Journal of Asthma and Allergy

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

Changes in and Potential Mechanisms of Circulating IgA+CD27-Class-Switched Memory B Cells in Patients With Allergic Rhinitis

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Background: The role of memory B cells and their subgroups in allergic rhinitis (AR) and allergen immunotherapy (AIT) remains unclear. This study aimed to investigate the characteristics of memory B cells in the circulation of patients with AR and those undergoing AIT, as well as their clinical significance.

Methods: This study involved a cohort comprising 32 healthy control subjects, 39 individuals diagnosed with AR, and 31 AR patients who had received AIT for over one year. Visual analog scale (VAS) scores were used for symptom assessment, and the serum concentrations of immunoglobulins and cytokines were quantified. This study evaluated alterations in the proportions of peripheral blood memory B cells and their subpopulations, plasma cells, and various T-cell subsets across the three participant groups.

Results: The proportion of IgA+CD27- class-switched memory B cells in the AR group significantly decreased compared to the control group, but significantly increased following AIT ($P < 0.05$). In AR patients, circulating IgA+CD27- class-switched memory B cells were significantly positively correlated with Treg cells, IL-10, and IL-4 and significantly negatively correlated with IFN-γ, total IgE, sIgE, and VAS scores ($P < 0.05$). After AIT, the number of circulating IgA+CD27- class-switched memory B cells in AR patients was significantly positively correlated with the number of Treg cells and IL-10 and significantly negatively correlated with the VAS score ($P < 0.05$).

Conclusion: The IgA+CD27- class-switched memory cell subset in human peripheral blood may serve as a potential biomarker for evaluating AR symptoms and treatment efficacy. Its mechanism may be associated with interactions between T and B cells.

Keywords: memory B cell, IgA memory B cell, allergic rhinitis

Introduction

Memory B cells are enduring, antigen-specific B cells that develop from naive B cells during the immune response or infection process. Upon initial exposure to an antigen, circulating naive B cells migrate to the lymphoid follicles in the spleen and lymph nodes, where they proliferate and differentiate into germinal center B cells, resulting in the formation of new germinal centers. Following processes such as immunoglobulin class switching, somatic hypermutation, and affinity maturation, memory B cells generated in these germinal centers enter the peripheral blood circulation and migrate to other lymphoid tissues, primarily the bone marrow, to establish long-term immune memory. Upon re-exposure to the same antigen, memory B cells rapidly proliferate and differentiate into plasma cells that secrete antibodies, producing a substantial quantity of specific antibodies to mediate the secondary immune response. Simultaneously, they form new germinal centers to renew immune memory.[1]

Received: 19 October 2024
Accepted: 8 January 2025
Published: 22 January 2025

Based on the expression of IgD, CD19+CD38dim memory B cells can be classified into nonswitched (IgD+) memory B cells and class-switched memory B cells (IgD-). Class-switched memory B cells can be further categorized into germinal center-derived subtypes (IgA+/IgE+/IgG+, CD27+) and nongerminal center-derived subtypes (IgA+/IgE+/IgG+, CD27-) depending on the expression of CD27 and membrane immunoglobulins (mIg), such as mIgA, mIgE, and mIgG.[2–4]

Allergic rhinitis (AR) is characterized as a hypersensitive response mediated by specific immunoglobulin E (sIgE), which occurs following sensitization to an allergen and subsequent exposure to the same allergen. Previous studies have demonstrated that CD19+ B cells are closely associated with the production of sIgE.[5,6] Recent research has indicated that the quantity of memory B cells and class switching are linked to the onset and progression of AR.[7] However, the specific mechanisms by which memory B cells contribute to the development and progression of AR have not yet been elucidated. Furthermore, allergen immunotherapy (AIT) can induce immune tolerance to allergens and is currently recognized as the most prevalent and effective therapeutic approach for AR in clinical practice.[8] Nevertheless, the understanding of the immunological mechanisms and efficacy biomarkers associated with AIT in the treatment of AR is limited. Recent studies have suggested that AIT influences the quantity and class switching of memory B cells in AR patients;[9,10] however, the underlying mechanisms responsible for these alterations remain incompletely understood.

This study aimed to compare healthy controls, patients with AR, and AR patients undergoing AIT. Indicators closely associated with the manifestation of AR in the peripheral blood of AR patients were analyzed. Furthermore, we investigated the role and potential mechanisms of memory B cells and their subtypes in the pathogenesis of AR, as well as the effects of AIT.

Methods

Subjects

The Ethics Committee of Renmin Hospital of Wuhan University approved this research protocol (WDRY2023-K143). Written informed consents were obtained from all participants, in accordance with the Declaration of Helsinki. A total of 102 subjects participated in this study, which was conducted from February 2023 to January 2024 at Renmin Hospital of Wuhan University. The cohort comprised 32 healthy controls (HC), 39 patients with untreated AR (AR), and 31 patients who had undergone AIT for over one year (AIT). Importantly, none of the patients had received immunosuppressive treatments, including inhaled corticosteroids, within four weeks prior to their enrollment in the study. The diagnosis of AR was established according to the guidelines on Allergic Rhinitis and Its Impact on Asthma (ARIA) guidelines,[11] and symptom severity was evaluated using a visual analog scale (VAS). The inclusion criteria for the AR group were as follows: 1. Symptoms: at least two of the following symptoms must be present: sneezing, runny nose, nasal itching, nasal congestion, etc., occurring for more than one hour daily or cumulatively, or accompanied by ocular symptoms such as itching, tearing, and redness; 2. For physical signs, a large number of clear water-like secretions observed in the nasal cavity, and the nasal mucosa was pale and swollen; 3. A serum-specific sIgE test ≥0.35 IU/mL; 4. Total IgE ≥30 IU/mL; 5. Absence of a history of malignant tumors or systemic diseases. The inclusion criterion for the AIT group was that participants be diagnosed with AR and have received regular subcutaneous injections of dust mite extract (Novo-Helisen-Depot, Merck & Co. Inc., Germany) according to medical prescription. All individuals in the AIT group had been undergoing AIT for a minimum of one year in the Department of Otorhinolaryngology, Head and Neck Surgery at Renmin Hospital of Wuhan University. Clinical information pertaining to each patient group is detailed in Table 1. The symptom scores of patients with different sIgE grades are shown in Table 2.

VAS

The VAS, based on the ARIA guidelines,[11] was used to evaluate the symptoms of the patients. Participants provided self-reports detailing their nasal allergy symptoms, which included sneezing, rhinorrhea, nasal pruritus, and nasal obstruction, in addition to ocular allergy symptoms, asthma-related allergic manifestations, and systemic allergic symptoms (inhalant allergies, food allergies, drug allergies, and contact allergies).[12] These symptoms were evaluated according to their severity. The total VAS score was calculated by summing these individual ratings. Additionally, a correlation analysis

Table 1 Demographic Data of the Human Subjects

Variables	HC	AR	AIT*
Sex (M/F)	17/15	18/21	14/17
Age (years), Mean (Median)± SD	30.3(31)±9.7	29.1(29)±11.2	32.1(32)±12.4
Allergen specific IgE (Grade)			
Mean ± SD	0	3.3±1.7	3.4±1.6
Median	0	3	3
Total IgE (IU/mL)			
Mean ± SD	0	716.1±758.9	867.7±594.9
Median	0	439	679.5
Visual analog scale, Mean (Median)± SD			
Total	0	17.2(17)±7.4	8.9(8)±4.0
Systemic allergy symptom scores	0	6.6(7)±2.6	3.7(4)±1.4
Nasal symptom scores	0	6.9(7)±2.8	2.9(3)±1.0
Ocular symptom scores	0	3.6(3)±3.0	2.3(2)±2.0
Asthma symptom scores	0	0	0

Notes: * For the AIT group, Allergen-specific IgE and total IgE were collected before receiving AIT, while VAS scores were collected after one year AIT treatment.
Abbreviations: HC, Healthy controls; AR, Patients with untreated allergic rhinitis; AIT, Allergic rhinitis patients who had undergone allergen immunotherapy for over one year; IgE, immunoglobulin E; SD, standard deviation.

Table 2 VAS Scores Corresponding to Each sIgE Grade in the AR and AIT Group

Grade of sIgE	Nasal Symptom Scores		Ocular Symptom Scores		Systemic Allergy Symptom Scores	
	AR	AIT	AR	AIT	AR	AIT
Grade 1	8	3.5	2	3	7	4.5
Grade 2	7	3	3	1	6	4
Grade 3	7.5	3	4	2	7.5	4
Grade 4	5.5	2	2	1	5.5	2
Grade 5	8	4	4.5	4	8	4.5
Grade 6	8	3	6.5	0	8	3

Note: Data are presented as the Median.
Abbreviations: sIgE, Allergen specific immunoglobulin E; AR, Patients with untreated allergic rhinitis; AIT, Allergic rhinitis patients who had undergone allergen immunotherapy for over one year.

was performed to investigate the relationship between the VAS score and the proportion of memory B cells present in the peripheral blood of patients.

Biological Sample Preparation

Peripheral blood samples, ranging from 2 to 3 mL, were collected from patients with AR and from healthy control subjects via K3-EDTA blood collection tubes (Becton Dickinson Biosciences, San Jose, CA, USA).

Flow Cytometry

A volume of 150 μL of peripheral blood was transferred into a designated flow cytometry tube, followed by the addition of 2 mL of red blood cell lysis buffer. The sample was incubated for 10 minutes to facilitate the lysis of red blood cells. Subsequently, 2 mL of phosphate-buffered saline (PBS) was added to terminate the lysis process. The mixture was then centrifuged at 1300 rpm for 6 minutes, and this step was repeated twice. After centrifugation, 300 μL of PBS was added to resuspend the cells. Following established flow cytometry staining protocols, 1 μL of specific cell surface staining antibodies, including CD4-KO525 (BD, USA, 582970), CD25-FITC (564467), CXCR5-PC5.5 (566469), CD19-APC-Cy7 (557791),

 https://doi.org/10.2147/JAA.S501775

CD38-PE-Cy7 (560677), and CD27-BV421 (562513), was used. The mixture was incubated on ice for 30 minutes. After surface staining, the cells were washed by adding 2 mL of PBS. Following washing, the sample was centrifuged at 1300 rpm for 6 minutes, after which the supernatant was discarded. Permeabilization buffer (Transcription Factor Buffer Set, 562574) was then added in accordance with the manufacturer's guidelines. After a 50-minute incubation on ice, 1 μL of intracellular antibodies, including FOXP3-APC (560401), PU-1-PE (86886s), T-bet-PB450 (563318), Rorγ-PE (563081), GATA3-PB450 (563349), IgD-BV510 (563034), IgE-BB700 (745980), IgM-PerCP-Cy5.5 (561285), IgG-APC (550931), and IgA-AlexaFlour647 (Santa Cruz, USA, sc-373823AF647), was added. After a second 50-minute incubation on ice, the cells were resuspended in 300 μL of PBS and subsequently analyzed via a Beckman flow cytometer (Beckman, USA, BC43326).

Cytokines and Immunoglobulins

A Cytokine Microsphere Array Kit (CBA) was used to detect changes in the concentrations of relevant cytokines and immunoglobulins, including IL-2, IL-4, IL-6, IL-10, TNF, and γ-IFN, as well as IgA, IgE, IgG, and IgM, in the participants' serum.

The ImmunoCAP system is utilized to measure specific IgE for various allergens in serum, including house dust mites, grass pollen, cat dander, dog dander, and certain food allergens. The levels of sIgE were measured in IU/mL and were estimated by a 6 grade EAST scale.[13] A serum allergen-specific IgE concentration below 0.35 IU/mL is classified as negative, whereas concentrations above 0.35 IU/mL are classified as positive. As per the method recommendation, results were divided into the following 6 grades: Grade 1: 0.35–0.70 IU/mL; Grade 2: 0.70–3.5 IU/mL; Grade 3: 3.5–17.5 IU/mL; Grade 4: 17.5–50.0 IU/mL; Grade 5: 50–100 IU/mL; and Grade 6: greater than 100 IU/mL.[14,15]

Data Analysis

Statistical analysis was conducted using GraphPad Prism software version 9.5 (GraphPad Software, Inc). Initially, all the data were subjected to descriptive analysis, normality tests, and variance analysis. For datasets that satisfied the criteria for a normal distribution and homogeneity of variance, comparisons between two groups were performed using an unpaired *t*-test, whereas comparisons among three groups were executed via one-way ANOVA. In cases where the data did not meet the assumptions of a normal distribution or homogeneity of variance, the Mann–Whitney *U*-test was applied. A P value of less than 0.05 was considered statistically significant. Additionally, correlation analyses between two groups were conducted using Pearson's correlation coefficient to evaluate the strength and direction of the relationship.

Results

In the Circulation of AR Patients, Th1 and Treg Cells Significantly Decrease, Whereas Th2 and Class-Switched Memory B Cells Significantly Increase. Following AIT, Th1, and Treg Cells Significantly Increase, Whereas Th2 and Class-Switched Memory B Cells Significantly Decrease

The flow cytometry gating strategy utilized in this investigation is depicted in Figure 1A and D. The results revealed no statistically significant differences in the proportions of Th9 cells, Th17 cells, Tfh cells, or plasma cells among the control, AR, and AIT groups (Figure 1B and C, $P > 0.05$). Compared with the control group, the proportions of Th1 cells and Treg cells in the AR group significantly decreased, whereas the proportion of Th2 cells significantly increased, demonstrating a clear Th2 bias (Figure 1B and C, $P < 0.05$). Further analysis revealed that the proportions of memory B cells, nonswitched memory B cells, and overall switched memory B cells in the AR group were significantly greater than those in the control group, whereas the proportion of plasma cells was not significantly different (Figure 1E, $P>0.05$).

Compared with the AR group, the proportion of Th1 cells and Treg cells in the AIT group significantly increased, whereas the proportion of Th2 cells significantly decreased (Figure 1B and C, $P < 0.05$). These findings indicate a reversal of the Th1/Th2/Treg imbalance. Additionally, the total number of memory B cells in the AIT group did not significantly differ from that in the AR group; however, the number of nonswitched memory B cells increased further (Figure 1E, $P < 0.05$). Notably, AIT significantly reduced the number of overall class-switched memory B cells

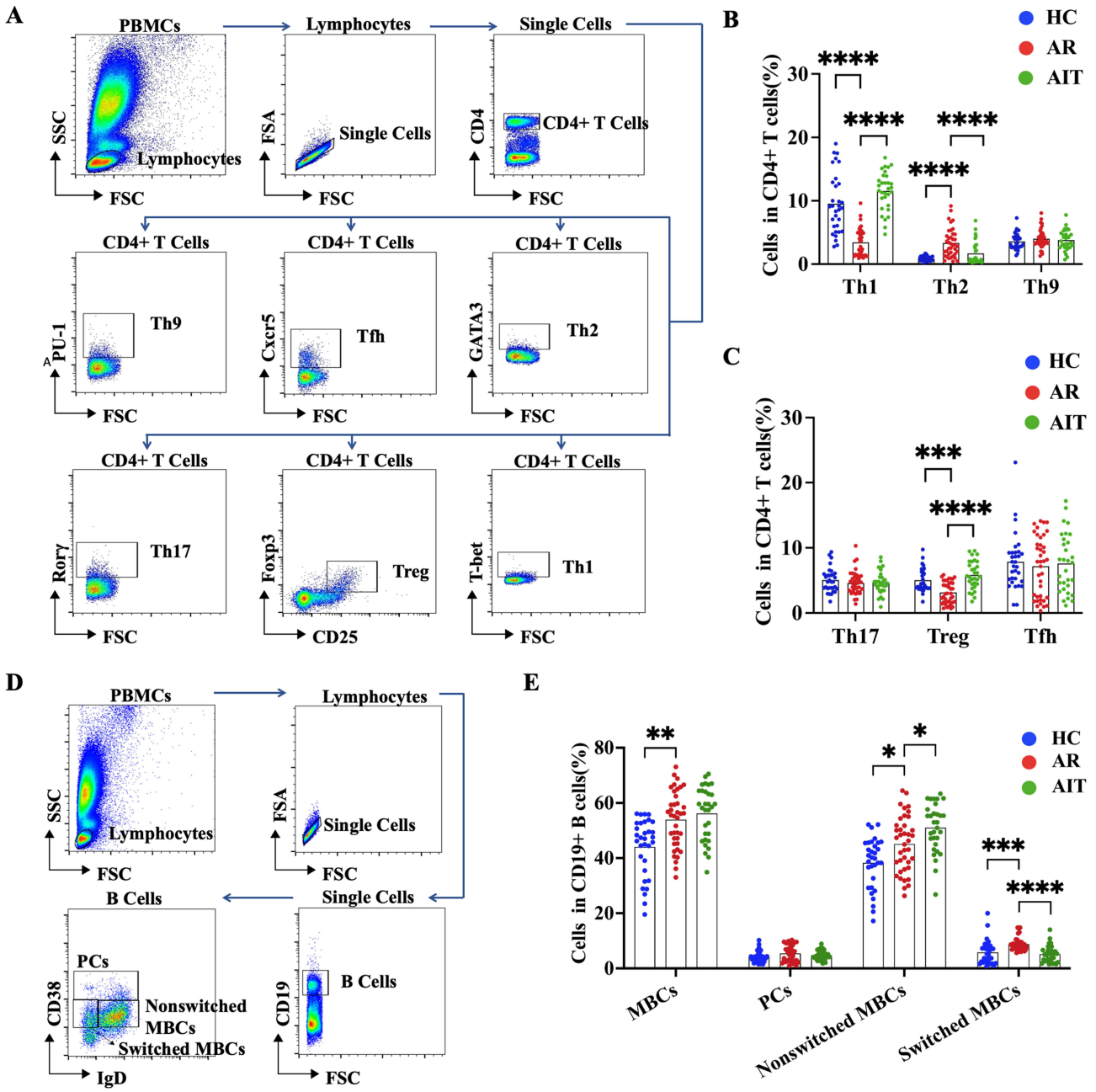

Figure 1 Flow cytometry analysis of the frequencies of CD4+ T-cell subsets and CD19+ B-cell subsets across each group. (**A**) Flow cytometry gating strategy for CD4+ T cells. (**B**) Bar graph illustrating the proportions of Th1, Th2, and Th9 cells in each group. (**C**) Bar graph depicting the proportions of Th17, Treg, and Tfh cells in each group. (**D**) Flow cytometry gating strategy for CD19+ B cells. (**E**). Bar graph representing the proportions of total memory B cells, plasma cells, nonswitched memory B cells, and class-switched memory B cells. *P < 0.05,**P < 0.01, ***P < 0.001, ****P < 0.0001.

(Figure 1E, P < 0.05). These results suggest that AIT may exert its therapeutic effect by decreasing the number of overall class-switched memory B cells.

In AR Patients, the Population of IgA+CD27- Class-Switched Memory B Cells Significantly Decreases. However, Following AIT, There Was a Significant Increase in These Cells

To further investigate the role of class-switched memory B-cell subtypes in the occurrence, development, and treatment of AR, we analyzed the changes in three subtypes based on the expression of IgA, IgG, and IgE across the three groups.

https://doi.org/10.2147/JAA.S501775

The flow cytometry gating strategy is illustrated in Figure 2A. The results indicated that there was no statistically significant difference in the proportion of IgE+ class-switched memory B cells among the control group, AR group, and AIT group (Figure 2B, P > 0.05). Compared with the control group, the proportion of IgA+ class-switched memory B cells in the AR group significantly decreased (Figure 2B, P < 0.05), whereas the proportion of IgG+ class-switched memory B cells increased; however, this difference was not statistically significant (Figure 2B, P = 0.19). Compared with the AR group, the proportion of IgA+ class-switched memory B cells in the AIT group significantly increased, whereas the proportion of IgG+ class-switched memory B cells significantly decreased (Figure 2B, P < 0.05). In summary, there are significant differences in IgA+ class-switched memory B cells before and after AIT in AR patients, and the trend of change is inversely related to that of IgG+ memory B cells.

To gain a deeper understanding of the role of class-switched memory B cells in the occurrence and treatment of AR, we further categorized the three types of class-switched memory B-cell subsets based on CD27 expression into six subgroups (Figure 2A) and analyzed their variations among the three groups. The results indicated that IgA+CD27- class-switched memory B cells constituted the highest proportion in peripheral blood, and the trend of change was the most pronounced. Compared the control group, the proportion of IgA+CD27- class-switched memory B cells in the AR group was significantly lower. Compared with the AR group, the proportion of IgA+CD27- class-switched memory B cells in the AIT group significantly increased after AIT (Figure 2C, P < 0.05).

Moreover, we observed that the proportion of IgA+CD27+ class-switched memory B cells in the peripheral blood of AR patients was significantly greater than that in the control group (Figure 2C, P < 0.05). However, there was no significant change following AIT (Figure 2C, P > 0.05). Additionally, the proportions of IgG+CD27+ and IgG+CD27- class-switched memory B cells in AR patients did not differ significantly from those in the control group (Figure 2C, P > 0.05), although AIT led to a significant reduction in their proportions (Figure 2C, P < 0.05). The differences in the proportions of IgE+CD27+ and IgE+CD27- class-switched memory B cells among the control, AR, and AIT groups were not statistically significant (Figure 2C, P > 0.05). These results suggest that among the subtypes of class-switched

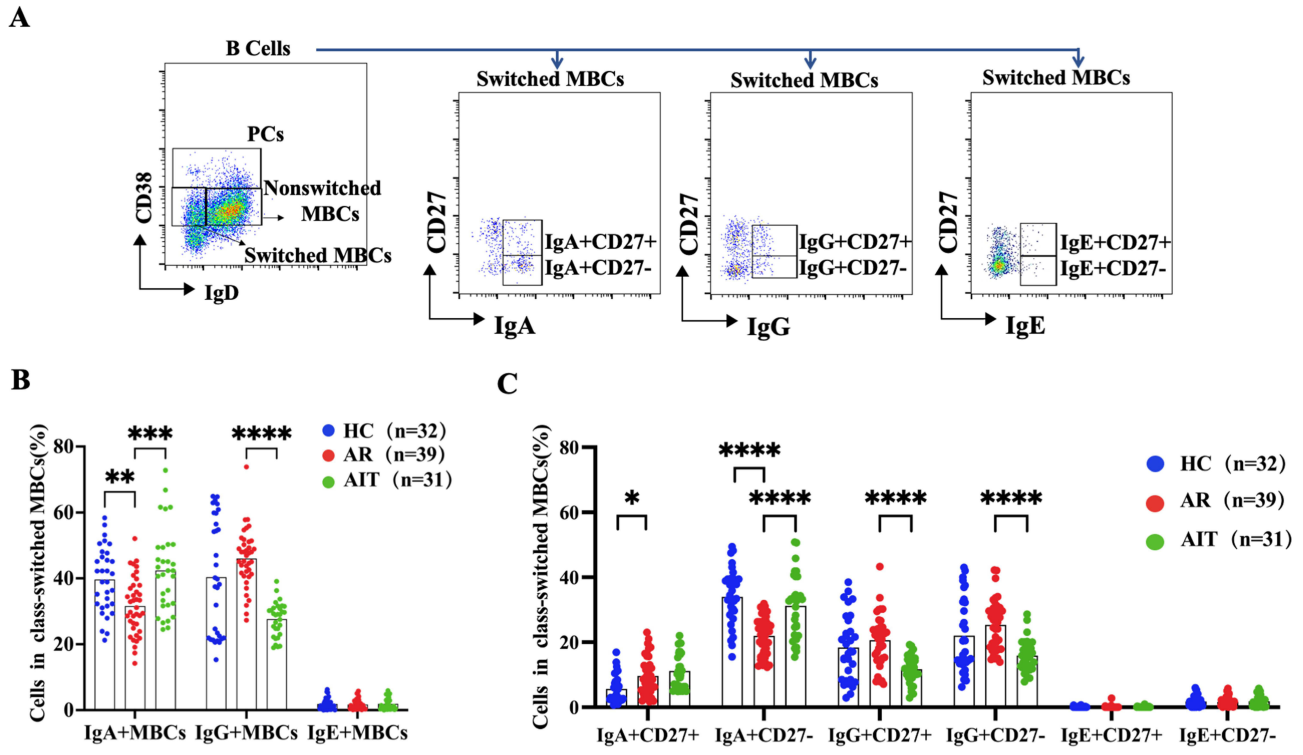

Figure 2 Changes in the frequency of memory B-cell subtypes following class switching, as detected by flow cytometry. (**A**) Flow cytometry gating strategy for identifying memory B cells. (**B**). Changes in the proportions of three subtypes of memory B cells after class switching (IgA+, IgG+, and IgE+). (**C**). Changes in the proportions of six subtypes of memory B cells after class switching (IgA+CD27+, IgA+CD27-, IgG+CD27+, IgG+CD27-, IgE+CD27+, and IgE+CD27-). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

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memory B cells, IgA+ class-switched memory B cells—particularly IgA+CD27- class-switched memory B cells—play crucial roles in the pathogenesis of AR and the response to AIT.

Relationships Between IgA+CD27- Class-Switched Memory B Cells and Treg Cells in the Circulation of AR Patients

This study revealed that IgA+CD27- class-switched memory B cells are closely associated with the onset and efficacy of AR. To investigate their role in the development of AR, we performed a correlation analysis with CD4+ T-cell subsets (Figure 3A). Our results indicated that IgA+CD27- class-switched memory B cells in the circulation of AR patients were significantly positively correlated with Treg cells, showing the highest correlation (Figure 3B, $P < 0.01$).

Furthermore, we observed that as the proportion of memory B cells in the IgG+CD27- category increased, the proportion of Treg cells in AR patients decreased, demonstrating a significant negative correlation between the two (Figure 3C, $P < 0.01$). These results indicate that the conversion of memory B cells to IgA+CD27- and IgG+CD27- cells significantly affects the proportion of Treg cells, which may be associated with T-B-cell interactions.

The Proportion of IgA+CD27- Class-Switched Memory B Cells in the Circulation of AR Patients Is Significantly Positively Correlated With IL-10 Levels

This study revealed that the proportion of IgA+CD27- class-switched memory B cells in the circulation of AR patients is positively correlated with the proportion of Treg cells. To further investigate the underlying mechanism of this relationship, we conducted a correlation analysis of B-cell subsets and T-cell-related cytokines (Figure 4A). Our analysis revealed that the proportion of IgA+CD27- class-switched memory B cells in the circulation of AR patients exhibited the strongest correlation with the level of the Treg cell cytokine IL-10, indicating a positive correlation. Specifically, as the proportion of IgA+CD27- class-switched memory B cells increased, the IL-10 levels in AR patients also increased (Figure 4B, $P < 0.001$).

Additionally, we observed that the proportion of IgA+CD27- class-switched memory B cells in the circulation of AR patients was negatively correlated with the level of the Th1 cytokine IFN-γ (Figure 4C, $P < 0.05$) and positively correlated with the level of the Th2 cytokine IL-4 (Figure 4E, $R = 0.3945$, $P < 0.05$). As the proportion of IgA+CD27- class-switched memory B cells increased, the IFN-γ levels in AR patients decreased, whereas the IL-4 levels increased. Furthermore, the proportion of class-switched memory B cells in the circulation of AR patients was positively correlated with the level of the Th2 cytokine IL-4. With the increase in the proportion of class-switched memory B cells, the IL-4 levels in AR patients also increased (Figure 4D, $P < 0.01$). These results suggest that IgA+CD27- class-switched memory B cells may be associated with the production of various cytokines, including IL-10, IFN-γ, and IL-4, with the most significant effect on IL-10.

In AR Patients, Class-Switched Memory B Cells That are IgA+CD27- are Associated With Total IgE and sIgE Levels

To investigate the role of B-cell subtypes in the circulating immune response of AR patients, we performed a correlation analysis between B-cell subsets and immunoglobulin levels in AR patients (Figure 5A). Our findings revealed that the proportion of class-switched memory B cells in the circulation of AR patients was positively correlated with total serum IgE levels (Figure 5B, $P < 0.01$).

In addition, the increase in the proportion of IgA+CD27- class-switched memory B cells was associated with a decrease in the serum level of sIgE in patients with AR (Figure 5C, $P < 0.05$), as was the reduction in the total serum IgE level (Figure 5D, $P < 0.05$). Furthermore, there was no significant correlation between the proportion of IgA +CD27- memory B cells and the proportion of plasma cells (Figure 5E, $P > 0.05$). These findings suggest that, in contrast to class-switched memory B cells, an increase in IgA+CD27- class-switched memory B cells may negatively impact IgE production, with this effect being more pronounced for sIgE.

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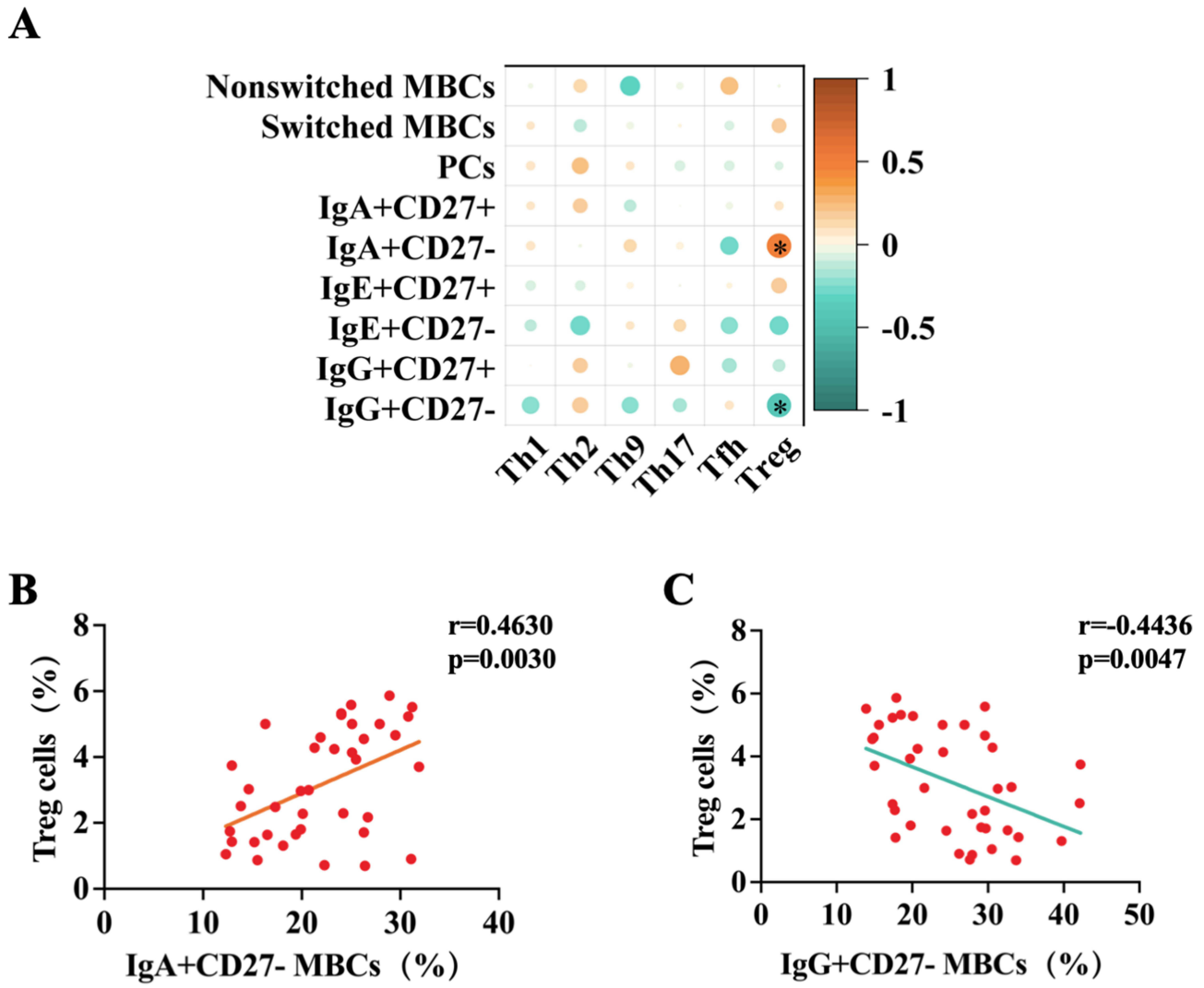

Figure 3 Correlation analysis of memory B-cell subsets and CD4+ T-cell subsets in AR patients. (**A**) Data were analyzed using Pearson's correlation coefficient and are presented in a correlation matrix. The matrix illustrates the strength of both positive and negative correlations between each pair of parameters, as indicated by the color scale on the right. (**B**) Correlation analysis of IgA+CD27- switched memory B cells with Treg cells. (**C**) Correlation analysis of IgG+CD27- switched memory B cells with Treg cells. *P < 0.05.

In Patients With AR, IgA+CD27- Class-Switched Memory B Cells, Including Total Score, Allergic Symptoms, and Nasal Symptoms, are Associated With the VAS Score

This study revealed that memory B cells and their subgroups are associated with various indicators in the circulation of patients with AR. To further investigate the impact of these cells on AR clinical symptoms, we analyzed their correlation with the VAS score, which includes total scores, allergic symptoms, and nasal symptoms (Figure 6A). Our findings indicate that the proportion of IgA+CD27- class-switched memory B cells in the circulation of AR patients is negatively correlated with the VAS score (total score, allergic symptoms, and nasal symptoms). Specifically, as the proportion of IgA+CD27- memory B cells increased, the VAS score (total score, allergic symptoms, and nasal symptoms) decreased (Figure 6B–D; P < 0.05). In addition, we found no direct correlation between sIgE and symptom scores (Supplementary Figure 1). These results suggest that IgA+CD27- class-switched memory B cells are closely related to the severity of clinical symptoms in AR patients.

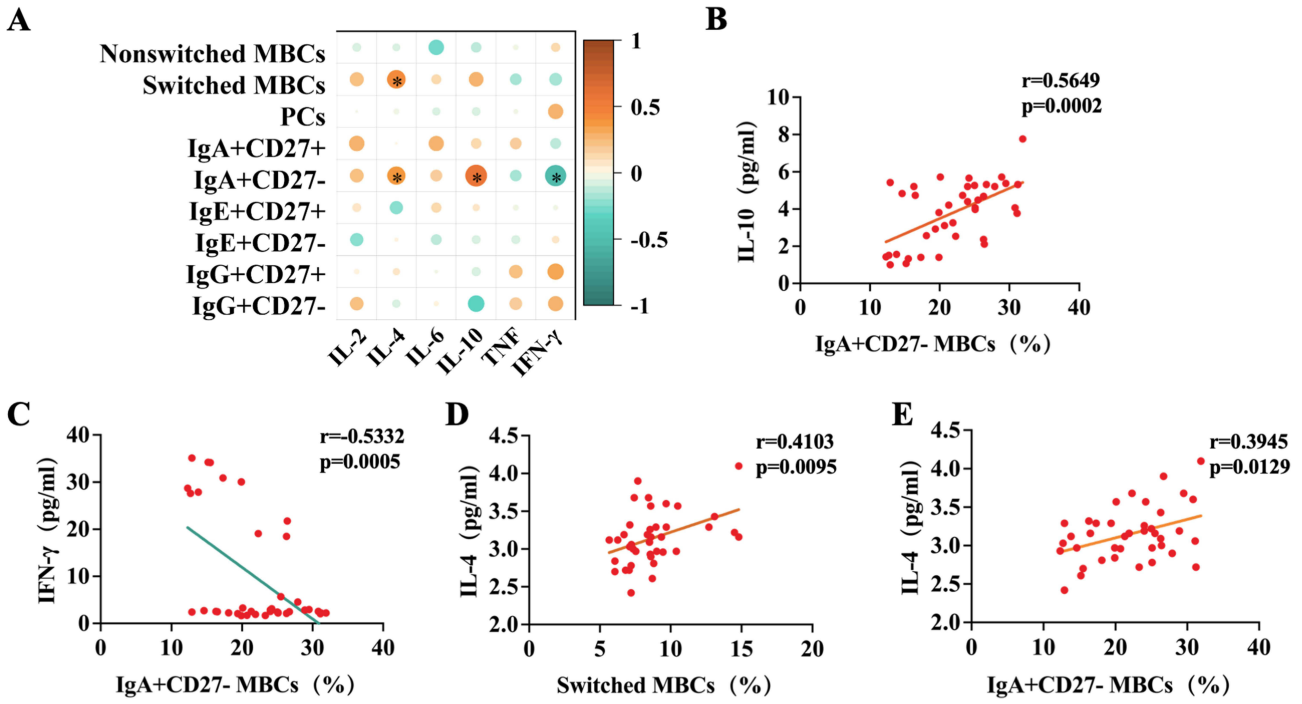

Figure 4 Correlation analysis of memory B-cell subsets and CD4+ T-cell subsets in AR patients. (**A**) Data were analyzed using Pearson's correlation coefficient and are presented in a correlation matrix. This matrix illustrates the strength of both positive and negative correlations between each pair of parameters, as indicated by the color scale on the right. (**B**) Correlation analysis of IgA+CD27- class-switched memory B cells with serum IL-10 levels. (**C**) Correlation analysis of IgA+CD27- class-switched memory B cells with IFN-γ levels. (**D**) Correlation analysis of class-switched memory B cells with IL-10 levels. (**E**). Correlation analysis of IgA+CD27- class-switched memory B cells with IL-4 levels. *P < 0.05.

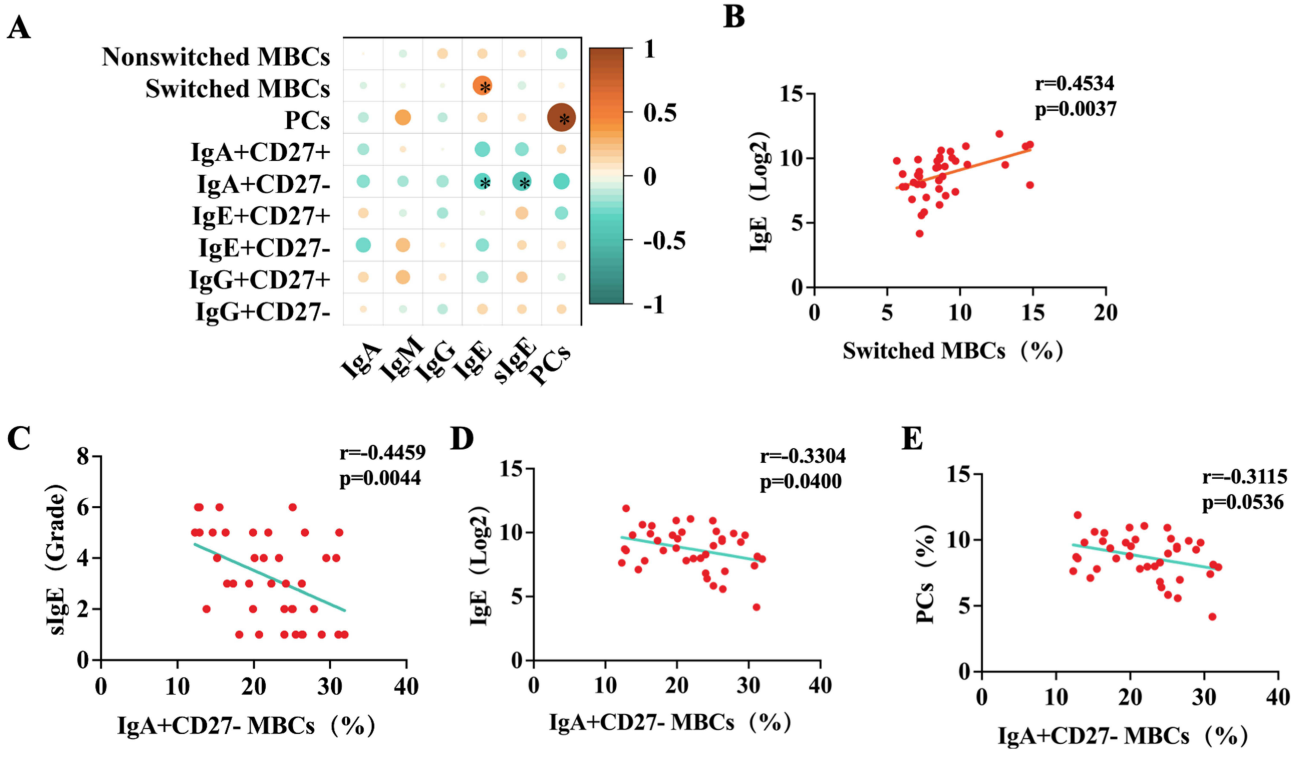

Figure 5 Correlation analysis of memory B-cell subsets and immunoglobulins in AR patients. (**A**) Data were analyzed using Pearson's correlation coefficient and are presented in a correlation matrix. This matrix illustrates the strength of both positive and negative correlations between each pair of parameters, as indicated by the color scale on the right. (**B**). Correlation analysis of class-switched memory B cells with total IgE levels. (**C**) Correlation analysis of IgA+CD27- class-switched memory B cells with sIgE levels. (**D**) Correlation analysis of class-switched IgA+CD27- memory B cells with total IgE levels. E. Correlation analysis of class-switched IgA+CD27- memory B cells with plasma cells. *P < 0.05.

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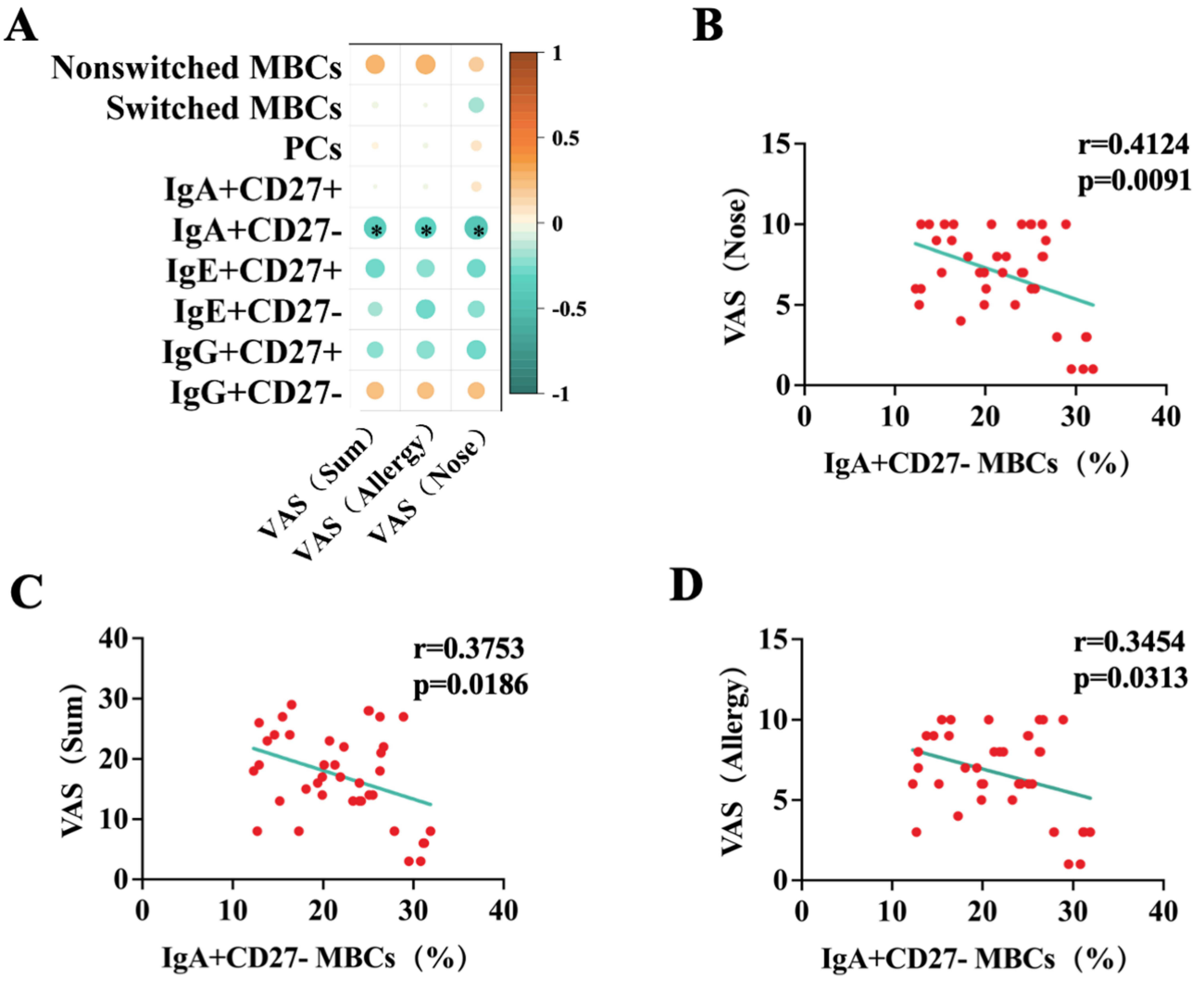

Figure 6 Correlation analysis between memory B-cell subsets and the VAS score in AR patients. (**A**) Data were analyzed using Pearson's correlation test and are presented in a correlation matrix. This matrix illustrates the strength of both positive and negative correlations between each pair of parameters, as indicated by the color scale on the right. (**B**) Correlation analysis between IgA+CD27- class-switched memory B cells and the VAS score (nasal symptoms). (**C**) Correlation analysis between IgA+CD27- class-switched memory B cells and the VAS (total score). (**D**) Correlation analysis between IgA+CD27- class-switched memory B cells and the VAS score (allergic symptoms). *P < 0.05.

In AR Patients Treated With AIT for More Than One year, the Conversion of Circulating IgA+CD27- Class-Switched Memory B Cells Is Associated With Treg Cells, IL-10, and the Total VAS Score

To further elucidate the relationships between memory B-cell subtypes and the efficacy of AIT, as well as to investigate their underlying mechanisms, we validated previously identified correlations in AR patients who had undergone AIT for more than one year. The flow cytometry gating strategy is illustrated in Figure 7A. Our findings indicate that in the circulation of AR patients receiving AIT for more than one year, IgA+CD27- class-switched memory B cells were significantly positively correlated with Treg cells (Figure 7B, $P < 0.05$). In contrast, IgG+CD27- class-switched memory B cells did not significantly correlate with Treg cells (Figure 7C, $P > 0.05$). Additionally, IgA+CD27- class-switched memory B cells were significantly positively correlated with IL-10 (Figure 7D, $P < 0.01$), whereas no significant correlations were observed with IFN-γ or IL-4 ($P > 0.05$).

In addition, we found that in AR patients who received AIT for more than one year, an increase in the proportion of IgA+CD27- class-switched memory B cells was associated with a decrease in the total VAS score (Figure 7E, $P < 0.05$).

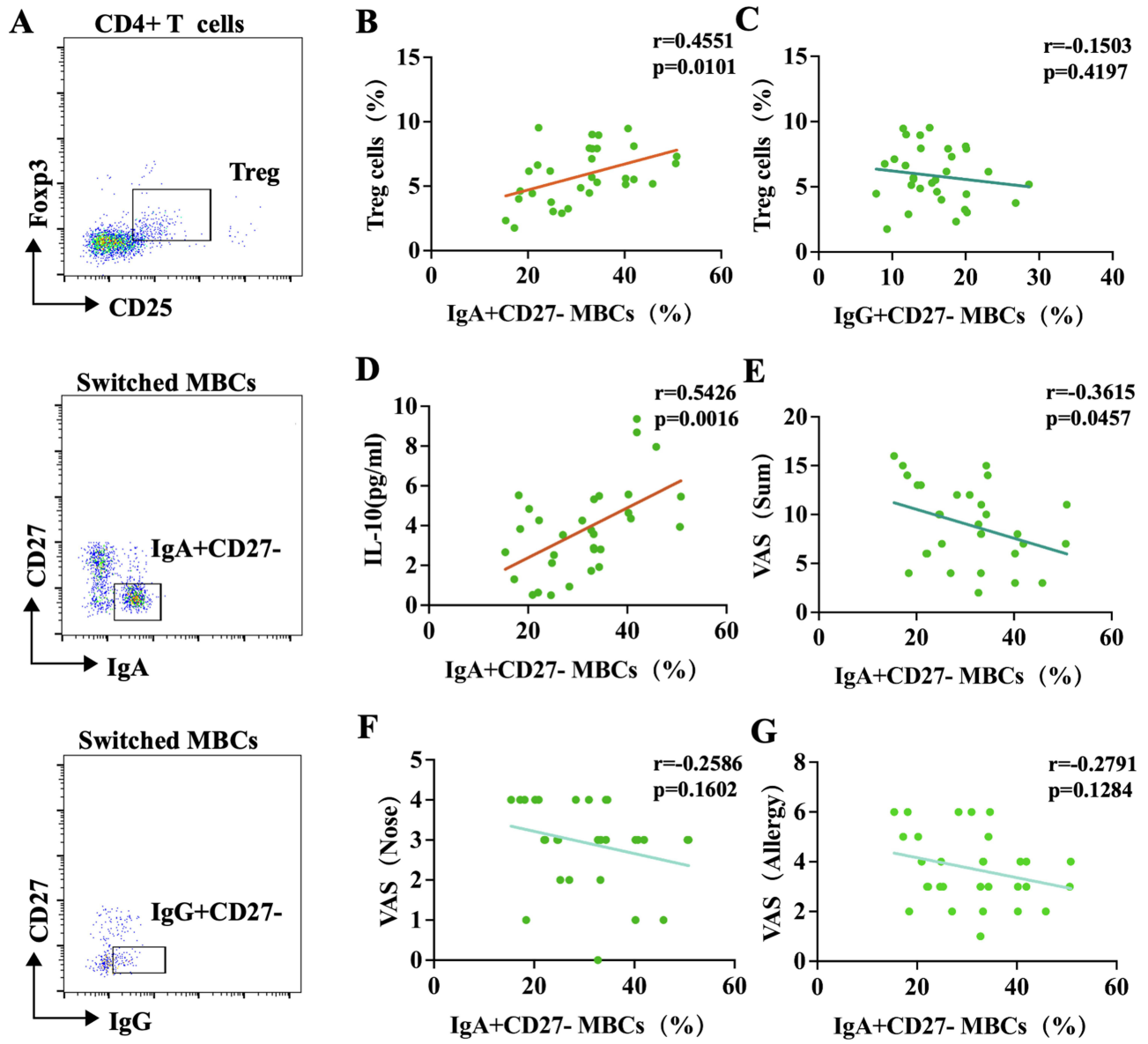

Figure 7 Pearson correlation analysis of memory B-cell subtypes and related indicators in the circulation of AR patients who underwent AIT for more than one year. (**A**) Flow cytometry gating strategy for Treg cells, IgA+CD27- class-switched memory B cells, IgG+CD27- class-switched memory B cells. (**B**) Correlation analysis between IgA +CD27- class-switched memory B cells and Treg cells. (**C**) Correlation analysis between IgG+CD27- class-switched memory B cells and Treg cells. (**D**) Correlation analysis between IgA+CD27- class-switched memory B cells and serum IL-10. (**E**) Correlation analysis between IgA+CD27- class-switched memory B cells and the VAS total score. (**F**) Correlation analysis between IgA+CD27- class-switched memory B cells and the VAS score for nasal symptoms. (**G**) Correlation analysis between IgA+CD27- class-switched memory B cells and the VAS score for allergic symptoms.

However, the VAS scores for nasal symptoms and allergic symptoms did not significantly change (Figure 7F and G, $P > 0.05$). These results indicate that in AR patients who have undergone AIT for more than one year, circulating IgA+CD27- class-switched memory B cells can significantly influence Treg and IL-10 levels and are closely related to the severity of AR symptoms.

Discussion

In this study, we employed two widely accepted gating strategies for T cells and B cells: CD25/Foxp3/T-bet/GATA3/PU-1/Rorγ/CXCR5 for T cells and CD38/CD27/IgD/IgA/IgG/IgE for B cells. These strategies were used to conduct a comprehensive analysis of T-cell and B-cell subsets in patients with AR.[16,17] In addition to confirming previous reports of increased peripheral blood memory B cells and decreased Treg cells, as well as an imbalance in the Th1/Th2

https://doi.org/10.2147/JAA.S501775

ratio among AR patients,[6] we discovered that the proportion of overall class-switched memory B cells was significantly higher in AR patients than in healthy controls. Following AIT, this proportion significantly decreased, indicating that overall class-switched memory B cells are closely associated with the onset and treatment of AR. Based on these findings, we further analyzed three subtypes of class-switched memory B cells: IgA+, IgG+, and IgE+. We observed that the changes in the proportion of IgA+ class-switched memory B cells among the three groups exhibited an inverse trend compared with that of the overall class-switched memory B cells. Specifically, the proportion of IgA+ class-switched memory B cells decreased in the peripheral blood of AR patients and significantly increased after AIT. Although no statistically significant difference was found in the number of IgG+ class-switched memory B cells, the trend of change mirrored that of the overall class-switched memory B cells.

Previous studies have demonstrated that IgG+ class-switched memory B cells represent the primary subset of class-switched memory B cells and function as storage units for immune memory.[18,19] During an allergic reaction, IgG+ class-switched memory B cells are the first to differentiate into plasma cells, releasing large amounts of sIgE.[20,21] Consequently, IgG+ class-switched memory B cells are key contributors to the development of AR. This study revealed that while the proportion of IgG+ class-switched memory B cells in AR patients was greater than that in normal controls, the difference was not statistically significant. IgG+ memory B cells can be categorized into four subsets based on the expression of mIgG: IgG1+, IgG2+, IgG3+, and IgG4+, with IgG1+ memory B cells being the predominant subset. These cells primarily store immune memory and produce IgE+ plasma cells, thereby facilitating the onset of AR.[22–24] In contrast, IgG4+ memory B cells can inhibit the activity of IgE,[25] which is contrary to the function of IgG1+ memory B cells. Therefore, the absence of a significant increase in IgG+ class-switched memory B cells in AR patients may be due to differing trends among these subtypes, warranting further investigation.

Importantly, this study revealed that among the three subtypes of class-switched memory B cells, the changes in IgA+ class-switched memory B cells were the most pronounced, exhibiting a trend opposite to that of IgG+ class-switched memory B cells. Research indicates that the gene expression profile of IgA+ class-switched memory B cells is highly similar to that of IgG+ class-switched memory B cells,[26] suggesting that IgA+ class-switched memory B cells have the potential to convert into IgG+ class-switched memory B cells. Therefore, we propose that class switching in the memory B cells of AR patients has shifted from IgA to IgG, and that AIT can significantly reverse this change.

Previous studies have demonstrated that IgE+ memory B cells are closely associated with the production mechanisms of plasma cells that secrete sIgE.[19] However, this study did not identify a correlation between IgE+ class-switched memory B cells and AR. This lack of association may be attributed to the rarity of IgE+ memory B cells in circulation, which complicates the assessment of their changes through statistical analysis. This perspective aligns with findings from earlier research.[27]

CD27 is a crucial immune molecule that is generally associated with the maturation and differentiation status of B cells, as well as the affinity for antibody production. Through further analysis of the subgroups of class-switched memory B cells based on CD27 expression, we discovered that the proportion of IgA+CD27- class-switched memory B cells in the peripheral blood of AR patients was significantly reduced. In contrast, AR patients who had undergone AIT for more than a year presented a significant increase in IgA+CD27- class-switched memory B cells. CD27- memory B cells are often considered memory B cells that do not participate in germinal center reactions,[28] and the antibodies produced following their further proliferation and differentiation typically exhibit lower affinity and specificity. Circulating CD27- memory B cells can enter the germinal center with the assistance of T cells and subsequently differentiate into CD27+ memory B cells.[29,30] Class-switched CD27+ memory B cells can further differentiate into plasma cells and produce high-affinity antibodies upon re-exposure to antigens.[31] During allergic reactions, the immune system may cause memory B cells to aggregate in the germinal center, potentially leading to a relative decrease in the proportion of CD27- B cells in the circulation. Therefore, we speculate that the reduced number of IgA+CD27- class-switched memory B cells in AR patients may be linked to the activation of immune responses and the redistribution of immune cells.

Further correlation analysis revealed that IgA+CD27- memory B cells in the circulation of AR patients were significantly positively correlated with Treg cells and their cytokine IL-10, but significantly negatively correlated with sIgE and VAS scores. Previous studies have indicated that the number of Treg cells in the peripheral blood is closely

associated with the occurrence, severity of symptoms, and treatment efficacy of AR.[12] IL-10 can activate the signal transducer and activator of transcription 3 (STAT3) pathway by phosphorylating IL-10R on Treg cells through Janus kinase 1 (JAK1), thereby promoting the proliferation and differentiation of Treg cells.[32] Research conducted by Ticha et al has demonstrated that CD27 class-switched memory B cells are closely linked to the production of Breg cells, which are a primary source of IL-10.[33,34] Additionally, Breg cells can maintain immune tolerance to allergens through antigen-specific interactions with Treg cells and the production of antigen-specific IgG4.[35] We speculate that the reduction in IgA +CD27- class-switched memory B cells in AR patients may lead to a decrease in Breg cells and their production of IL-10, thereby affecting the proliferation and activation of Treg cells. In our study, we observed a significant association of memory B cells with sIgE and symptom scores. However, we did not find a direct correlation between sIgE and symptom scores, which is consistent with the research results of Hasegawa et al and Angjeli et al.[36,37] We propose that memory B cells could act as a link between allergen sensitization and clinical impact. Furthermore, memory B cells in AR patients may be more prone to accumulate in germinal centers for further activation and differentiation, whereas their class-switching direction shifts from IgA to IgG, resulting in increased IgE production.[38] These changes may exacerbate the allergic inflammatory response, contributing to the progression and worsening of allergic diseases.

After AIT, IgA+CD27- class-switched memory B cells in the circulation of AR patients continued to exhibit a significant positive correlation with Treg cells and IL-10, and a significant negative correlation with the VAS total score. This finding further supports our hypothesis. Notably, after AIT, IgA+CD27- class-switched memory B cells no longer demonstrated a significant correlation with VAS scores (which encompass both allergic and nasal symptoms). This change may be attributed to a reduction in interindividual symptom variability resulting from the improvement in AR symptoms following AIT.

The limitations of this study stem from the observational nature of cross-sectional research, which prevents us from determining whether the changes in memory B cells and their subsets in AR patients are a cause of AR or merely a coincidental phenomenon. Future studies should aim to confirm this relationship and evaluate the potential of memory B-cell subsets as biomarkers for the onset and progression of AR, as well as the efficacy of AIT.

Owing to challenges in sample collection, the samples used in this study were obtained from excess peripheral blood following the clinical examination of the patients. Consequently, we were unable to further analyze immune cells in nasal mucosal tissue and immune organs, such as the tonsils. Localized Allergic Rhinitis (LAR) is a subtype of AR. Patients diagnosed with LAR present with nasal symptoms characteristic of AR, in addition to localized allergic reactions within the nasal region. Nevertheless, these patients do not demonstrate any abnormalities in serum sIgE or total IgE levels.[39] Consequently, the diagnostic criteria for AR employed in this study may have resulted in the exclusion of this particular patient group. The changes of local memory B cells and their subtypes in the nasal cavity of AR patients deserve further investigation. In addition, future research should incorporate nasal lavage fluid or nasal mucosal scrapings, along with optimal immune organ tissues, and be supported by experimental mouse models to investigate the association of memory B cells in local tissues and blood.

Conclusion

In summary, IgA+CD27- class-switched memory B cells circulating in peripheral blood may represent an unactivated state of immune cells. Among the various subtypes of memory B cells, changes in these cells during the onset and treatment of AR are particularly significant. These genes are strongly correlated with the ratio of Treg cells in AR patients, sIgE levels, and VAS scores. Therefore, we propose that IgA+CD27- memory B cells may serve as crucial relay points in the interaction between T and B cells, sIgE production, and the development of symptoms in AR patients. Additionally, they could act as a link between allergen sensitization and clinical impact, and may also serve as potential biomarkers for evaluating AR symptoms and treatment efficacy.

Abbreviations

AIT, allergen immunotherapy; ARIA, Allergic Rhinitis and Its Impact on Asthma; AR, allergic rhinitis; HC, healthy controls; IgE, immunoglobulin E; LAR, Localized Allergic Rhinitis; MBCs, memory B

cells; PCs, plasma cells; sIgE, allergen specific immunoglobulin E; VAS, visual analog scale.

Data Sharing Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Statement

The Ethics Committee of Renmin Hospital of Wuhan University approved this research protocol (WDRY2023-K143). Written informed consents were obtained from all participants, in accordance with the Declaration of Helsinki.

Consent for Publication

All authors have confirmed that all data from this study is publishable and have seen the final version of the article for publication. Written informed consent for publication was obtained from all participants.

Acknowledgments

We thank Origin for their figure services (Figure 3A, 4A, 5A and 6A by Origin) (Licence: KE4D1F0801170130).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the National Natural Science Foundation of China [82371116, 82201249, 82301274]; the Fundamental Research Funds for the Central Universities (2042024YXB008 and 2042023kf0013); The Open Project of Hubei Key Laboratory of Renmin Hospital of Wuhan University (2023KFH001); The medical and health research project of Zhejiang province (2023KY172).

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Inoue T, Kurosaki T. Memory B cells. *Nat Rev Immunol*. 2024;24:5–17.
2. Weisel NM, Joachim SM, Smita S, et al. Surface phenotypes of naive and memory B cells in mouse and human tissues. *Nat Immunol*. 2022;23(1):135–145. doi:10.1038/s41590-021-01078-x
3. Good-Jacobson KL. Strength in diversity: phenotypic, functional, and molecular heterogeneity within the memory B cell repertoire. *Immunol Rev*. 2018;284(1):67–78. doi:10.1111/imr.12663
4. Phad GE, Pinto D, Foglierini M, et al. Clonal structure, stability and dynamics of human memory B cells and circulating plasmablasts. *Nat Immunol*. 2022;23(7):1076–1085. doi:10.1038/s41590-022-01230-1
5. Colas L, Magnan A, Brouard S. Immunoglobulin E response in health and disease beyond allergic disorders. *Allergy*. 2022;77(6):1700–1718. doi:10.1111/all.15230
6. Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. *J Allergy Clin Immunol*. 2015;135(3):626–635. doi:10.1016/j.jaci.2014.11.001
7. Luo J, Guo H, Liu Z, et al. Analysis of Peripheral B Cell Subsets in Patients With Allergic Rhinitis. *Allergy Asthma Immunol Res*. 2018;10(3):236–243. doi:10.4168/aair.2018.10.3.236
8. Zhang Y, Lan F, Zhang L. Update on pathomechanisms and treatments in allergic rhinitis. *Allergy*. 2022;77(11):3309–3319. doi:10.1111/all.15454
9. Yao Y, Wang N, Chen CL, et al. CD23 expression on switched memory B cells bridges T-B cell interaction in allergic rhinitis. *Allergy*. 2020;75(10):2599–2612. doi:10.1111/all.14288
10. McKenzie CI, Varese N, Aui PM, et al. RNA sequencing of single allergen-specific memory B cells after grass pollen immunotherapy: two unique cell fates and CD29 as a biomarker for treatment effect. *Allergy*. 2023;78(3):822–835. doi:10.1111/all.15529
11. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63(86):8–160. doi:10.1111/j.1398-9995.2007.01620.x
12. Bousquet J, Bewick M, Arnavielhe S, et al. Work productivity in rhinitis using cell phones: the MASK pilot study. *Allergy*. 2017;72(10):1475–1484. doi:10.1111/all.13177
13. Nikolov G, Todordova Y, Emilova R, Hristova D, Nikolova M, Petrunov B. Allergen-Specific IgE and IgG4 as Biomarkers for Immunologic Changes during Subcutaneous Allergen Immunotherapy. *Antibodies*. 2021;10(4). doi:10.3390/antib10040049

14. Niggemann B, Nilsson M, Friedrichs F. Paediatric allergy diagnosis in primary care is improved by in vitro allergen-specific IgE testing. *Pediatr Allergy Immunol*. 2008;19(4):325–331. doi:10.1111/j.1399-3038.2007.00651.x
15. Letrán A, García I, Espinazo-Romeu M, Moreno-Aguilar C, Moreno F. Cut-off value of D. pteronyssinus specific IgE in double negative patients Der p 1 and Der p 2 and its clinical repercussion. *Sci Rep*. 2021;11(1):23585. doi:10.1038/s41598-021-03005-4
16. Qiao YL, Jiao WE, Xu S, et al. Allergen immunotherapy enhances the immunosuppressive effects of Treg cells to alleviate allergic rhinitis by decreasing PU-1+ Treg cell numbers. *Int Immunopharmacol*. 2022;112:109187. doi:10.1016/j.intimp.2022.109187
17. Looman KIM, van Meel ER, Grosserichter-Wagener C, et al. Associations of Th2, Th17, Treg cells, and IgA + memory B cells with atopic disease in children: the Generation R Study. *Allergy*. 2020;75(1):178–187. doi:10.1111/all.14010
18. Asrat S, Kaur N, Liu X, et al. Chronic allergen exposure drives accumulation of long-lived IgE plasma cells in the bone marrow, giving rise to serological memory. *Sci Immunol*. 2020;5(43). doi:10.1126/sciimmunol.aav8402
19. He JS, Subramaniam S, Narang V, et al. IgG1 memory B cells keep the memory of IgE responses. *Nat Commun*. 2017;8(1):641. doi:10.1038/s41467-017-00723-0
20. Schmitt MER, Lutz J, Haase P, et al. The B-cell antigen receptor of IgE-switched plasma cells regulates memory IgE responses. *J Allergy Clin Immunol*. 2020;146(3):642–651.e645. doi:10.1016/j.jaci.2020.02.015
21. Jiménez-Saiz R, Ellenbogen Y, Koenig JFE, et al. IgG1 + B-cell immunity predates IgE responses in epicutaneous sensitization to foods. *Allergy*. 2019;74(1):165–175. doi:10.1111/all.13481
22. Saunders SP, Ma EGM, Aranda CJ, de Lafaille MA C. Non-classical B Cell Memory of Allergic IgE Responses. *Front Immunol*. 2019;10:715. doi:10.3389/fimmu.2019.00715
23. Ota M, Hoehn KB, Ota T, et al. The memory of pathogenic IgE is contained within CD23 (+) IgG1 (+) memory B cells poised to switch to IgE in food allergy. *bioRxiv*. 2023;2023:1.
24. Turqueti-Neves A, Otte M, Schwartz C, et al. The Extracellular Domains of IgG1 and T Cell-Derived IL-4/IL-13 Are Critical for the Polyclonal Memory IgE Response In Vivo. *PLoS Biol*. 2015;13(11):e1002290. doi:10.1371/journal.pbio.1002290
25. Dodev TS, Bowen H, Shamji MH, et al. Inhibition of allergen-dependent IgE activity by antibodies of the same specificity but different class. *Allergy*. 2015;70(6):720–724. doi:10.1111/all.12607
26. Habener A, Grychtol R, Gaedcke S, et al. IgA + memory B-cells are significantly increased in patients with asthma and small airway dysfunction. *Eur Respir J*. 2022:60.52102130. doi:10.1183/13993003.02130-2021.
27. Berkowska MA, Heeringa JJ, Hajdarbegovic E, et al. Human IgE(+) B cells are derived from T cell-dependent and T cell-independent pathways. *J Allergy Clin Immunol*. 2014;134(3):688–697.e686. doi:10.1016/j.jaci.2014.03.036
28. Grimsholm O. CD27 on human memory B cells-more than just a surface marker. *Clin Exp Immunol*. 2023;213(2):164–172. doi:10.1093/cei/uxac114
29. Bernasconi NL, Onai N, Lanzavecchia A. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. *Blood*. 2003;101(11):4500–4504. doi:10.1182/blood-2002-11-3569
30. Marasco E, Farroni C, Cascioli S, et al. B-cell activation with CD40L or CpG measures the function of B-cell subsets and identifies specific defects in immunodeficient patients. *Eur J Immunol*. 2017;47(1):131–143. doi:10.1002/eji.201646574
31. Davey AM, Pierce SK. Intrinsic differences in the initiation of B cell receptor signaling favor responses of human IgG(+) memory B cells over IgM (+) naive B cells. *J Immunol*. 2012;188(7):3332–3341. doi:10.4049/jimmunol.1102322
32. Song J, Lin Z, Liu Q, et al. MiR-192-5p/RB1/NF-κBp65 signaling axis promotes IL-10 secretion during gastric cancer EMT to induce Treg cell differentiation in the tumour microenvironment. *Clin Transl Med*. 2022;12(8):e992. doi:10.1002/ctm2.992
33. Ticha O, Slanina P, Moos L, Stichova J, Vlkova M, Bekeredjian-Ding I. TNFR2 expression is a hallmark of human memory B cells with suppressive function. *Eur J Immunol*. 2021;51(5):1195–1205. doi:10.1002/eji.202048988
34. Noh J, Noh G, Kim HS, Kim AR, Choi WS. Allergen-specific responses of CD19(+)CD5(+)Foxp3(+) regulatory B cells (Bregs) and CD4(+)Foxp3 (+) regulatory T cell (Tregs) in immune tolerance of cow milk allergy of late eczematous reactions. *Cell Immunol*. 2012;274(1–2):109–114. doi:10.1016/j.cellimm.2012.01.005
35. Cantaert T, Doorenspleet ME, Francosalinas G, et al. Increased numbers of CD5+ B lymphocytes with a regulatory phenotype in spondylarthritis. *Arthritis Rheum*. 2012;64(6):1859–1868. doi:10.1002/art.34364
36. Hasegawa A, Utsumi D, Lund K, Okano M, Ohashi-Doi K, Okubo K. Correlation between sensitization to house dust mite major allergens, age, and symptoms in Japanese house dust mite allergic subjects. *Int Immunopharmacol*. 2022;107:108640. doi:10.1016/j.intimp.2022.108640
37. Angjeli E, Gomes P, Lane KJ, Stein L, Abelson MB. Technical and clinical validation of the Allergen BioCube ® for timothy grass. *Immun Inflamm Dis*. 2017;5(1):78–84. doi:10.1002/iid3.143
38. Seifert M, Przekopowitz M, Taudien S, et al. Functional capacities of human IgM memory B cells in early inflammatory responses and secondary germinal center reactions. *Proc Natl Acad Sci U S A*. 2015;112(6):E546–555. doi:10.1073/pnas.1416276112
39. Berghi O, Dumitru M, Cergan R, Musat G, Serboiu C, Vrinceanu D. Local Allergic Rhinitis-A Challenge for Allergology and Otorhinolaryngology Cooperation (Scoping Review). *Life*. 2024;14:1.