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Title 論文題目	Activated circulating T follicular helper cells and skewing of T follicular helper 2 cells are down-regulated by treatment including an inhaled corticosteroid in patients with allergic asthma (アトピー型喘息患者では吸入ステロイドを含む治療によって末梢血濾胞ヘルパーT 細胞の活性化ならびに２型濾胞ヘルパーT 細胞の偏りが減少する)
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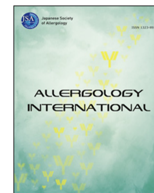

Original Article

Activated circulating T follicular helper cells and skewing of T follicular helper 2 cells are down-regulated by treatment including an inhaled corticosteroid in patients with allergic asthma

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AA, atopic asthma; ACQ, asthma control questionnaire; AR, allergic rhinitis; BCL, B cell lymphoma; Breg, regulatory B; cT_{FH}, circulating T follicular helper; CSR, class switch recombination; FeNO, fractional exhaled nitric oxide; GC, germinal center; HDM, house dust mite; HV, healthy volunteers; ICOS, inducible costimulator; IgG4-RD, IgG4 related disease; IL, interleukin; ILC2, type 2 innate lymphoid cell; LABA, long-acting β2 agonist; LTRA, leukotriene receptor antagonist; LN, lymph node; PBMC, peripheral blood mononuclear cell; PD-1, programmed death 1; PFAS, pollen food allergy syndrome; SHM, somatic hypermutation; T_H2, type 2 helper T; Treg, regulatory T

A B S T R A C T

Background: $CXCR5^+$ T follicular helper (T_{FH}) cells primarily promote B cells to produce an antigen-specific antibody through germinal centers (GCs). T_{FH} cells exist in circulation, and circulating(c) $T_{FH}2$ cells, a subset of cT_{FH} cells, are able to help naïve B cells produce IgE in healthy individuals. Conversely, IL-10-producing regulatory B (Breg) cells inhibit an accelerated immune response.
Methods: We investigated the roles of cT_{FH} cells and cBreg cells based on a T_H2 response in patients with atopic asthma (AA). Thirty-two patients with AA and 35 healthy volunteers (HV) were enrolled. We examined cT_{FH} cells including their subsets, their expression of ICOS and PD-1, and cBreg cells by flow cytometry and their associations with clinical biomarkers. Plasma levels of CXCL13, which is a counterpart of CXCR5, were also measured using ELISA.
Results: In patients with AA, $cT_{FH}2$ cells were increased and $cT_{FH}1$ cells were decreased compared with those in HV. The expression levels of ICOS on cT_{FH} and their subset cells were elevated and Breg cells were greatly decreased. The plasma levels of CXCL13 in patients with AA were significantly elevated and correlated well with the $cT_{FH}2$/cBreg ratio. These cells were examined in 10 patients AA before and after inhaled corticosteroid (ICS) treatment. Interestingly, the percentages and numbers of $T_{FH}2$ and $ICOS^+$ cT_{FH} cells declined after ICS treatment together with improvements in symptoms and clinical biomarkers.
Conclusions: The percentages and numbers of $cT_{FH}2$ and $ICOS^+$ cT_{FH} cells might be useful as biomarkers of T_H2 typed airway inflammation in patients with AA.
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Introduction

Asthma is the most common chronic lung disease affecting about 300 million people in the world. It has long been evident that the severity of asthma varies greatly among patients, but this phenotypic heterogeneity has recently been investigated in more systematic ways. Cluster analyses of large numbers of highly characterized individuals with asthma have shown that they can be grouped into several phenotypic clusters.[1–3] Allergic asthma, which is one of these clusters, is based on type 2 immune responses to allergens and other environmental factors, including type 2 helper T (T_H2) cell cytokine responses and production of IgE antibodies. T_H2-type $CD4^+$ T cells and type 2 innate lymphoid cells (ILC2s), which produce interleukin (IL)-4, IL-5 and IL-13 have been considered major players in directing the pathophysiology of allergic asthma, including airway eosinophilia, mucosal hyperplasia, and airway remodeling.[4,5]

T_H2 cells have been thought to play a critical role in allergic asthma because they produce IL-4, a key cytokine that robustly regulates IgE class switching. However, T follicular helper (T_{FH}) cells, which are a specialized subset of $CD4^+$ T cells, primarily help B cells undergo class switch recombination (CSR), somatic hypermutation (SHM) and affinity maturation through germinal center (GC) reactions in lymphatic tissues. T_{FH} cells express a chemokine receptor of CXCR5, which facilitates their interaction with B cells, and they secrete IL-4 and IL-21 to promote B-cell differentiation. In GCs, T_{FH} cells also express various costimulatory molecules including inducible costimulator (ICOS) and programmed death 1 (PD-1) of an immune-regulatory molecule, and B cell lymphoma (BCL)-6 as a transcription factors.[6] It later became evident that $CD4^+CXCR5^+$ T cells exist in human peripheral blood (circulating T follicular helper cells; cT_{FH} cells). These cells are classified into three distinct subsets, $T_{FH}1$, $T_{FH}2$, and $T_{FH}17$ cells, according to the expression profiles of CXCR3 and CCR6. $T_{FH}1$, $T_{FH}2$, and $T_{FH}17$ cells secrete restricted repertories of cytokines such as interferon (IFN)-γ, IL-4, and IL-17, respectively, as do T_H1, T_H2 and T_H17 cells. Furthermore, $T_{FH}2$ cells have the capacity to help naïve B cells produce IgE and IgG.[7,8] A recent study in mice suggested that T_{FH} cells, but not T_H2 cells, initially induced by exposure to house dust mites (HDMs) and produce IL-4 and promote the production of HDMs-specific IgE from B cells in the GCs (sensitization phase) and that upon re-exposure of HDMs (allergen), IL-4-committed $T_{FH}2$ cells could differentiate into IL-4 and IL-13-double-producing T_H2 cells that preferentially migrate to the affected allergic lesions of lung (effector phase).[9] These results indicate that IgE is produced as a consequence of the cognate interaction between T_{FH} cells and B cells and that the plasticity between T_{FH} cells and T_H2 cells is closely related to be the pathogenesis of allergic asthma. For these reasons, T_{FH} cells are considered to a key player in the specific immune responses underlying allergic inflammation. We have already reported the skewing of circulating $T_{FH}2$ cells in patients with allergic rhinitis (AR) with or without asthma.[10] Most cT_{FH} cells are considered to be central memory T cells except for some cT_{FH} cells that express ICOS and/or PD-1.[8] These cT_{FH} cells were compared with GC T_{FH} cells by gene profile, cytokine profile and functional properties, and the results were almost the same as those for GC T_{FH} cells.[11] Therefore, T_{FH} cells derived from GC in LNs of the affected lesion may be flowed into the circulating blood stream through lymphatics.

On the other hand, there has been accumulating evidence supporting a suppressive function of B cells. These B cells, which are called regulatory B cells (Breg cells), can suppress immune responses in mice and humans.[12,13] The absence or loss of these Breg cells exacerbates disease symptoms in patients with allergic and autoimmune diseases.[14–17] A population of Breg cells showing specific surface markers has not been reported, but several populations showing different surface markers have been reported.[18] Breg cells inhibit T cell-mediated immunity by producing IL-10, IL-35 and TGF-β and inducing regulatory T cells (Treg) cells.[12] Iwata *et al.* reported that $CD24^{hi}CD27^+$ B cells exhibited a regulatory function in human autoimmune diseases.[19]

Inhaled corticosteroid (ICS) administration is a most effective treatment for asthma. In this study, to investigate the therapeutic efficacy in cT_{FH} cells based on T_H2 response, we examined the proportion and number of cT_{FH} cells including their subsets and expression of ICOS and PD-1 in patients with AA, and we also compared patients before and after treatment patients with ICS administration. CXCL13 is a counterpart for CXCR5 and their expression in B and T_{FH} cells is necessary for migration to B cell follicles. Therefore, CXCL13 plasma level reported to be a marker of the activity of GC reactions including the interaction between antigen-presented T_{FH} cells and B cells.[20] We measured plasma CXCR13 levels in patients with AA and HV. The proportion and number of (IL-10-producing) $CD24^{hi}CD27^+$ cBreg cells, which inhibit immune responses in patients with AA, were also investigated. The results suggest the usefulness of cT_{FH} cells as a biomarker and therapeutic importance in patients with AA.

Methods

Study population

In this study, 32 AA patients and 35 HV were enrolled. The characteristics of the patients with AA and HV are summarized in Table 1. The diagnosis of AA was performed on the basis of the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2017, 2018 and Japan Respiratory Society Guidelines.[21] Subjects with AA were diagnosed by a questionnaire (asthma control questionnaire (ACQ),[22] physical examination, spirometry, airway reversibility and hyperreactivity tests and measurements of specific IgEs and fractional exhaled nitric oxide (FeNO). FeNO was measured by NIOX VERO (CHEST M.I., Tokyo, Japan). Serum levels of specific IgEs to allergens and non-specific (total) IgE were measured by Immuno-CAP (Thermo Fisher Scientific; Waltham, MA, USA). All of the patients with AA had several or multiple specific IgEs to allergens. Seventeen of the AA patients also had other allergic diseases such as AR, allergic conjunctivitis, atopic dermatitis, pollen food allergy syndrome (PFAS) and food allergy (Table 1). Ten patients with AA (three patients with mild AA, two patients with moderate AA and five patients with severe AA) were already being treated with an ICS ± long -acting β2 agonist (LABA) ± leukotriene receptor antagonist (LTRA) or other drugs, and the other 22 patients had a no history of allergen-specific immunotherapy and had not been

Table 1
Characteristics of subjects.

	Atopic asthma patients	Healthy volunteers
	(n = 32)	(n = 35)
Age (years)	46.8 ± 16.0	43.8 ± 13.0
No.male/female	11/21	17/18
Smoking history	never:19/ex:8/current:5	
IgE (IU/ml)	654 ± 1404*	45 ± 54
Blood eosinophils (number/μl)	483 ± 752*	147 ± 120
FEV1% pred (%)	77.0 ± 18.6	
FeNO (ppb)	63.2 ± 44.6	
Other allergic complications	AR; 14, AC; 4, PFAS; 2, food allergy; 2	

AR, allergic rhinitis; AC, allergic conjunctivitis; PFAS, pollen food allergy syndrome. *$p < 0.01$. Mean ± SD.

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treated with drugs for three years prior to this study. None of the HV had abnormal physical or chest X-ray findings and they were all negative for the Immuno-CAP test. All of the subjects were non-smokers. Written informed consent was obtained from all subjects according to the Declaration of Helsinki. All of the protocols were approved by the Institutional Review Boards of Sapporo Medical University Hospital in Japan.

Antibodies

Panels of directly conjugated anti-human monoclonal antibodies were used to measure lymphocyte species of T_{FH} cells (anti-CD3-APC, anti-CD4-APC-Cy7, anti-CD45RA-BV510 and anti-CXCR5-PerCP-Cy5.5), T_{FH} cell subsets (anti-CD3-FITC, anti-CD4-APC-Cy7, anti-CD45RA-BV510, anti-CXCR5-PerCP-Cy5.5, anti-CCR6-APC, and anti-CXCR3-PE-Cy7), T_H2 subset (anti-CCR4-BV510), and activation marker (anti−ICOS−BV421, anti-PD-1-PE) gated by cells negative to anti-CD3-APC and Breg cells (anti-CD19-APC-Cy7, anti-CD24-PerCP-Cy5.5, and anti-CD27-FITC) gated by cells negative to anti-CD3-APC. These monoclonal antibodies were purchased from BD Biosciences (San Jose, CA, USA).

Flow cytometry

Heparinized PBMCs were isolated from fresh blood specimens by centrifugation over a discontinuous density gradient (Lympholyte-H; Cedarlane, Burlington, ON, Canada). Cell staining and flow cytometry using FACSCanto II (BD Biosciences) were performed as previously described.[23]

ELISA

The Human CXCL13 Quantikine ELISA Kit obtained from R&D Systems (Minneapolis, MN, USA) was used for the plasma samples as described in the instructions.

Statistical analysis

All data are shown as means ± SD. Significant differences in results were determined by using Student's *t*-test or the Mann−Whitney U test. Wilcoxon's rank sum test was used for analysis of data before and after treatment. Correlations were determined by Pearson's correlation coefficient. Probability values less than 0.05 were considered significant.

Results

cT_{FH} cells and their subsets in patients with AA and HV

First, we examined the percentages and absolute numbers of cT_{FH} ($CD3^+CD4^+CD45RA^-CXCR5^+$) cells in all $CD4^+$ T cells ($CD3^+CD4^+$) of patients with AA and HV (FACS procedure of cT_{FH} and other cT_H cell subsets is shown in Fig. 1A and Supplementary Fig. 1). The percentage and number in AA patients was significantly larger than those in HV (AA, 20.7 ± 6.0%; HV, 17.0 ± 4.1%, $p = 0.0038$, and AA, 154 ± 70/μl; HV, 117 ± 45/μl, $p = 0.0127$, Fig. 1B). Interestingly, the percentage and absolute number of $cT_{FH}2$ cells in AA patients were further increased compared to those in HV (AA, 41.6 ± 6.1%; HV, 34.0 ± 5.7%, $p < 0.0001$, and AA, 62 ± 24/μl, HV; 39 ± 15/μl, $p < 0.0001$, Fig. 1C) and the percentage and absolute number of $T_{FH}1$ cells in patients with AA were conversely decreased compared to those in HV (AA, 17.7 ± 5.3%; HV, 20.3 ± 5.8%, $p = 0.0524$, AA, 28 ± 19/μl; HV, 24 ± 13/μl, $p = 0.2660$, Fig. 1C). The percentage and absolute number of $T_{FH}17$ cells were almost the same in the two groups. Therefore, the ratio of % T_{FH} 2 cells to % T_{FH} 1 cells was significantly elevated in AA patients compared to that in HV (AA, 2.6 ± 0.9%; HV, 1.8 ± 0.6%, $p = 0.0001$, Fig. 1D).

ICOS and PD-1 expression on c-T_{FH} cells and their subsets in patients with AA and HV

We next assessed the expression of ICOS and PD-1 on cT_{FH} cells in patients with AA and HV (Fig. 2A). The expression was examined in all AA patients and in 22 of the 35 HV in whom the expression levels were measured. The percentages and absolute numbers of $ICOS^+$ and PD-1^+ cT_{FH} cells in AA patients were significantly increased compared with those in HV ($ICOS^+$ cT_{FH} cells: AA, 9.1 ± 4.8%; HV 6.4 ± 2.7%, $p = 0.0123$ and AA, 14 ± 10/μl, HV 7 ± 3/μl, $p = 0.0010$, PD-1^+ cT_{FH} cells: AA. 22.5 ± 7.7%; HVs, 16.3 ± 5.7%, $p = 0.0014$ and AA, 34 ± 16/μl, HV 18 ± 7/μl, $p < 0.0001$, $ICOS^+$PD-1^+ cT_{FH} cells: AA, 4.3 ± 2.9%; HV 2.7 ± 1.5%, $p = 0.0109$ and AA, 6 ± 5/μl, HVs 3 ± 2/μl, $p = 0.0011$, Fig. 2B). We also examined the expression of ICOS and PD-1 on cT_{FH} cell subsets. The percentages and absolute numbers of $ICOS^+$ $cT_{FH}2$ and $cT_{FH}1$ cells in AA patients were much larger than those in HV. The percentage of $ICOS^+$ $cT_{FH}17$ cells in AA patients was higher, but not significantly higher, than that in HV, but the absolute number was significantly lager in AA patients ($ICOS^+$ $cT_{FH}2$ cells: AA, 8.4 ± 4.7%; HV, 6.0 ± 3.1%, $p = 0.0248$ and AA, 5 ± 4/μl, HV, 2 ± 1/μl, $p = 0.0001$, $ICOS^+$ $cT_{FH}1$ cells: AA, 12.8 ± 7.6%; HV, 8.5 ± 4.5%, $p = 0.0131$ and AA, 4 ± 3/μl, HV, 2 ± 1/μl, $p = 0.0089$, $ICOS^+$ $cT_{FH}17$ cells; AA, 9.3 ± 6.3%: HVs 6.7 ± 4.4%, $p = 0.0906$ and AA, 5 ± 4/μl, HV 3 ± 2/μl, $p = 0.0301$, Fig. 2C). In contrast, the percentages and absolute numbers of three subsets of PD-1^+ cT_{FH} cells in AA patients were larger (but not significantly larger) than those in HV except for the number of $T_{FH}2$ cells (Supplementary Fig. 2). The percentages and absolute numbers of subsets of $ICOS^+$PD-1^+ cT_{FH} cells were the same as those of subsets of PD-1^+ cT_{FH} cells (data not shown). These results indicated that some cT_{FH} cells, especially cells with higher ICOS expression, were activated in patients with AA, whereas most of the cT_{FH} cells are thought to be quiescent.

cBreg cells in patients with AA

We then examined cBreg cells ($CD3^-CD19^+CD24^{hi}CD27^+$ cells), as negative regulators of immune response, in patients with AA (Fig. 3A). The percentage and absolute number of cBreg cells in AA patients were significantly smaller than those in HV (AA, 16.8 ± 9.4% and 25 ± 28/μl; HV, 30.2 ± 10.2% and 37 ± 22/μl, $p < 0.0001$, Fig. 3B).

CXCL13 in patients with AA

The CXCL13-CXCR5 chemokine axis plays a major role in organizing both B cell follicles and GCs. Plasma CXCL13 has been shown to be a biomarker of HIV^+ patients who have broad antibodies to HIV virus,[20] and it has been reported that CXCL13 levels were elevated in bronchio-alveolar lavage (BAL) fluid in AA patients[24] and that IL-17 induced CXCL13 expression, which mediated B cell infiltration in inflamed tissues of asthma patients.[25] CXCL13 is a counterpart of CXCR5 on T_{FH} cells, and we therefore examined the plasma CXCL13 levels in patients with AA by ELISA. CXCL13 levels were measurable in 26 AA patients and 22 HV. The plasma levels of CXCL13 in AA patients were significantly elevated compared to those in HV (AA, 59.8 ± 41.9 pg/ml; HV, 39.4 ± 21.0 pg/ml, $p =$

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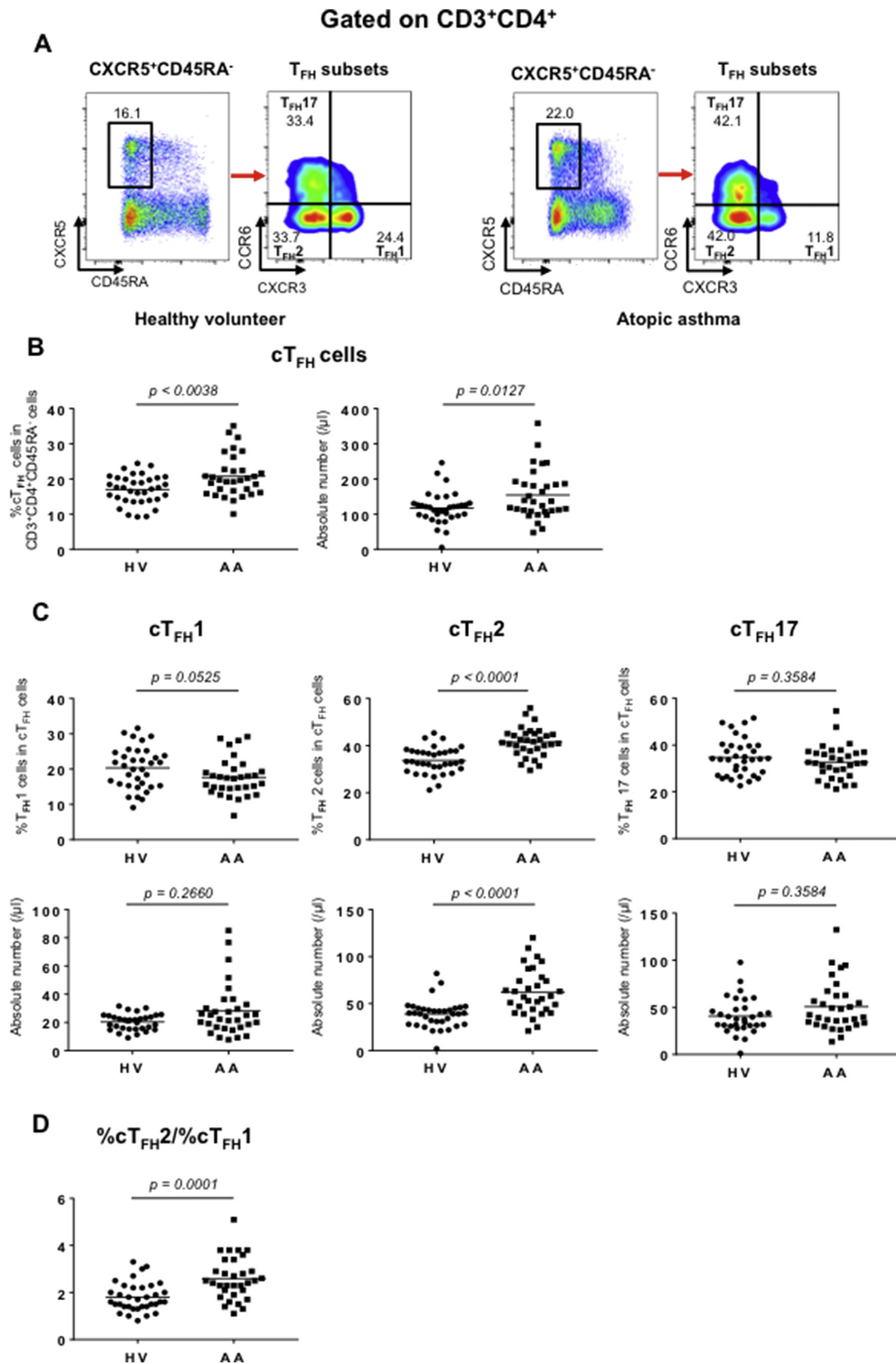

Fig. 1. Percentages and absolute numbers of cT_{FH} cells and their subsets in patients with AA and HV. **(A)** Representative FACS profiles for T_{FH} cells subsets in peripheral blood from patients with AA and HV are shown. Numbers indicate percentage of cells in the gate. **(B–D)** Percentages and absolute numbers of cT_{FH} cells and their subsets in patients with AA and HV.

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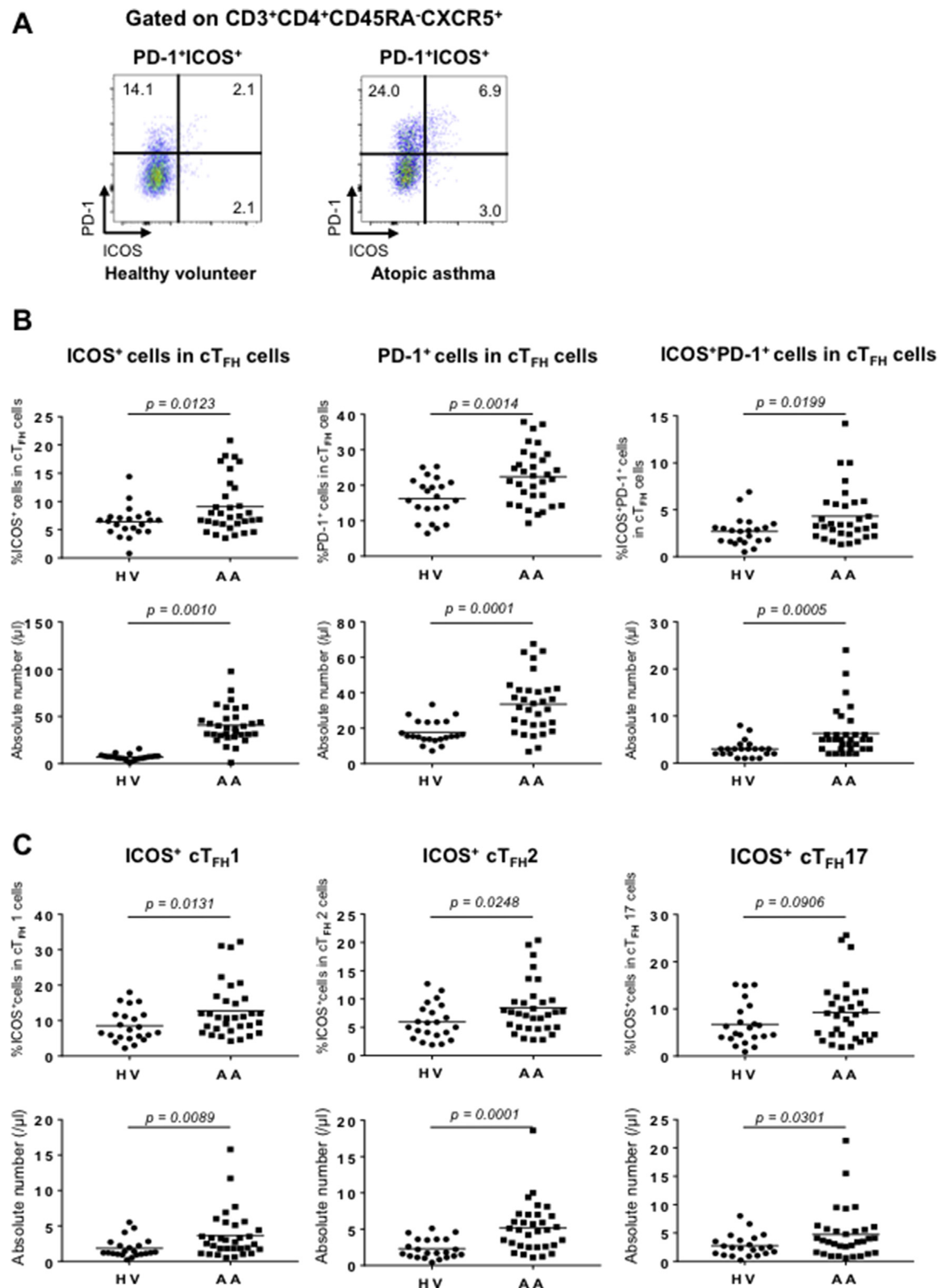

Fig. 2. Percentages and absolute numbers of ICOS+ and/or PD-1+cT$_{FH}$ cells and their subsets of patients with AA and HV. **(A)** Representative FACS profiles for ICOS+ and/or PD-1+ T$_{FH}$ cells subsets in peripheral blood from patients with AA and HV are shown. Numbers indicate percentage of cells in the gate. **(B)** Percentages and absolute numbers of ICOS+ and/or PD-1+cT$_{FH}$ cells. **(C)** Percentages and absolute numbers of ICOS+cT$_{FH}$ subset cells.

0.0369, Fig. 4A). Moreover, the plasma levels of CXCL13 in AA patients were significantly correlated to % cT$_{FH}$2 cell/% cBreg cell (cT$_{FH}$2/cBreg) ratio ($r = 0.3993$, $p = 0.0433$, Fig. 4B). If this ratio is assumed to be an immune balance maker between T$_{FH}$2 cells under the condition of an accelerated allergic response and Breg cells having immune suppressive functions, these results indicate that plasma CXCL13 level as a marker is affected by the T$_{FH}$2 cell and Breg cell balance.

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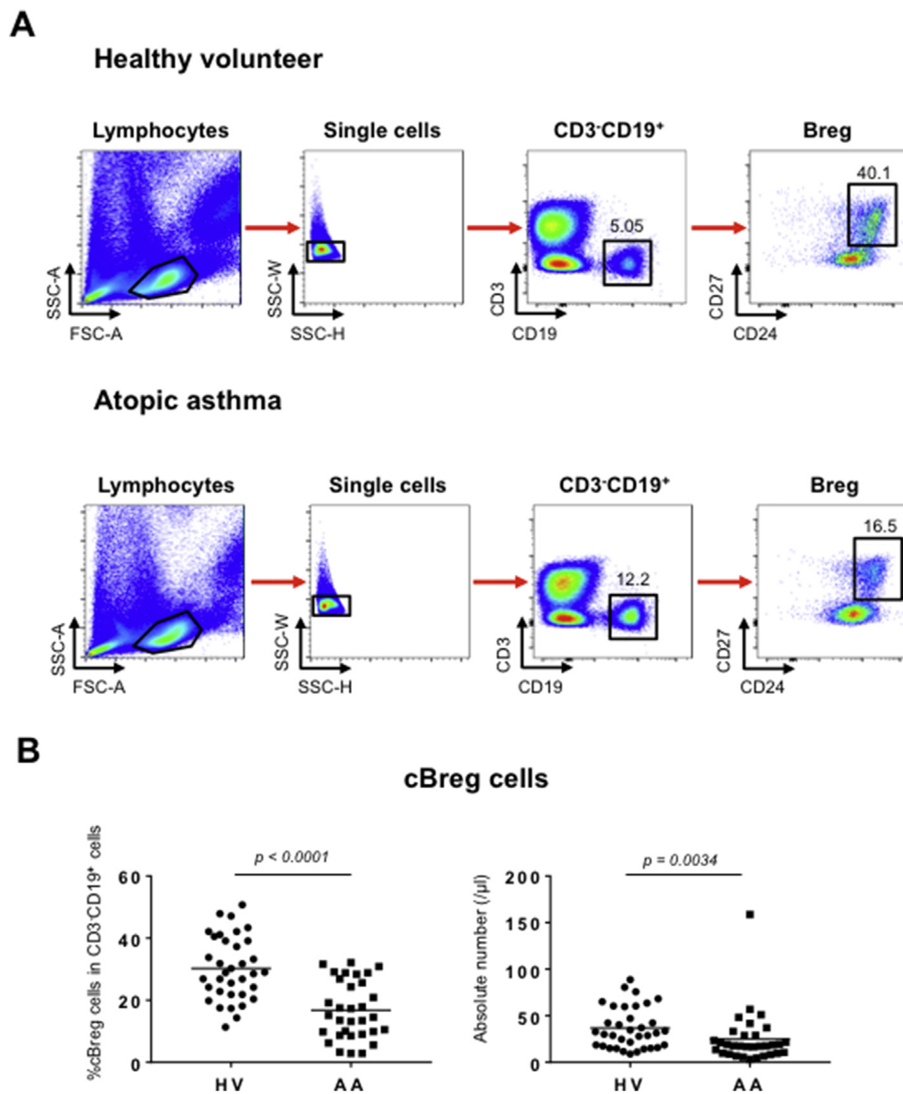

Fig. 3. Percentage and absolute number of Breg cells in peripheral blood from patients with AA and HV. **(A)** Gating strategy for Breg cells in peripheral blood from patients with AA and HV. Representative FACS profiles for Breg cells in peripheral blood from patients with AA and HV are shown. Numbers indicate percentage of cells in the gate. **(B)** Percentage and absolute numbers of cBreg cells in patients with AA and HV.

Relationships between cT_{FH} cells and clinical biomarkers

The results of this study suggested that T_{FH} 2 cell skewing with activated T_H2 response plays an important role in allergic airway disease. Therefore, we investigated the relationship between T_{FH} 2 cells and clinical markers. The serum total IgE level (based on T_H2 response) was correlated with percentage of $cT_{FH}2$ cells (IgE vs % $cT_{FH}2$ cells: $r = 0.4088$, $p = 0.0202$, Fig. 5A). And then, the absolute number of $cT_{FH}2$ and $ICOS^+$ $cT_{FH}2$ cells also showed significant correlation to the level of FeNO, which is a biomarker of airway eosinophilic inflammation in pretreated non-smoker patients with AA (n = 14, FeNO vs $cT_{FH}2$ cells: $r = 0.5474$, $p = 0.0427$ and FeNO vs $ICOS^+$ $cT_{FH}2$ cells: $r = 0.5783$, $p = 0.0303$, Fig. 5B). A possible reason for this is that IL-4 produced by activated $T_{FH}2$ cells may indirectly affect airway eosinophilic inflammation. Forthermore, $cT_{FH}2$ cells exhibited the good correlation with the numbers of eosinophil in examined AA patients (eosinophil vs cT_{FH} 2 cells: $r = 0.4915$, $p = 0.0043$, Fig. 5C). The results suggest that $cT_{FH}2$ cell skewing may play a role in IgE production and subsequently cause eosinophilic inflammation in the allergic airway.

Change in cT_{FH} cells and their subsets after asthma treatment

All of the patients with AA were treated by ICS ± LABA inhalation, LTRA (and other drugs) according to the GINA guideline. The percentage of cT_{FH} cells, subsets of cT_{FH} cells and cBreg cells were determined in ten patients after treatments. These patients were improved or remised by these treatments, especially by ICS administration to airway. The ACQ scores and the FeNO levels were significantly decreased, and the values of $\%FEV_{1.0}$ were increased (Supplementary Table 1).

The percentage of cT_{FH} cells was significantly increased after treatment, but there was no difference in the percentage of cBreg cells (data not shown) before and after treatment (Fig. 6A). The percentage of $cT_{FH}2$ cells was decreased ($cT_{FH}2$ cells before treatment: 41.4 ± 4.4%; $cT_{FH}2$ cells after treatment: 37.8 ± 5.6%, $p = 0.0284$) and the percentage of $cT_{FH}1$ cells was conversely increased ($cT_{FH}1$ cells before treatment: 15.9 ± 6.8%; $cT_{FH}1$ cells after treatment: 19.9 ± 7.8%, $p = 0.0190$), and the ratio of $\%cT_{FH}2$ cells to % $cT_{FH}1$ cells was therefore significantly decreased after treatment ($cT_{FH}2/cT_{FH}1$ ratio before treatment: 3.5 ± 2.6; $cT_{FH}2/cT_{FH}1$ ratio

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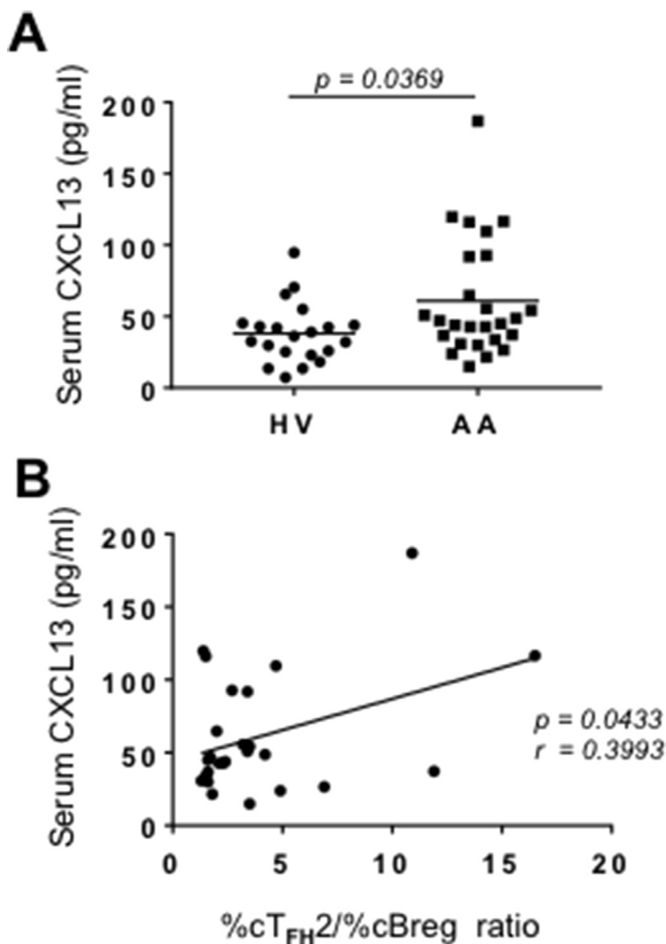

Fig. 4. Plasma CXCL13 levels in patients with AA and HV. **(A)** Plasma CXCL13 levels in patients with AA and HV. **(B)** Correlation between plasma CXCL13 level in patients with AA and ratio of %cT_{FH}2 cells/%cBreg cells.

after treatment: 2.7 ± 2.8, $p = 0.0127$) (Fig. 6B, C). These results indicated that cT_{FH}2 skewing was improved.

Next, we examined cT_{FH} cells expressing ICOS and/or PD-1 (Fig. 7A). The percentage of $ICOS^+$ cT_{FH} cells was decreased after treatment, but there was no difference in the percentage of PD-1^+ cT_{FH} cells before and after treatment ($ICOS^+$ cT_{FH} cells before treatment: 10.9 ± 6.2%; $ICOS^+$ cT_{FH} cells after treatment: 4.5 ± 2.0%, $p = 0.0069$ and PD-1^+ cT_{FH} cells before treatment: 10.6 ± 8.8%; $PD1^+$ cT_{FH} cells after treatment: 11.2 ± 7.0%, $p = 0.7643$ and $ICOS^+$PD-1^+ cT_{FH} cells before treatment: 5.2 ± 3.1%; $ICOS^+$ PD-1^+ cT_{FH} cells after treatment: 3.2 ± 3.8%, $p = 0.0367$, Fig. 7B). The expression of ICOS was greatly decreased in all subsets of $ICOS^+$ cT_{FH} cells including c-T_{FH}17 cells ($ICOS^+$ cT_{FH}2 cells before treatment: 9.9 ± 6.2%; $ICOS^+$ cT_{FH}2 cells after treatment: 4.1 ± 2.5%, $p = 0.0117$ and $ICOS^+$ cT_{FH}1 cells before treatment: 16.5 ± 9.2%; $ICOS^+$ cT_{FH}1 cells after treatment: 5.4 ± 3.0%, $p = 0.0077$ and $ICOS^+$ cT_{FH}17 cells before treatment: 12.5 ± 8.1%; $ICOS^+$ cT_{FH}17 cells after treatment: 3.5 ± 1.9%, $p = 0.0077$, Fig. 7C). The expression levels in all subsets of PD-1^+ cT_{FH} cells, however, were not different after treatment (Supplementary Fig. 3). The expression of ICOS on cT_{FH} cells may be related to the activity in the T_H2-dominant airway inflammation in patients with AA. The results indicate that the T_H2-dominant and activated T_{FH} reaction in the asthmatic airway with its lymphatic tissues was improved by the local ICS treatment and that this improvement was related to the circulating profile of cT_{FH} cells. It is interesting that the change of T_H2-dominant airway inflammation is result in the change in profile of systemic T_{FH} cell circulation. cT_{FH}2 and $ICOS^+$ cT_{FH} cells may be a useful marker of the activity in T_H2 and T_{FH}2-dominant airway inflammation with BALT.

Discussion

In this study, we examined cT_{FH} cells and their subsets in patients with AA in order to clarify the role of T_{FH} cells based on T_H2 response. We found that the percentage and absolute number of cT_{FH} cells and the expression levels of ICOS and PD-1 on cT_{FH} cells were increased in the patients with AA. We also found that the percentage and absolute number of cT_{FH}2 cells were increased within the cT_{FH} cell subset (T_{FH}2 skewing) and that the percentage of T_{FH}2 cells was correlated with serum IgE level. The levels of plasma CXCL13, which reflect GC activity, were increased in patients with AA compared with the levels in HV. On the other hand, the percentage and absolute number of cBreg cells were decreased in patients with AA compared with that in HV. Interestingly, the plasma level of CXCL13 in patients with AA was correlated with the %cT_{FH2}/%cBreg ratio. We also examined cT_{FH} cells after treatment with ICS (±LABA ± LTRA), and we found that cT_{FH}2 skewing was improved and the percentage of $ICOS^+$ cT_{FH} cells was decreased. Thus, cT_{FH}2 skewing toward normalization and decrease in activated cT_{FH} cells, were related to local improvement of airway inflammation based on T_H2 reaction and clinical conditions.

The role of T_{FH} cells is primarily helping B cells undergo CSR and affinity maturation through GC reactions in lymphoid tissues. In pathogenic conditions regarding infection, autoimmune disorder, immune response to cancer, T_{FH} cells play an important role through induction of specific antibody production from B cells and/or plasma cells.[26] Recently, there have been some reports about the cT_{FH} cells in allergic diseases of human. We previously showed the cT_{FH}2 cell skewing in AR patients with or without asthma.[10] Szabó *et al.* reported the expansion of $ICOS^+$PD-1^+cT_{FH} cells in patient with childhood atopic dermatitis.[27]

The origin of human cT_{FH} subset cells remains unknown. In addition to the report by Locci *et al.*,[11] He *et al.* showed that human cPD-$1^+$$CCR7^{lo}$ T_{FH} cells rapidly differentiate into $ICOS^+$PD-1^+mature T_{FH} cells upon antigen encounter in order to promote responses of antibodies and that they provide a biomarker to monitor protective antibody responses during infection or vaccination and pathogenic antibody responses in autoimmune diseases.[28] Moreover, in chronic SIV infection of rhesus macaques, Velu *et al.* found that CXCR3(Th1)-T_{FH} cells were dominant, that there was no change in CCR4 (Th2)-T_{FH} cells and that CCR6 (Th17)-T_{FH} cells were decreased compared to those in SIV negative rhesus macaques in GC-T_{FH} cells.[29] The results obviously indicated the existence of a subset of T_{FH} cells in GCs like a subset of cT_{FH} cells in human. Thus, cT_{FH} cells and their subsets including $ICOS^+$ and/or PD-1^+ T_{FH} cells may reciprocally influx and efflux from the follicles including GC sites of affected lymphoid tissues to circulation.

We showed that percentage of cT_{FH}2 cells was correlated with serum IgE levels in patients with AA. There have been few evidences between T_{FH} cells and Ig production including IgE in human inflamed lesions. Kamekura *et al.*[23] reported infiltration of T_{FH} cells with high expression levels of ICOS and PD-1 in submandibular glands of patients with IgG4-related disease (IgG4-RD). These T_{FH} cells also had a high expression level of Bcl-6 and helped B cells to produce IgG4. Regarding cT_{FH} cells in patients with AA, Gong *et al.* reported that there was an increase in IL-4^+IL-21^+ cT_{FH} cells and that IgE production was accelerated in co-culture with $CD19^+$ B cells.[30] On the other hand, other than the report by Ballesteros-Tato *et al.*, Kobayashi *et al.*,[31] found that T_{FH} cells in OVA -sensitized mice supported the sustained production of IgE antibody *in vivo* in the absence of other T cell lineages, even when T_H2 cell function was

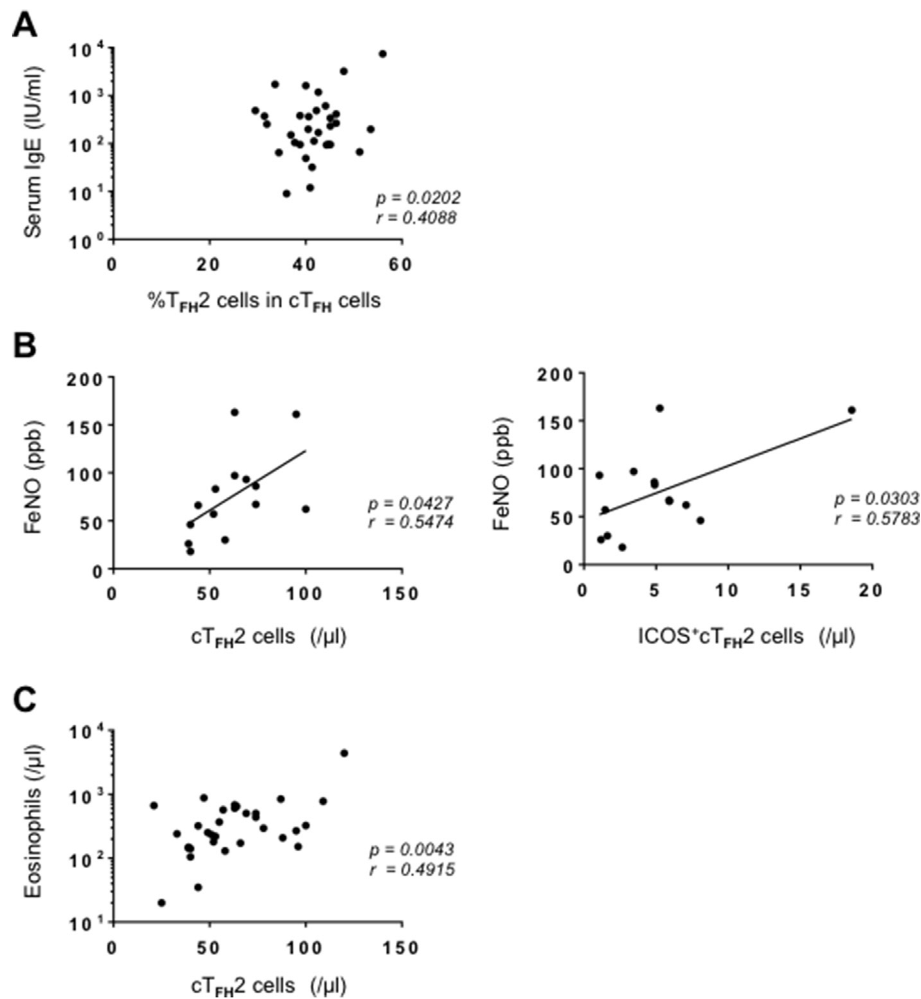

Fig. 5. Relationships between $cT_{FH}2$ cells and clinical biomarkers. **(A)** Correlation between $\%cT_{FH}2$ cells and serum IgE. **(B)** Correlation between the number of $cT_{FH}2$ cells and FeNO level, and between the number of $ICOS^{+}cT_{FH}2$ cells and FeNO level in patient with untreated and non-smoker patients with AA. **(C)** Corprelation between the number of $cT_{FH}2$ cells and blood eosinophiles.

severely compromised. Conditional deficiency of the master regulator *Bcl6* in $CD4^{+}$T cells resulted in a marked reduction in T_{FH} cells and IgE antibody levels. We showed the $cT_{FH}2$ cell skewing and correlation between the $T_{FH}2$ cells and serum levels of IgE, it is speculated that $T_{FH}2$ cells stimulated with allergic antigens were activated and proliferated, accelerating IgE production from mature B cells and PCs in allergic lung lymphoid regions, and some of these T_{FH} cells flowed into circulation.

The mechanism of human IgE production has not been fully elucidated. Some mouse models have shown that IgE memory B cells from GCs transiently disappear, except for IgE-producing plasma cells, upon antigen recall, and IgG1 memory B cells convert IgE memory B cells through CSR.[32] On the other hand, it was reported that there was local IgE production in bronchial and nasal biopsy tissues from patients with asthma and AR.[33,34] As a result of CSR and SHM from IgM and IgG in these tissues, IgE is produced from B cells. However, it is unclear whether these results are responsible for T_{FH} cells. Zang Y—N *et al.* reported that IL-$4^{+}CXCR5^{+}CD4^{+}$ T_{FH} cell count correlated with local IgE production in a patient with chronic rhinosinusitis with a polyp. IL-4^{+}Bcl-$6^{+}CD4^{+}$ T_{FH} cells were identified in the ectopic lymphoid structure in the polyp, and it was evident that T_{FH} cells were involved in local IgE production in the nasal polyp.[35] It has been ethically difficult to perform invasive biopsy of the airway with lymphoid tissues in patients with AA, therefore, we need to elucidate the pathophysiology in T_{FH} cells of inflamed lesions using the BALF and induced sputa in patients with AA.

As shown in Figure 3, cBreg cells in patients with AA were greatly decreased compared to those in HV. Although the mechanism of the decrease in Breg cells was not determined, Van der Vlugt *et al.* showed that Breg cell was decreased and caused dysfunction to lipopolysaccharide ex vivo in patients with AA.[36] Anchour *et al.* found that human IL-10 and TGF-β-producing Breg cells controlled T_{FH} cell maturation, increased follicular regulatory T (Tfr) cells, and inhibited T_{FH} cell-mediated antibody secretion.[37] These findings indicated that the cause of accelerated T_{FH} response in patients with AA may be partially due to the substantial decline and dysfunction of Breg cells. These facts indicate that an equilibrium between T_{FH} cells and Breg cells is very important, and therefor, $\%cT_{FH}2/\%cBreg$ ratio may be a marker in disease activity of IgE-mediated allergic diseases. This index was already used as significant correlations with clinical data in ref. 10.

Except for HIV disease,[20] there are some reports in autoimmune diseases that there were elevated levels of CXCL13 in plasma or serum and CXCL13 level showed the disease activity.[38,39] Thus, our result showing increased CXCL13 levels in patients with AA are considered that T_{FH} cells and B cells were infiltrated and interacted

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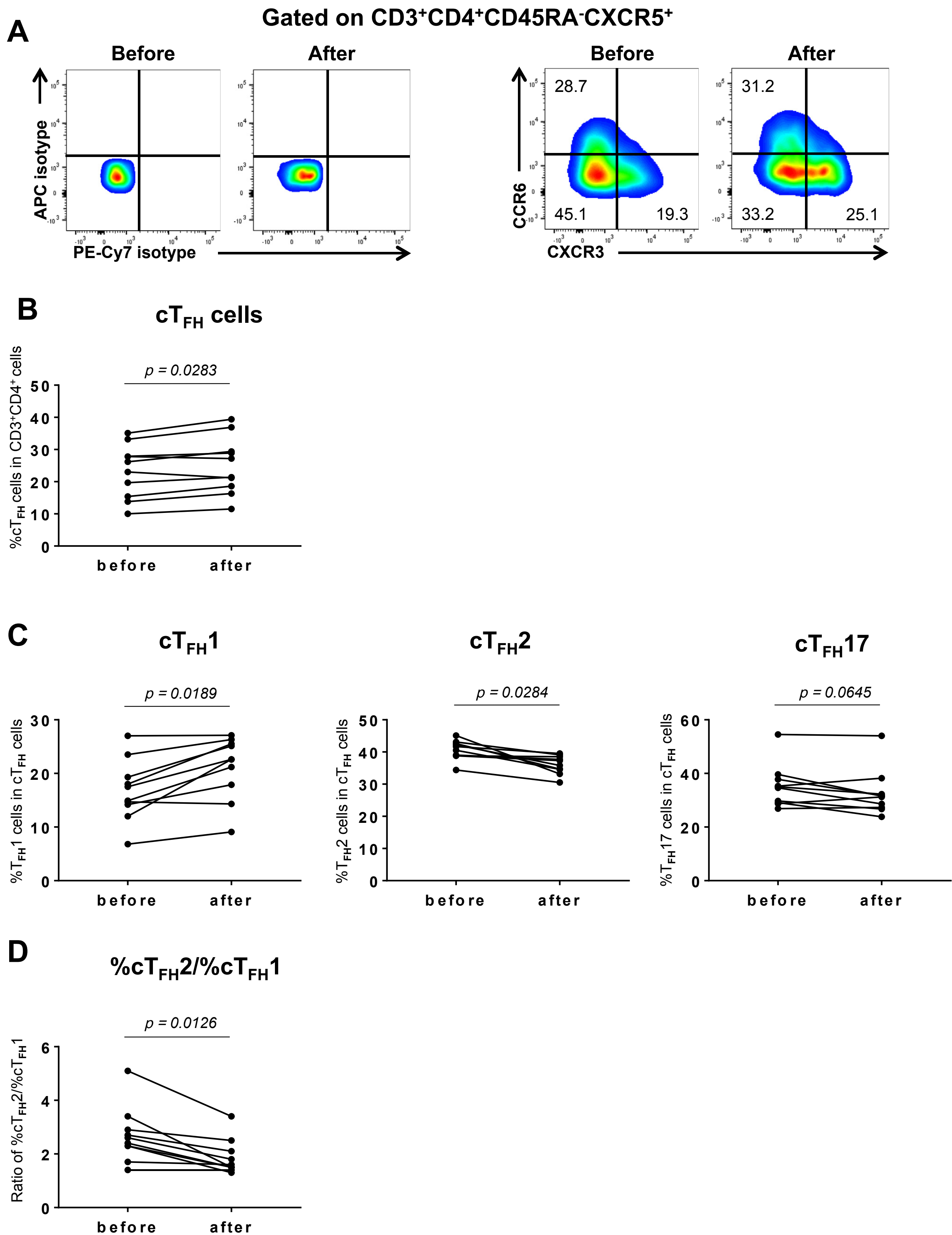

Fig. 6. Change in cT_{FH} cells and their subsets after treatment. **(A)** Representative FACS profile indicating circulating $CD3^+CD4^+CD45RA^-CXCR5^+$ (cT_{FH}) and their subsets cells before and after treatment. **(B)** Change in cT_{FH} cells before and after treatment. **(C)** Change in cT_{FH} subset cells before and after treatment. **(D)** Change in $\%cT_{FH}2/\%cT_{FH}1$ subset cells before and after treatment.

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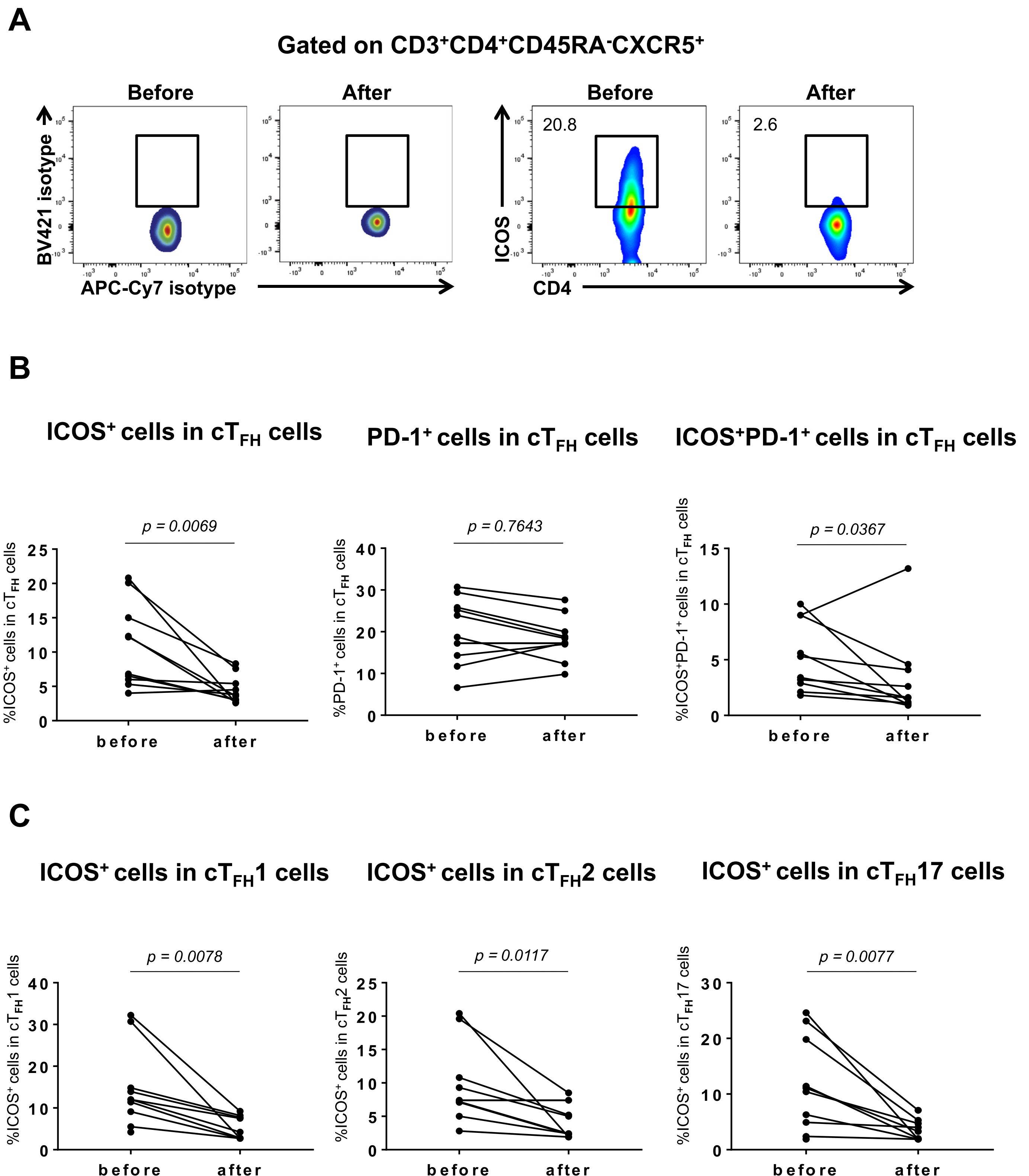

Fig. 7. Change in $ICOS^{+}cT_{FH}$ cells and their subsets after treatment. **(A)** Representative FACS profile indicating $CD3^{+}CD4^{+}CD45RA^{-}CXCR5^{+}ICOS^{+}$cells before and after treatment. **(B)** Change in $ICOS^{+}$ and/or $PD\text{-}1^{+}cT_{FH}$ cells before and after treatment. **(C)** Change in $ICOS^{+}cT_{FH}$ subsets cells before and after treatment.

in the B cell follicles of airways with BALT. It is reasonable that plasma CXCL13 levels correlated well with the ratio of $\%cT_{FH}2/\%$ cBreg in patients with AA, and it is possible that plasma CXCL13 level is also a biomarker of active T_{FH} cells in $T_{H}2$-mediated allergic diseases. Our results are the first results for plasma CXCL13 levels in patients with AA.

In our study, we found that $cT_{FH}2$ skewing within cT_{FH} cells was improved and that ICOS expression declined with ICS

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administration. It has been reported that immunological disorder involving T_{FH} cells was improved by systemic administration of a corticosteroid in patients with IgG4-RD.[23,40] However, ICS administration in our patients with AA was only by local inhalation to the inflamed airway, and there would have been little corticosteroid influx into blood stream and thus little systemic effect of the corticosteroid. Nevertheless, local administration of ICS ameliorated airway T_H2 inflammation including T_{FH} cells with improved symptoms and clinical biomarkers, interestingly, this result caused change profile of cT_{FH} cell. Measurement of $cT_{FH}2$ and $ICOS^+$ cells may be useful as markers of allergic responses in patients with AA. Indeed, blockade of ICOS signaling after allergic airway disease establishment successfully depleted T_{FH} cells, however, did not affect the differentiation of other $CD4^+$ T cell subsets in Balb/c mice.[41]

Our study showed that ICOS expression, but not PD-1 expression, on c-T_{FH} cells was down-regulated by treatment with ICS in patients with AA. Upon antigen encounter, T_{FH} cells express ICOS and Bcl-6, and ICOS promotes the development and functions of T_{FH} cells into activated state.[41,42] On the other hand, Shi *et al.* showed that PD-1 inhibits the recruitment of T_{FH} cells to follicles, promotes T_{FH} cell concentration through CXCR3 expression and the stringency of GC affinity selection.[43] Thus, there is a functional difference between ICOS and PD-1 in T_{FH} cells, but they are essential for the maintenance of T_{FH} cell function. The different action between ICOS and PD-1 expression by ICS treatment have been not determined. This will be investigated in a future study; however, PD-1 ligand and PD-1-related transcription factors of T_{FH} cells may not be influenced by corticosteroid action.

In conclusion, we demonstrated that the plasma CXCL13 levels were elevated and were significantly correlated with the %$cT_{FH}2$/%cBreg ratio in patients with AA. Local treatment of the allergic airway by administration of ICS results in improvement of $cT_{FH}2$ skewing and decline in $ICOS^+$ cT_{FH} cells. cT_{FH} cells and CXCL13 in patients with AA may become useful biomarkers and therapeutic targets in the future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2019.08.008.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

MS, KS, and RK equally designed the study, performed the experiments and discussed the experiment results. HT contributed to ELISA measurement of CXCL13. HY, II, YA and HN contributed to healthy volunteer collection. HC, HT and SI contributed to advice for this study.

References

1. Bel EH. Clinical phenotypes of asthma. *Curr Opin Pulm Med* 2004;**10**:44–50.
2. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;**181**:315–23.
3. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;**18**:716–25.
4. Fahy JV. Type 2 inflammation in asthma - present in most, absent in many. *Nat Rev Immunol* 2015;**5**:57–65.
5. Wynn TA. Type 2 cytokines: mechanisms and therapeutic strategies. *Nat Rev Immunol* 2015;**15**:271–82.
6. Crotty S. T follicular helper cell differentiation, function, and roles in disease. *Immunity* 2014;**41**:529–42.
7. Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, et al. Human blood $CXCR5^+CD4^+$ T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* 2011;**34**:108–21.
8. Ueno H, Banchereau J, Vinuesa GC. Pathophysiology of T follicular helper cells in humans and mice. *Nat Immunol* 2015;**16**:142–52.
9. Ballesteros-Tato A, Randall TD, Lund FE, Spolski R, Leonard WJ, León B. T follicular helper cell plasticity shapes pathogenic T helper 2 cell-mediated immunity to inhaled house dust mite. *Immunity* 2018;**44**:259–73.
10. Kamekura R, Shigehara K, Miyajima S, Jitsukawa S, Kawata K, Yamashita K, et al. Alteration of circulating type 2 follicular helper T cells and regulatory B cells underlies the comorbid association of allergic rhinitis with bronchial asthma. *Clin Immunol* 2015;**158**:204–11.
11. Locci M, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn C, et al. Human circulating $PD\text{-}1^+CXCR3^-CXCR5^+$ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. *Immunity* 2013;**39**:758–69.
12. Braza F, Chesne J, Castagnet C, Magnan A, Brouard S. Regulatory functions of B cells in allergic diseases. *Allergy* 2014;**69**:1454–63.
13. Palomares O, Martin-Fontecha M, Lauener R, Traidl-Hoffmann C, Cavkaytar O, Akdis M, et al. Regulatory T cells and immune regulation of allergic diseases: roles of IL-10 and TGF-β. *Genes Immun* 2014;**15**:511–20.
14. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J Clin Invest* 2008;**118**:3420–30.
15. Hartung HP, Kieseier BC. Atacicept: targeting B cells in multiple sclerosis. *Ther Adv Neurol Disord* 2010;**3**:205–16.
16. Mauri C, Gray D, Mushtaq N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med* 2003;**197**:489–501.
17. Goetz M, Atreya R, Ghalibafian M, Galle PR, Neurath MF. Exacerbation of ulcerative colitis after rituximab salvage therapy. *Inflamm Bowel Dis* 2007;**13**: 1365–8.
18. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. *Immunity* 2015;**42**:607–12.
19. Iwata Y, Matsushita T, Horikawa T, DiLillo DJ, Yanaba K, Venturi GM, et al. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 2011;**17**:530–41.
20. Havenar-Daughton C, Lindqvist M, Heit A, Wu JE, Reiss Sm, Kendric K, et al. CXCL13 is a plasma biomarker of germinal center activity. *PNAS* 2016;**113**: 2702–7.
21. Kohno S, Ishida T, Uchida Y, Kishimoto H, Sasaki H, Shioya T, et al. Committee for the Japanese respiratory society guidelines for management of cough. The Japanese respiratory society guidelines for management of cough. *Respirology* 2006;**11**(Suppl 4):S135–86.
22. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;**14**: 902–7.
23. Kamekura R, Takano K, Yamamoto M, Kawata K, Shigehara K, Jitsukawa S, et al. Cutting edge: a critical role of lesional T follicular helper cells in the pathogenesis of IgG4-related disease. *J Immunol* 2017;**199**:2624–9.
24. Baay-Guzman GJ, Huerta-Yepez S, Vega MI, Aguilar-Leon D, Campillos M, Blake J, et al. Role of CXCL13 in asthma : novel therapeutic target. *Chest* 2012;**141**:886–94.
25. Al-Kufaidy R, Vazquez-Tello A, BaHammam AS, Al-Muhsen S, Hamid S, Halwani R. IL-17 enhances the migration of B cells during asthma by inducing CXCL13 chemokine production in structural lung cells. *J Allergy Clin Immunol* 2016;**14**:696–9.
26. Vinuesa CG, Linterman MA, Yu D, MacLennan CM. Follicular helper T cells. *Annu Rev Immunol* 2016;**34**:335–68.
27. Szabó K, Gáspár K, Dajnoki Z, Papp G, Fábos B, Szegedi A, et al. Expansion of circulating follicular T helper cells associates with disease severity in childhood atopic dermatitis. *Immunol Lett* 2017;**189**:101–8.
28. He J, Tsai LM, Leong YA, Hu X, Ma C S, Chevalier N, et al. Circulating precursor $CCR7^{lo}PD\text{-}1^{hi}$ $CXCR5^+$ $CD4^+$ T cell indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity* 2013;**39**:770–81.
29. Velu V, Mylvaganam G, Ibegbu C, Amara RR. Tfh1 cells in germinal centers during chronic HIV/SIV infection. *Front Immunol* 2018;**9**:1272.
30. Gong F, Zhu HY, Zhu J, Dong QJ, Huang X, Jiang DJ. Circulating $CXCR5^+CD4^+$ T cells participate in the IgE accumulating in allergic asthma. *Immunol Lett* 2018;**197**:9–14.

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31. Kobayashi T, Iijima K, Dent AL, Kita H. Follicular helper T (Tfh) cells mediate IgE antibody response to airborne allergens. *J Allergy Clin Immunol* 2017;**139**:300–13.
32. Wu LC, Zarrin AA. The production and regulation of IgE by the immune system. *Nat Rev Immunol* 2014;**14**:247–59.
33. Takhar P, Corrigan CJ, Smurthwaite L, O'Connor BJ, Durham SR, Lee TH, et al. Class switch recombination to IgE in the bronchial mucosa of atopic and nonatopic patients with asthma. *J Allergy Clin Immunol* 2007;**119**:213–8.
34. Coker HA, Harries HE, Banfield GK, Carr VA, Durham SR, Chevretton E, al at. Biased use of V_H IgE-positive B cells in the nasal mucosa in allergic rhinitis. *J Allergy Clin Immunol* 2005;**116**:445–52.
35. Zhang YN, Song J, Wang H, Wang H, Zeng M, Zhai GT, et al. Nasal IL-4^+CXCR5$^+$CD4$^+$ T follicular helper cell counts correlate with local IgE production in eoshinophilic nasal polyps. *J Allegy Clin Immunol* 2016;**137**:462–73.
36. van der Vlugt LE, Mlejnek E, Ozir-Fazalalikhan A, Janssen Bonas M, Dijksman TR, Labuda LA, et al. CD24^{hi}CD27$^+$ B cells from patients with allergic asthma have impaired regulatory activity in response to lipopolysaccharide. *Clin Exp Allergy* 2013;**44**:517–28.
37. Achour A, Simon Q, Mohr A, Seite JF, Youinou P, Bendaoud B, et al. Human regulatory B cells control the the T_{FH} cell response. *J Allergy Clin Immunol* 2017;**140**:215–22.
38. Lee HT, Shiao YM, Wu TH, Chen WS, Hsu YH, Tsai SF, et al. Serum BLC/CXCL13 concentrations and renal expression of CXCL13/CXCR5 in patients with systemic lupus erythematosus and lupus nephritis. *J Rheumatol* 2010;**37**:45–52.
39. Klimatcheva E, Pandina T, Reilly C, Torno S, Bussler H, Scrivens M, et al. CXCL13 antibody for the treatment of autoimmune disorders. *BMC Immunol* 2015;**16**:6.
40. Akiyama M, Yasuoka H, Yamaoka K, Suzuki K, Kaneko Y, Kondo H, et al. Enhanced IgG4 production by follicular helper 2 T cells and the involvement of follicular helper 1 T cells in the pathogenesis of IgG4-related disease. *Arthritis Res Ther* 2016;**18**:167–80.
41. Uwadiae FI, Pyle CJ, Walker SA, Lloyd CM, Harker JA. Targeting the ICOS/ICOS-L pathway in a mpuse of established allergic asthma disrupts T follicular helper cell response and ameliorates disease. *Allergy* 2019;**74**:650–62.
42. He J, Tsai LM, Leong YA, Hu X, Ma C S, Chevalier N, et al. Circulating precursor CCR7loPD-1^{hi} CXCR5$^+$ CD4$^+$ T cells indicate Tfh cell activity and promote antibody responses upon antigen re-exposure. *Immunity* 2013;**39**:770–81.
43. Shi J, Hou S, Fang Q, Liu X, Liu X, Qi H. PD-1 controls follicular T helper cell positioning and function. *Immunity* 2018;**49**:264–74.

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