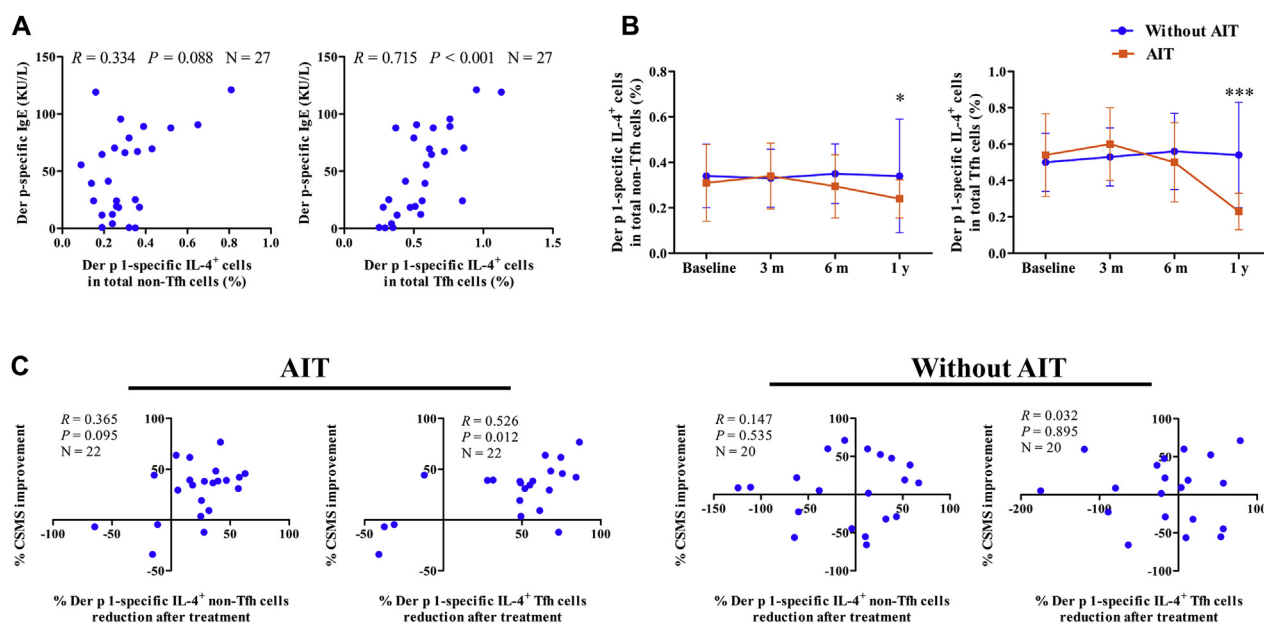


FIG 2. Decrease in numbers of circulating allergen-specific IL-4⁺CXCR5⁺CD4⁺ T_H cells after AIT. **A**, Correlations between frequencies of Der p 1-specific IL-4⁺ non-T_H and T_H cells with *D pteronyssinus*-specific IgE levels in patients with AR. **B**, Changes in percentages of Der p 1-specific IL-4⁺ cells within non-T_H and T_H cells in patients with AR with (n = 22) and without (n = 20) AIT after treatment. Data are presented as means $\pm$ SDs. **C**, Correlations of reduction in numbers of Der p 1-specific IL-4⁺ non-T_H and T_H cells with CSMS improvement in patients with AR with and without AIT after treatment. * $P < .05$ and *** $P < .001$.

circulating T_H cells with autologous naive B cells, we found that T_{FH} cells from patients with AR, when compared with those from HC subjects, enhanced only production of IgE but not IgM, IgG, or IgA (Fig 1, C, and see Fig E2, A, in this article's Online Repository at www.jacionline.org). Consistent with the previous report that T_{FH2} cells are the T_{FH} subset particularly helping B cells to produce IgE,⁴ we discovered that T_{FH2} cells from patients with AR had a markedly enhanced capacity to induce IgE production than those from HC subjects (Fig 1, D, and see Fig E2, B). Type 2 cytokines promote IgE isotype switching and are secreted from T_{FH2} cells but not from T_{FH1} or T_{FH17} cells.⁴ In T-cell/B-cell cocultures we found that T_{FH2} cells from patients with AR secreted greater levels of IL-4 but comparable levels of IFN- γ , IL-17A, and IL-21 in comparison with those from HC subjects (see Fig E2, C). Furthermore, we found that blocking IL-4 diminished IgE production (Fig 1, E). Thus an upregulated T_{FH2} response can robustly promote IL-4-mediated IgE induction in patients with AR. As a control, we confirmed that non-T_{FH} cells induced low-level production of immunoglobulin, and no difference between patients with AR and HC subjects was observed (Fig 1, C and D, and see Fig E2, A and B).

We next examined allergen-specific circulating T_{FH} cells in patients with AR. We found that Der p 1-pulsed monocytes induced memory T_H cell proliferation. Although both non-T_{FH} and T_{FH} cells from patients with AR induced more proliferation than those from HC subjects, T_{FH} cells displayed a stronger phenotype than non-T_{FH} cells (see Fig E3, A, in this article's Online Repository at www.jacionline.org), indicating significant accumulation of Der p 1-specific T_{FH} cells in patients with AR. Moreover, we measured IL-4 expression on Der p 1 stimulation and found that numbers of Der p 1-specific IL-4⁺ non-T_{FH} and T_{FH} cells were markedly increased in patients with AR, with greater

expression of IL-4 detected again in T_{FH} cells than non-T_{FH} cells (see Fig E3, B). This greater expression of IL-4 in T_{FH} cells indicates that distinct signaling pathways might underlie IL-4 regulation in T_{FH} and non-T_{FH} cells. For example, the basic leucine zipper transcription factor ATF-like is important for IL-4 expression in T_{FH} cells rather than in canonical T_{H2} cells.⁷ There was no significant difference for expression of IFN- γ , IL-17A, and IL-21 in these CD4⁺ T-cell subsets after stimulation with Der p 1 (data not shown). Interestingly, Der p 1-specific IgE levels in patients with AR correlated positively only with the frequency of Der p 1-specific IL-4⁺ T_{FH} cells but not non-T_{FH} cells (Fig 2, A). We also measured allergen-specific T-cell numbers by analyzing expression of the activation markers CD137 and CD154 and found that the frequency of Der p 1-specific IL-4⁺CD154⁺CD137⁺ T_{FH} cells was increased in patients with AR compared with that in HC subjects (data not shown). Collectively, these data support the notion that allergen-specific T_{FH2} cells produce IL-4 and orchestrate sIgE production in patients with AR.

Finally, we explored the relationship between T_{FH} cells and the efficacy of AIT in patients with AR. Consistent with previous reports,⁸ compared with those without AIT, the AIT group demonstrated significant symptom improvement and a decrease in *Dermatophagoides pteronyssinus*-specific IgE levels after the 12-month treatment, which was accompanied by an increase in *D pteronyssinus*-specific IgG₄ levels (see Fig E4, A and B, in this article's Online Repository at www.jacionline.org). Moreover, compared with those without AIT, a significant reduction in numbers of both Der p 1-specific IL-4⁺ T_{FH} and non-T_{FH} cells was found in the AIT group after treatment with a more prominent reduction for the former (Fig 2, B). In contrast, no difference in other T_H cell subsets was recorded (see Fig E4, C-E).

Importantly, the reduction in numbers of Der p 1–specific IL-4⁺ T_{FH} cells correlated positively with the improvement in CSMS only in patients with AIT but not in the non-AIT control subjects (Fig 2, C). However, we did not observe a significant correlation of sIgE reduction with downregulation of Der p 1–specific IL-4⁺ T_{FH} cells or improvement in CSMS (see Fig E4, F). This suggests that other factors, such as regulatory follicular T cells and long-lived plasma cells, can also be involved in sIgE regulation during AIT. It also reflects the persistence of cell-bound sIgE in the nasal mucosa, which is important for symptom expression in patients with AR. Additionally, the mechanisms underlying the change in numbers of allergen-specific T_{FH} cells and its contribution to the rebalance of the immune system after AIT warrant further investigation.

Our study design did not focus on patients with asthma given the low frequency of asthma in our cohort. However, it will be interesting to compare differences between patients with AR with and without asthma in future studies. A recent study reported a reduction in numbers of total T_{FH} cells after AIT in patients with grass allergy; however, this report did not examine allergen-specific T_{FH} cells or the association between T_{FH} cell reduction and the efficacy of AIT.⁹ Our findings highlight an important role of allergen-specific T_{FH} cells in sIgE production and the development of AR.

Intriguingly, high expression of CCR2 and CCR5 was detected on allergen-specific non-T_{FH} cells (see Fig E5 in this article's Online Repository at www.jacionline.org), suggesting that these cells preferentially migrate to nonlymphoid tissues, such as the nasal mucosa, to mediate local inflammatory responses through producing type 2 cytokines. On the contrary, high CCR7 expression on allergen-specific T_{FH} cells indicates that these cells likely recirculate through secondary lymphoid organs, where they encounter antigens and participate in T-dependent antibody responses and support sIgE production by B cells (see Fig E5).

In summary, for the first time, our study provides evidence that allergen-specific IL-4⁺ T_{FH} cells can contribute to sIgE production and correlate with clinical efficacy of AIT in patients with AR. T_{FH}2 cells might be a promising therapeutic target and biomarker for AIT in patients with AR. However, our findings here need to be evaluated and validated in randomized, double-blind, placebo-controlled trials.

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Supported by National Natural Science Foundation of China (NSFC) grants 81325006, 81570899, and 81630024 (to Z.L.) and 81429003 (to D.Y.), Hubei Province Natural Science Foundation grant 2017CFA016 (to Z.L.), the Australian National Health and Medical Research Council (NHMRC) fellowship GNT1085509 (to D.Y.), and the National Basic Research Program of China 973 Program 2014CB541903 (to D.Y.).

Disclosure of potential conflict of interest: D. Yu received grant 81429003 from the National Natural Science Foundation of China, grant GNT1085509 from the Australian National Health and Medical Research Council (NHMRC) fellowship, and grant 2014CB541903 from the National Basic Research Program of China 973 Program for this work. Z. Liu received grants 81630024, 81570899, and 81325006 from the National Natural Science Foundation of China and grant 2017CFA016 from the Hubei Province Natural Science Foundation for this work. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online April 4, 2018.

<https://doi.org/10.1016/j.jaci.2018.03.008>

Serum from Asian patients with atopic dermatitis is characterized by T_H2/T_H22 activation, which is highly correlated with nonlesional skin measures



To the Editor:

Assessment of atopic dermatitis (AD) severity is largely limited to clinical disease measures, but the nature of the disease is such that it cannot be quantified accurately through clinical scores alone. Biomarkers of disease activity in the skin or blood might be useful adjuncts in clinical trials to follow disease severity by using objective criteria.¹ However, the cytokine profile of Asian patients with AD differs from that of European American (EA) patients with AD in the skin, representing a blend between EA patients with AD and patients with psoriasis and showing increased epidermal hyperplasia, greater T_H17/T_H22 and lower T_H1 skewing, and comparable T_H2 activation.² Currently, it is unknown how these differences translate to circulating biomarkers. Peripheral biomarker profiling is essential for precision therapeutic approaches and for potentially minimizing the need for biopsies. Although skin and serum data exist for EA patients with AD,^{1,3}

METHODS

Subjects

This study was approved by the Ethics Committee of Tongji Hospital and the Ethics Committee of China Resources & Wisco General Hospital, and written informed consent was obtained from each patient. Patients with AR and HC subjects were recruited from these 2 hospitals from April 2015 to April 2017. Forty-one patients with AR and 42 HC subjects participated in the phenotype and function study of circulating CD4⁺ T_H cells (Table E1). An additional 22 patients with AR with subcutaneous AIT were recruited to explore the effect of AIT on T_H cells, with 20 patients with AR without AIT as control subjects (Table E2).

All patients with AR fulfilled the following inclusion criteria: (1) age greater than 18 years, (2) diagnosis of AR according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines and presentation of clinical symptoms,^{E1} (3) greater than 1-year history of AR, and (4) positive skin prick test response to *D pteronyssinus* and levels of serum sIgE to *D pteronyssinus* levels of 0.7 KU/L or greater (Phadia ImmunoCAP, Uppsala, Sweden). Exclusion criteria were as follows: (1) patients allergic to other inhalant allergens than house dust mites (*D pteronyssinus* and *Dermatophagoides farinae*) according to skin prick tests; (2) patients with asthma or others allergic disease according to ARIA and Global Initiative for Asthma guideline^{E1,E2}; (3) patients with upper respiratory tract infections or chronic rhinosinusitis; (4) pregnant or breast-feeding patients; (5) patient having received previous treatment with immunotherapy; (6) patients with neurologic or psychiatric disorders, autoimmune diseases, or cancer; and (7) patients who used antihistamines or intranasal steroids in the past 1 week or oral steroids in the past 3 months before this study. All of the patients with AR in this study had persistent AR according to the ARIA classification.^{E1} Healthy subjects with negative skin prick test responses and without a history of allergy were recruited as HC subjects.^{E3} Skin prick tests with a panel of 19 inhalant allergens common in our region were performed, as previously described.^{E3}

For those participating in phenotype and function studies of circulating T_H cell subsets, peripheral blood was collected after enrollment. For those patients with AR participating in the treatment study, peripheral blood was collected at baseline (before treatment) and at 3, 6, and 12 months after treatment.

AIT and symptom assessment

The semidepot house dust mite allergen extracts (50% *D pteronyssinus* plus 50% *D farinae*; Allergopharma GmbH, Reinbek, Germany) were used for AIT with subcutaneous injections, as previously described.^{E4} Over the course of the study, all patients were also managed with standard pharmacotherapy, as recommended.^{E1}

Participants involved in a treatment study with or without AIT were provided with a daily record card for recording the 6 rhinoconjunctivitis symptom scores (nasal itching, sneezing, rhinorrhea, nasal obstruction, ocular itching, and ocular tearing) and rescue medication use (oral and/or topical nonsedative H₁-antihistamines, intranasal corticosteroids, and oral corticosteroids) during the previous month of scheduled visits (baseline and 3, 6, and 12 months).^{E5} Daily rhinoconjunctivitis symptom scores and rescue medication scores were evaluated and daily CSMS were calculated at scheduled visits, as previously described.^{E5} After 12 months of treatment, the improvement in CSMS was calculated as follows:

$$(\text{Baseline CSMS} - \text{CSMS at 12 months}) / (\text{Baseline CSMS}) \times 100\%.^{E6}$$

Flow cytometry

PBMCs were isolated, as previously mentioned.^{E7} Cells were stained with Fixable Viability Stain 700 (BD Biosciences, San Jose, Calif) to exclude dead cells. For cell-surface staining, primary antibodies were incubated with cells for 30 minutes at 25°C. For intracellular staining, cells were permeabilized with Cytofix/Cytoperm (BD Biosciences) for 20 minutes on ice after surface staining. Antibodies specific for intracellular cytokines were incubated with cells for 30 minutes at 4°C. Isotype controls were used as negative controls. Stained cells were analyzed with a BD LSRFortessa X-20 (BD Biosciences)

instrument and FlowJo software (TreeStar, Ashland, Ore).^{E8} The gating strategy is shown in Fig E1, A, as we previously described.^{E9} Antibodies used in flow cytometry are listed in Table E3.

Cell sorting

Monocytes were isolated from PBMCs by means of negative selection with a Monocyte Isolation Kit II (Miltenyi Biotec, Bergisch Gladbach, Germany), as previously described.^{E10}

Peripheral T-cell subsets and naive B cells were sorted from fluorescence-labeled PBMCs with a BD FACSARIA II Cell Sorter (BD Biosciences). Among CD4⁺ T cells, non-T_{FH} and T_{FH} cells were sorted as CD4⁺CD25^{−/low}CD127^{−/+}CD45RA^{low}CXCR5[−] and CD4⁺CD25^{−/low}CD127^{−/+}CD45RA^{low}CXCR5⁺ cells, respectively. T_{FH} and non-T_{FH} cells were further subgrouped into type 1 (CXCR3⁺CCR6[−]), type 2 (CXCR3[−]CCR6[−]), and type 17 (CXCR3[−]CCR6⁺) cell subsets. The type 1, 2, and 17 cell subsets in T_{FH} and non-T_{FH} cells were sorted. Naive B cells were sorted as CD3[−]CD19⁺IgD⁺CD27[−] cells. Purity of sorted cells was greater than 96%. Antibodies used in cell sorting are tabulated in Table E3.

Cell culture

For T-cell/B-cell coculture, sorted T cells (5×10^4 cells/well) were cultured with autologous naive B cells (2.5×10^4 cells/well) for 8 days in RPMI 1640 (Thermo Fisher Scientific, Waltham, Mas) supplemented with 10% heat-inactivated FBS (Thermo Fisher Scientific) and 1% penicillin/streptomycin (Sigma-Aldrich, St Louis, Mo) in the presence of staphylococcal enterotoxin B (1 μg/mL; Toxin Technology, Sarasota, Fla) in U-bottom 96-well plates, as previously described.^{E10} In blocking experiments anti-IL-4 mAb (1 μg/mL; R&D Systems, Minneapolis, Minn), IL-21 receptor-Fc (20 μg/mL; R&D Systems), or the corresponding control (mouse IgG_{2B} and IgG₁-Fc; R&D Systems) was added, as previously described.^{E7} Cytokine and immunoglobulin levels in culture supernatants were measured at days 2 and 8, respectively.

Carboxyfluorescein succinimidyl ester-labeled CXCR5[−] non-T_{FH} and CXCR5⁺ T_{FH} CD4⁺ T cells (1×10^5 cells/well) were cultured with autologous monocytes (1×10^5 cells/well) preincubated with Der p 1 (20 μg/mL; endotoxin <0.03 EU/μg; Indoor Biotechnologies, Manchester, United Kingdom) for 15 hours to examine allergen-specific circulating T_H cells.^{E10} At day 7, cells were stained with anti-CD4 and anti-CD14, and proliferation of CD4⁺ T cells was assessed by using flow cytometry. PBMCs (5×10^6 cells) stimulated with 20 μg/mL Der p 1 for 18 hours at 37°C and 1.7 ng/mL monensin (eBioscience, San Diego, Calif) were added for the last 2 hours to directly illustrate the cytokine production by allergen-specific T cells.^{E11,E12} After stimulation, the cells were underwent surface and intracellular staining and were subjected to flow cytometry, as mentioned above.

Allergen-specific T cells were also measured based on expression of the activation markers CD137 and CD154, as previously described.^{E12} PBMCs (5×10^6 cells) were stimulated with 20 μg/mL Der p 1 (Indoor Biotechnologies) in the presence of 1 mg/mL anti-CD40 (Miltenyi Biotec) and 1 mg/mL anti-CD28 antibody (eBioscience) for 7 hours at 37°C. Monensin (1.7 ng/mL) was added for the last 2 hours. IL-4⁺CD154⁺CD137[−] allergen-specific T-cell counts were determined.

For each stimulation, background enriched from unstimulated control was subtracted. For allergen-specific and cytokine-secreting T cells, a detection limit was defined as greater than 1.5-fold background cell numbers (unstimulated control).^{E12}

Cytokine and immunoglobulin quantification

Serum total IgE and sIgE levels to *D pteronyssinus* were measured by using ImmunoCAP (Phadia), and sIgG₄ levels to *D pteronyssinus* were measured by using ELISA (Dr. Focke Laboratorien GmbH, Neuss, Germany). IgG, IgM, IgA, IgE, IFN-γ, IL-4, IL-17A, and IL-21 levels in culture supernatants were detected by using commercial ELISA kits (immunoglobulin, IL-17A, and IL-21 ELISA kits from Thermo Fisher Scientific and IFN-γ and IL-4 ELISA kits from R&D Systems).^{E10}

Statistical analysis

All statistical analyses were carried out with GraphPad Prism Software 5.0 (GraphPad Software, La Jolla, Calif) or SPSS 19.0 software (IBM, Armonk, NY). For continuous variables, data are presented as means $\pm$ SDs or means $\pm$ SEMs, and unpaired 2-tailed *t* tests or Mann-Whitney *U* tests were used to compare paired sets of data. For categorical data, χ^2 or Fisher exact tests were performed to determine the difference between groups. Spearman rank correlation analysis was used to analyze the associations. *P* values of less than .05 were considered statistically significant.

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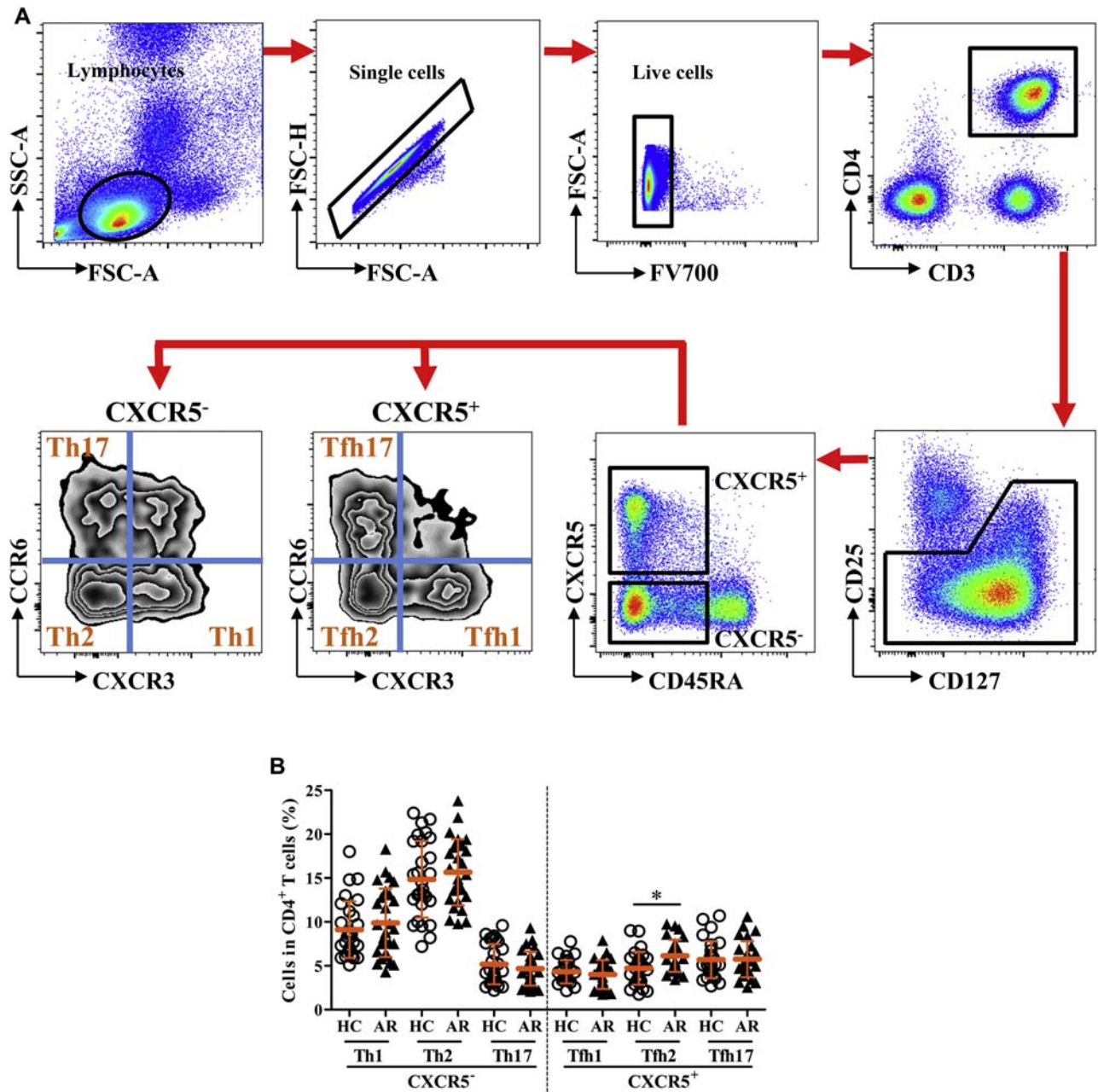


FIG E1. Peripheral T_{FH} and non-T_{FH} cells among total CD4⁺ T cells. **A**, Gating strategy for CXCR5⁻ non-T_{FH} cells and CXCR5⁺ T_{FH} cells and their subsets in peripheral blood. *FSC*, Forward scatter; *FV700*, fixable viability stain 700; *SSC*, side scatter. **B**, Percentages of subsets of T_{FH} and non-T_{FH} cells in total CD4⁺ T cells (*n* = 28 for HC subjects and *n* = 27 for patients with AR). Data are presented as means ± SDs. **P* < .05.

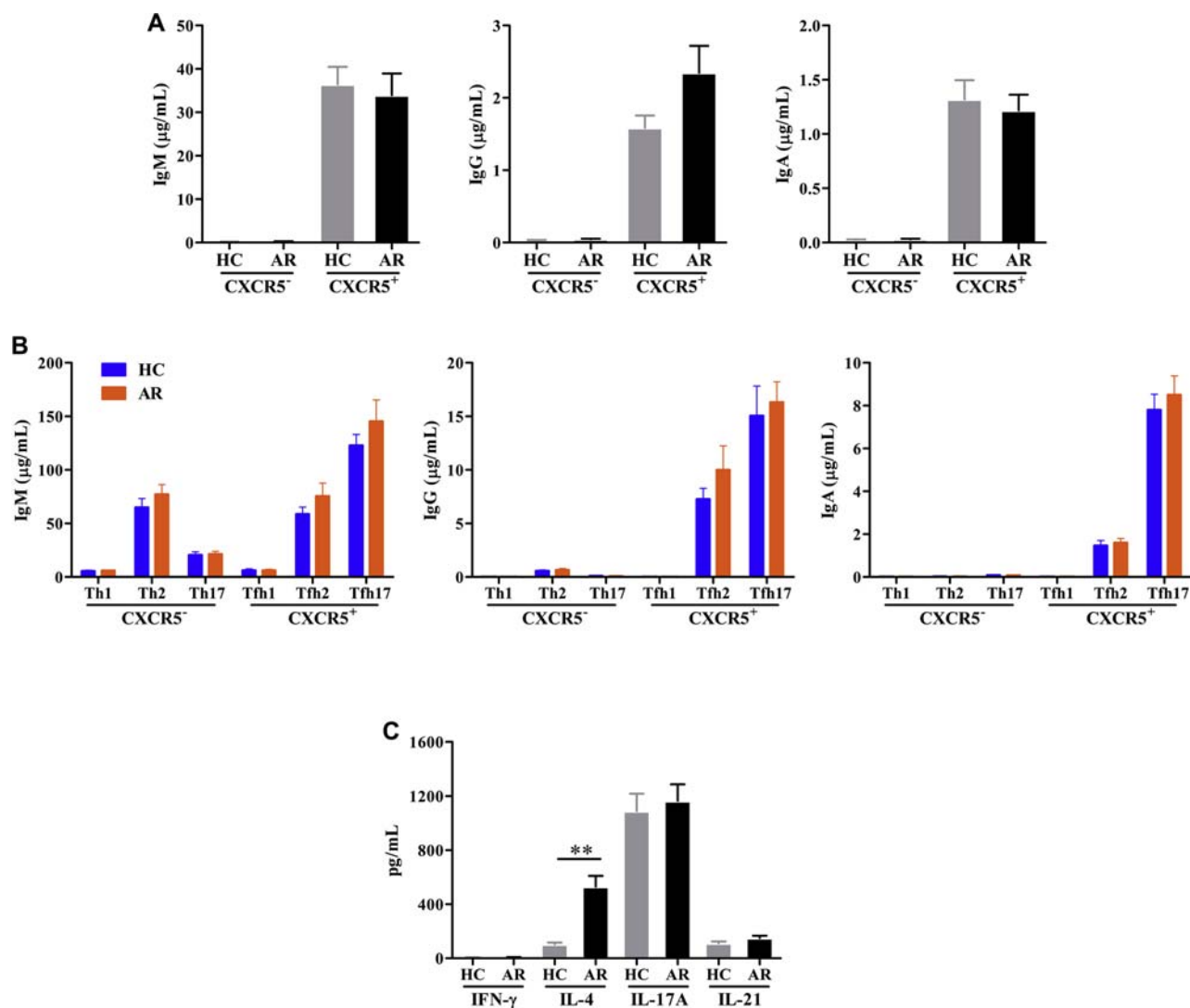


FIG E2. Immunoglobulin-inducing function of circulating T_{FH} and non-T_{FH} cells. **A**, Non-T_{FH} and T_{FH} cells from HC subjects or patients with AR were cocultured with autologous naive B cells, and immunoglobulin levels were detected at day 8 (n = 8). **B**, T_{FH} and non-T_{FH} cell subsets from HC subjects or patients with AR were cocultured with autologous naive B cells, and immunoglobulin levels were detected at day 8 (n = 6). **C**, T_{FH}2 cells from patients with AR were cocultured with autologous naive B cells, and cytokine levels were detected at day 2 (n = 6). Data are presented as means ± SEMs. **P < .01.

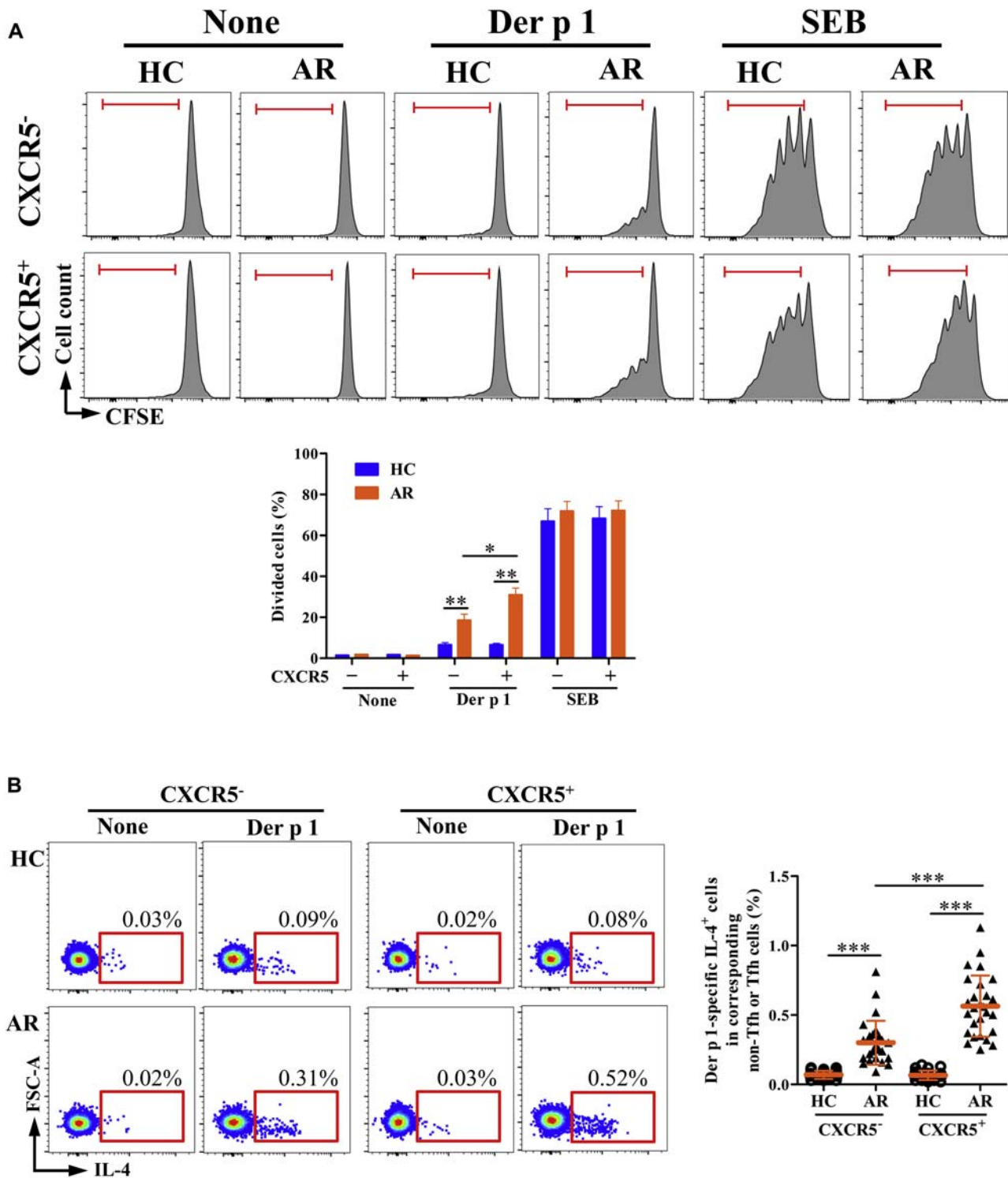


FIG E3. Allergen-specific circulating T_{FH} cells in patients with AR. **A**, Carboxyfluorescein succinimidyl ester (CFSE)-labeled circulating CXCR5⁻ non-T_{FH} and CXCR5⁺ T_{FH} cells were cultured with autologous monocytes, which were pretreated with Der p 1 or staphylococcal enterotoxin B. Cell proliferation was analyzed by using flow cytometry at day 7 (n = 6). Representative histograms are shown. Data are expressed as means ± SEMs. **B**, Percentages of Der p 1-specific IL-4⁺ non-T_{FH} and T_{FH} cells within corresponding total non-T_{FH} and T_{FH} cells. Representative flow cytometric plots are shown. Data are presented as means ± SDs (n = 27 for patients with AR and n = 25 for HC subjects). *P < .05, **P < .01, and ***P < .001.

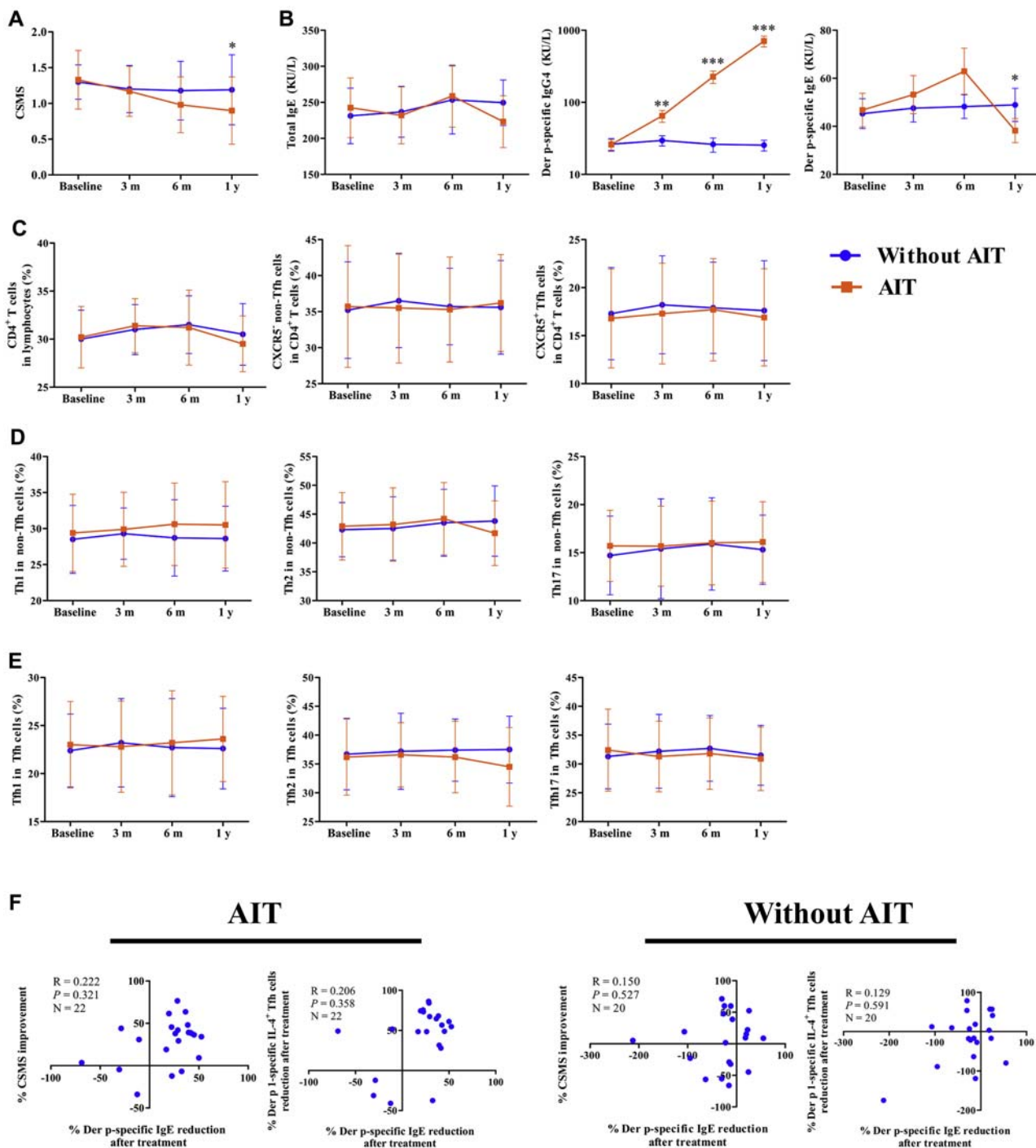


FIG E4. Changes in immunoglobulin levels and T_H cell counts in patients with AR with or without subcutaneous AIT after treatment. **A**, Changes in CSMS. Data are expressed as means $\pm$ SDs. **B**, Changes in total IgE, *D pteronyssinus*-specific IgE, and *D pteronyssinus*-specific IgG₄ levels. Data are expressed as means $\pm$ SEMs. **C-E**, Changes in T_H cell (Fig E4, C), CXCR5⁺ non-T_H cell subset (Fig E4, D), and CXCR5⁺ T_H cell subset counts (Fig E4, E; $n = 22$ for patients with AIT and $n = 20$ for patients without AIT). **F**, Correlations of reductions in *D pteronyssinus*-specific IgE levels with improvement in CSMS and decrease in Der p 1-specific IL-4⁺ T_H cell counts. Data are expressed as means $\pm$ SDs. * $P < .05$, ** $P < .01$, and *** $P < .001$.

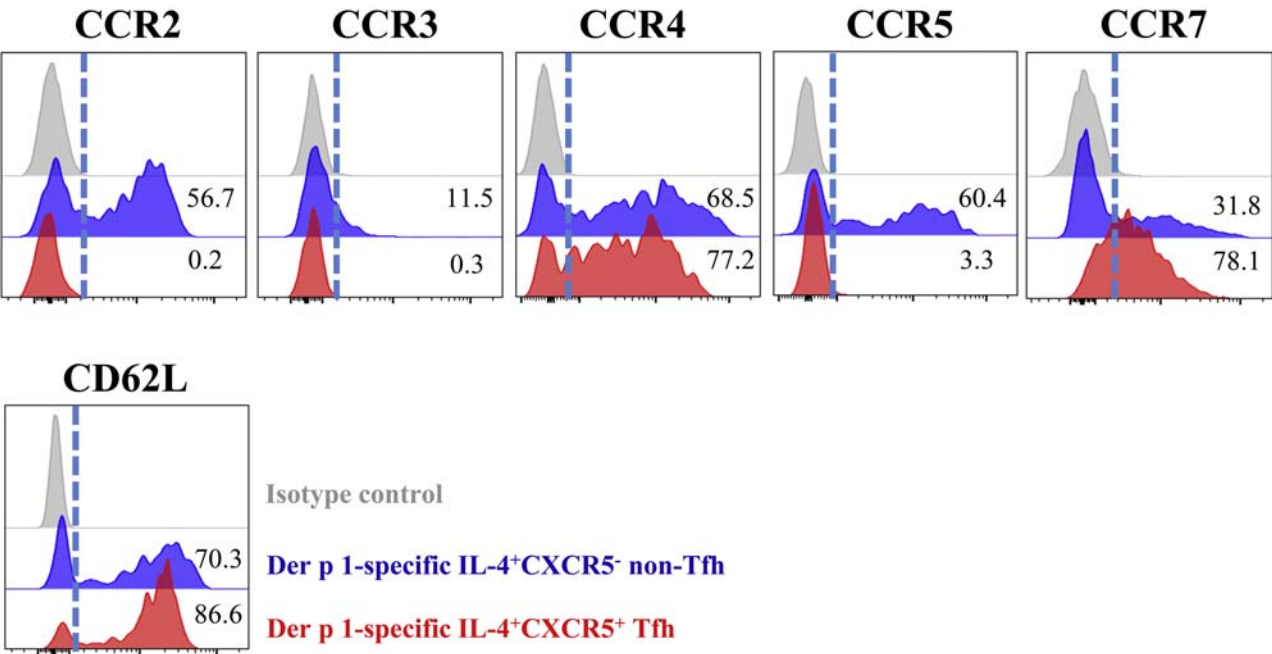


FIG E5. Patterns of chemokine receptor expression in Der p 1-specific IL-4⁺ T_{FH} and Der p 1-specific IL-4⁺ non-T_{FH} cells in patients with AR. Representative histograms are shown. Numbers in figures refer to percentages of positive cells (n = 2).

TABLE E1. Demographic characteristics of subjects in T_H cell phenotype and function study

	HC subjects	Patients with AR	P value
Total subjects (no.)	42	41	
Sex (male/female)	28/14	25/16	.651
Age (y), mean $\pm$ SD	31.5 $\pm$ 10.6	32.7 $\pm$ 12.4	.650
SPT response to <i>D pteronyssinus</i>	Negative	Positive	
Total IgE (KU/L), mean $\pm$ SD	20.9 $\pm$ 18.2	322.5 $\pm$ 250.3	<.001
Specific IgE to <i>D pteronyssinus</i> (KU/L), mean $\pm$ SD	0.14 $\pm$ 0.1	46.7 $\pm$ 33.4	<.001

SPT, Skin prick test.

TABLE E2. Baseline characteristics of patients with AR in the treatment study before treatment

	With AIT	Without AIT	P value
Total subjects (no.)	22	20	
Sex (male/female)	13/9	10/10	.757
Age (y), mean $\pm$ SD	30.5 $\pm$ 10.9	34.2 $\pm$ 10.6	.277
SPT response to <i>D pteronyssinus</i>	Positive	Positive	
Total IgE (KU/L), mean $\pm$ SD	242.5 $\pm$ 194.5	231.2 $\pm$ 186.4	.890
Specific IgE to <i>D pteronyssinus</i> (KU/L), mean $\pm$ SD	46.8 $\pm$ 32.6	45.3 $\pm$ 31.6	.871
Specific IgG ₄ to <i>D pteronyssinus</i> (KU/L), mean $\pm$ SD	26.0 $\pm$ 18.6	26.3 $\pm$ 21.5	.835
CSMS, mean $\pm$ SD	1.3 $\pm$ 0.4	1.3 $\pm$ 0.3	.684

SPT, Skin prick test.

TABLE E3. Antibodies used in flow cytometry and cell sorting

Antibody	Parameter	Clone ID	Source	Isotype	Manufacturer	Dilution
CD3	Brilliant Violet 510	UCHT1	Mouse	IgG ₁ , κ	BD Biosciences, San Jose, Calif	1:200
CD4	APC-Cy7	RPA-T4	Mouse	IgG ₁ , κ	BD Biosciences	1:200
CXCR5	BB515 (FITC)	RF8B2	Rat	IgG _{2b} , κ	BD Biosciences	1:100
CD25	PE-Cy7	M-A251	Mouse	IgG ₁ , κ	BD Biosciences	1:100
CD127	Brilliant Violet 421	HIL-7R-M21	Mouse	IgG ₁ , κ	BD Biosciences	1:100
CD45RA	Brilliant Violet 605	HI100	Mouse	IgG _{2b} , κ	BD Biosciences	1:100
CXCR3	APC	IC6/CXCR3	Mouse	IgG ₁ , κ	BD Biosciences	1:50
CCR6	PerCP-Cy5.5	11A9	Mouse	IgG ₁ , κ	BD Biosciences	1:100
CCR2	Alexa Fluor 647	SA203G11	Rat	IgG _{2b} , κ	BioLegend, San Diego, Calif	1:100
CCR3	PerCP-Cy5.5	5E8	Mouse	IgG _{2b} , κ	BioLegend	1:100
CCR4	PE	L291H4	Mouse	IgG ₁ , κ	BioLegend	1:100
CCR5	PE	J418F1	Rat	IgG _{2a} , κ	BioLegend	1:100
CCR7	Alexa Fluor 647	3D12	Rat	IgG _{2a} , κ	BD Biosciences	1:100
CD62L	PE	DREG-56	Mouse	IgG ₁ , κ	BD Biosciences	1:200
IL-4	APC	8D4-8	Mouse	IgG ₁ , κ	BD Biosciences	1:100
IL-4	PE-Cy7	8D4-8	Mouse	IgG ₁ , κ	BD Biosciences	1:100
CD137	PE	4B4-1	Mouse	IgG ₁ , κ	BioLegend	1:100
CD154	APC	TRAP1	Mouse	IgG ₁ , κ	BD Biosciences	1:100
CD3	FITC	UCHT1	Mouse	IgG ₁ , κ	BD Biosciences	1:200
CD19	APC-Cy7	SJ25C1	Mouse	IgG ₁ , κ	BD Biosciences	1:100
CD27	PE-Cy7	M-T271	Mouse	IgG ₁ , κ	BD Biosciences	1:100
IgD	PerCP-Cy5.5	IA6-2	Mouse	IgG _{2a} , κ	BD Biosciences	1:100
CD14	APC	M5E2	Mouse	IgG _{2a} , κ	BD Biosciences	1:100

APC, Allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein complex.