SLAM Negatively Regulates IL-21 Production in Tfh-Like Cells from Allergic Rhinitis Patients

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Research

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Abstract

Background: Allergic rhinitis (AR) is characterized by type I hypersensitivity that is mediated by IgE-induced humoral responses. It is known that follicular helper T cells (Tfh) comprise the key Th cell subset that promotes antibody production. Signaling lymphocytic activation molecule (SLAM) participates in the regulation of the differentiation and function of Tfh cells, but whether this regulation is involved in the pathogenesis of AR is unknown.

Methods: CD4⁺CXCR5⁺ Tfh-like cells from peripheral blood were detected by flow cytometry. The levels of IL-21 and IgE in serum were tested by ELISA. Blood CD4⁺CXCR5⁺ Tfh-like cells were sorted and cultured with anti-SLAM mAb in vitro.

Results: The frequencies of circulating CD4⁺CXCR5⁺ Tfh-like cells appeared virtually unchanged in AR patients, but the expression of SLAM and SLAM-associated protein (SAP) on circulating Tfh-like cells was significantly decreased. Meanwhile, the level of serum IL-21 was increased in AR patients, and there was a negative correlation between the levels of IL-21 and the expression of SLAM or SAP in CD4⁺CXCR5⁺ T cells. Treatment with anti-SLAM mAb resulted in the reduced production of IL-21 by Tfh-like cells in vitro. Additionally, the expression of SLAM on B cells significantly decreased, although the percentages of B cells were increased in AR patients.

Conclusions: SLAM negatively regulates the production of IL-21 in CD4⁺CXCR5⁺ Tfh-like cells, which contributes to the pathogenesis of AR.

Background

Allergic rhinitis (AR) is one of the most common upper respiratory diseases and is characterized by the clinical symptoms of sneezing, pruritis, nasal congestion, and rhinorrhea. Allergic rhinitis is also characterized by type I hypersensitivity mediated by IgE. The imbalance between the Th1 and Th2 immune responses plays a significant role in AR. The proportion of Th2 cells and the levels of Th2 cytokines are increased, including the level of IL-4, a switch factor for IgE, and IL-5, an eosinophil growth factor [1, 2]. According to recent studies and advances in the understanding of the mechanisms of AR, other subsets of CD4⁺ T cells and related cytokines take part in the pathologic process of AR, including IL-17-producing Th17 cells [3] and follicular helper T cells (Tfh). [4].

Tfh cells have been described as a new subset of CD4⁺ Th cells that can provide significant help to B cells. A distinguishing feature of Tfh cells is the high expression of CXCR5, programmed cell death protein (PD-1), inducible costimulator (ICOS), and transcription factor (Bcl-6). IL-21 is the most effective cytokine secreted by Tfh cells [5]. Tfh cells participate in the formation and maintenance of the germinal center (GC), where the processes of B cell affinity maturation, class switch recombination, plasma cell differentiation and memory B cell differentiation predominantly occur. The major cytokine IL-21 is important for optimal GC B cell proliferation [6] and can potently drive plasma cell differentiation in both

humans and mice through the activation of STAT3 [7–9]. Recently, Morita R and colleagues that CD4 + CXCR5 + T cells in human blood shared markers with GC Tfh cells and could induce the differentiation of naive B cells into plasmablasts via IL-21, which suggested that peripheral blood CD4 + CXCR5 + T cells may represent the circulating compartment of Tfh-like cells[10]. Many studies have focused on circulating Tfh cells and have indicated that circulating Tfh cells are produced in autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, and Graves' disease, and are related to serum autoantibodies [11, 12].

Signaling lymphocytic activation molecule (SLAM) is a transmembrane protein and a cell surface glycoprotein expressed on immune cells, such as T and B lymphocytes and dendritic cells. SLAM is a self-ligand that can promote the interaction between T cells and B cells or other antigen-presenting cells that express SLAM [13]. SLAM-associated protein (SAP) is a kind of intracellular adapter protein that can bind to the immunoreceptor tyrosine-based switch motif (ITSM) present in the cytoplasmic portion of SLAM to deliver the signal [14]. SAP functions in the stage of Tfh cell full polarization and is indispensable for GC Tfh cell development [15, 16]. GC Tfh cells express high levels of SAP. SAP deficiency can cause human X-linked lymphoproliferative disease (XLP), which is characterized by impaired GC formation and antibody responses [17, 18]. SLAM and SAP play critical roles in Tfh cell development and function. First, SAP is required for the formation and maintenance of T-B conjugates [16] through SLAM self-combination, which can regulate the capability of Tfh cells to help GC B cells. Second, SAP may modulate the crosstalk between SLAM and T cell receptor (TCR)[19, 20] by engaging with APCs (B cells). Third, it has been reported that SLAM can induce GC Tfh cells to synthesize IL-4 through SAP in a Th2-independent manner, suggesting the important role of SLAM in the regulation of cytokine expression in Tfh cells [21].

Given the pivotal role of Tfh cells in humoral immunity, it is worth determining whether CD4⁺CXCR5⁺ Tfh-like cells and SLAM/SAP in Tfh cells are involved in the progression of AR, which is an immune disorder disease accompanied by high levels of IgE. We documented that the decreased SLAM expression on CD4 + CXCR5 + T cells might upregulate the expression of IL-21, which may be involved in the pathogenesis of allergic rhinitis.

Materials And Methods

Individuals and samples

Twenty-two patients with AR, including 7 males and 15 females, who were treated in the Department of Otorhinolaryngology-Head were involved in this study. The diagnosis of AR was based on the commonly accepted clinical criteria, including nasal congestion, nasal drainage, sneezing, itching and pale nasal mucosa. None of the patients were being treated with oral or topical therapy. Twenty healthy subjects who exhibited no symptoms or history of allergic diseases were included as controls. Peripheral venous blood samples were obtained from all patients and healthy controls. The serum IgE levels were tested and were found to be significantly higher in AR patients than in healthy controls ($p < 0.05$). The

characteristics of the patients and controls are shown in Table 1. All samples were obtained in accordance with the regulations and approval of the Affiliated People's Hospital of Jiangsu University.

Cell isolation and purification

Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation with Ficoll-Hypaque solution (Haoyang Biological Technology Co., Tianjin, China). CD4⁺ T cells were isolated from PBMCs through immunomagnetic cell sorting using negative selection kits, and CXCR5⁺ cells were purified from CD4⁺ T cells by FITC-conjugated anti-human CXCR5 mAb and anti-FITC microbeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's instructions.

CD4⁺CXCR5⁺ T cell culture and stimulation

Ninety-six-well cell culture plates were precoated with 0.5 µg/ml monoclonal anti-CD3 (Miltenyi Biotec GmbH) for 2 h at 37°C. First, the sorted CD4⁺CXCR5⁺ cells (1×10⁵/well) were cultured in RPMI 1640 medium supplemented with 10% FBS and anti-SLAM or control IgG (BioLegend, San Diego, CA) for the indicated times at 37°C in 5% CO₂. Second, 50 ng/ml phorbol myristate acetate (PMA) and 1.0 µg/ml ionomycin (Sigma-Aldrich St. Louis, MO) were added to the cell culture plates for 5 h. Then, the supernatants were collected, and IL-21 was detected by ELISA.

ELISA

IL-21 in cell culture supernatant and serum and IgE in serum were detected by ELISA according to the manufacturer's instructions (eBioscience, San Diego, CA).

Flow cytometry

Cells were washed and immunostained with PE-Cy5-conjugated anti-CD3, PE-conjugated anti-SLAM, PE-conjugated anti-SAP (eBioscience, San Diego, CA), APC-conjugated anti-CD4, FITC-conjugated anti-CD19 (BioLegend, San Diego, CA), and Alexa Fluor 488-conjugated anti-CXCR5 (Becton Dickinson, San Jose, CA) mAbs against the human cell surface. Isotype-matched Ab controls were used in all procedures. The whole staining process was performed according to the manufacturer's protocol. The stained cells were analyzed on a FACSCalibur flow cytometer with CELLQUEST software (Becton Dickinson, Sparks, MD).

Statistics

Statistical significance was determined by the unpaired t-test (two-tailed with an equal SD), and correlations between variables were determined by calculation of Spearman's correlation coefficient. All data were analyzed with GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA). A p-value <0.05 was considered statistically significant.

Results

Decreased expression of SLAM and SAP in circulating Tfh-like cells in AR patients

First, we investigated the percentages of circulating Tfh-like cells in AR patients. However, there were no significant differences in the frequencies of CD4⁺CXCR5⁺ T cells in the peripheral blood in AR patients compared with those in healthy controls (Fig 1 A, B, C).

Next, we focused on SLAM and the adapter protein SAP, which are important for Tfh cell development. Compared with that in healthy individuals, lower expression of SLAM (Fig 1. D, E, F) and SAP (Fig 1. G, H) in CD4⁺CXCR5⁺ T cells was detected in AR patients according to flow cytometric analysis. Meanwhile, a positive correlation between the expression of SLAM and SAP in CD4⁺CXCR5⁺ T cells from AR patients (Fig 1. I) suggested that SLAM and SAP decreased at an equal rate. Additionally, the expression of SLAM and SAP were negatively correlated with the serum IgE levels in AR patients (Fig 1 J, K).

High levels of IL-21 in AR patients

As a functional cytokine of Tfh cells, IL-21 has the most potent capacity to drive plasma cell differentiation. Notably, the IL-21 levels in serum were significantly increased in AR patients (Fig 2 A), and there was a positive correlation between the expression of IL-21 and IgE (Fig 2 B). Interestingly, we found a negative correlation between the levels of IL-21 and the expression of SLAM or SAP in CD4⁺CXCR5⁺ T cells (Fig 2 C and D).

SLAM stimulation reduced the production of IL-21 in CD4⁺CXCR5⁺ Tfh-like cells

Since the data shown above indicated that serum IL-21 levels were negatively correlated with the expression of SLAM in CD4⁺CXCR5⁺ T cells, we wanted to determine whether SLAM expression on CD4⁺CXCR5⁺ T cells contributed to the reduced production of IL-21. CD4⁺CXCR5⁺ cells were isolated from PBMCs (Fig 3A) and stimulated with anti-SLAM mAb. There was a significant decrease in IL-21 production compared with that of control IgG (Fig 3B). The experiment showed that the change occurred after 48 h (Fig 3C) and was dose-dependent to some extent (Fig 3D).

Decreased expression of SLAM on CD19⁺ B cells in AR patients

As B cells and Tfh cells are the most important cells that participate in humoral immunity, their development is tightly interrelated. B cells can not only receive signals from Tfh cells to differentiate into plasma cells to produce immunoglobulin but can also deliver signals to Tfh cells as APCs. SLAM could homotypically bind and function as a costimulatory molecule [22, 23] and could be expressed on the surfaces of both T and B cells. Therefore, we further explored the changes in B cells. As shown in Fig 4A and B, we observed an increased percentage of B cells in patients with AR. However, the expression of SLAM on the B cell surface was decreased in AR patients compared to that in healthy controls (Fig 4. C and D).

Discussion

The dysregulation of Th1/Th2 cells has predominated investigations of the mechanism of allergic disease over the past 20 years. We sought to add an additional layer of complexity to the regulation of allergic inflammation. Recently, an increasing number of studies have already reported that the frequencies of circulating Tfh cells are increased in autoimmune diseases, which is accompanied by high levels of IL-21 and autoantibodies. However, the role of Tfh cells in IgE-mediated diseases is not well understood.

In the present study, we found no significant differences in the percentages of circulating CD4⁺CXCR5⁺ Tfh-like cells in peripheral blood between AR patients and healthy controls. This might be due to the predominately localized immune response in AR patients. Recently, Zhang Y N et al. confirmed that CD4⁺CXCR5⁺ Tfh cells accumulated and expanded in nasal polyp tissue and could promote IgG, IgA, and IgE production by naive B cells, and this could be blocked by depletion of IL-21 to some extent [24]. Blood CD4⁺CXCR5⁺ Tfh-like cells were shown to be circulating and to not fully reflect the local immunity. Another explanation was that blood CD4⁺CXCR5⁺ T cells were Tfh-like cells, and they were suggested to be resting cells with a memory phenotype [25]. Only a small proportion of blood CD4⁺CXCR5⁺ T cells expressed the activation molecules (ICOS and CD69) that are expressed by conventional follicular Tfh cells. Another recent study demonstrated that circulating CD4⁺CXCR5⁺ T cells can be defined as three subsets according to their differential expression of CXCR3 and CCR6 [10]. Only Th2-like CXCR5⁺ T cells (CXCR3⁻CCR6⁻) could induce naive B cells to produce IgE. Therefore, additional molecular markers could be used to define CD4⁺CXCR5⁺ T cells more precisely. The percentages of activated CD4⁺CXCR5⁺ T cells or Th2-like CXCR5⁺ T cells might make a difference in the severity of AR.

Here, we documented that CD4⁺CXCR5⁺ T cells in AR patients displayed decreased expression of the surface molecules SLAM and SAP. The decreased degree of SLAM and SAP expression reflected the disease severity to some extent by being negatively correlated with serum IgE. Since SLAM was expressed at higher levels on Th1 cells than on Th2 cells and could induce IFN- γ production [26], it was used as a marker of the Th2 to Th1 shift. Previous studies showed increased SLAM expression in Th1-predominant autoimmune diseases, such as multiple sclerosis [27] and rheumatoid arthritis [28]. Additionally, diminished expression of allergen-induced SLAM mRNA in PBMCs was observed in allergic rhinitis, and SLAM expression was recovered after specific immunotherapy (SIT) [13]. These reports supported our observation of the decreased expression of SLAM on CD4⁺CXCR5⁺ Tfh-like cells in AR.

We also discovered the decreased expression of SLAM on B cells and an equal decrease in the expression of both SLAM and SAP on CD4⁺CXCR5⁺ Tfh-like cells. SLAM primarily allows Tfh cells to adhere to B cells, and Tfh cell differentiation is induced by signals conveyed by B cells that sustain prolonged T-B conjugation times via SAP-dependent SLAM adhesion. Therefore, our observations suggested that there was a decrease in the formation of Tfh-B conjugates of SLAM-SLAM-SAP, indicating a change in the signal passed between Tfh cells and B cells.

As the most functional cytokine in Tfh cells, IL-21 showed levels that were significantly increased in AR patients and positively correlated with IgE. The role of IL-21 in IgE-mediated allergic diseases remains enigmatic, as conflicting results have been reported thus far. Salzer et al. reported that homozygous loss-of-function mutations in the IL-21 gene were associated with reduced numbers of CD19⁺ B cells and decreased serum IgG levels but an increased IgE concentration [29]. However, some in vitro experiments have shown that IL-21 enhances the production of IgE and promotes the proliferation of CD19⁺ B cells when they are stimulated with anti-CD40 and IL-4 or IL-13[30]. These discrepancies might be due to the pleiotropic effects of IL-21 and the complexity of the immune environment in vivo. Here, we speculate that high levels of serum IL-21 lead to high levels of IgE, which predominates type 2 immunity, in AR, and that the Th2 cytokines IL-4 and IL-13 also showed high concentrations. In addition to high levels of IL-21 in serum, a previous study indicated that there were higher frequencies of IL-21⁺ CD4⁺CXCR5⁺ T cells in allergic asthma patients [31]. Here, we speculated that CD4⁺CXCR5⁺ Tfh-like cells in AR patients could be more capable of producing IL-21, although the percentages of CD4⁺CXCR5⁺ Tfh-like cells showed no changes.

Because the decreased expression of SLAM and SAP was negatively correlated with the enhanced expression of serum IL-21, we examined the influence of SLAM on IL-21 production by CD4⁺CXCR5⁺ T cells. We observed that the engagement of SLAM resulted in a significant decrease in IL-21 expression in CD4⁺CXCR5⁺ T cells. This reduction in expression occurred at 48 h after stimulation and was maintained for 72 h, which revealed the prolonged effects. Downregulation of IL-21 occurred in a dose-dependent manner; the stronger the stimulation by SLAM was, the greater the reduction in the expression of IL-21 was. Conversely, insufficient/weak stimulation of CD4⁺CXCR5⁺ Tfh-like cells by SLAM resulted in more IL-21 production. This finding was consistent with our observation of the decreased expression of SLAM and high levels of IL-21 in AR patients. Therefore, we speculated that the low SLAM expression on CD4⁺CXCR5⁺ Tfh-like cells might contribute to the high levels of serum IL-21 in AR patients. Additionally, we reported increased percentages of B cells. This was in line with the high levels of IL-21 and IgE in serum in AR patients. IL-21 could drive more B cells to differentiate into plasma cells to secrete immunoglobulin, including IgE, which likely contributes to the pathogenesis of AR.

Based on a literature review, this report takes the initiative to focus on the role of SLAM on CD4⁺CXCR5⁺ Tfh-like cells. Our study provided evidence that decreased SLAM expression on CD4⁺CXCR5⁺ Tfh-like cells might upregulate the production of IL-21 to contribute to the pathogenesis of allergic rhinitis. Future studies need to be performed 1) to investigate SLAM expression on CD4⁺CXCR5⁺ Tfh-like cells in different allergic diseases, 2) to investigate SLAM expression on CD4⁺CXCR5⁺ Tfh-like cells in local tissues, and 3) to analyze the involvement of downstream factors that account for SLAM suppression of IL-21 expression. In terms of the value of this research, the data in the study extend the understanding of the pathogenesis of AR and identify a potential biomarker and therapeutic target.

Conclusion

In this study, we report for the first time that the decreased expression of SLAM on CD4⁺CXCR5⁺ Tfh-like cells contributes to enhance IL-21 production in AR patients, which might be further validated as a potential therapeutic strategy.

Abbreviations

AR: Allergic rhinitis;

GC: germinal center;

IL-21: interleukin 21;

PBMCs: peripheral blood mononuclear cells;

SAP: SLAM-associated protein;

SLAM: Signaling lymphocytic activation molecule;

Tfh: follicular helper T cells;

Th: helper T cells

Declarations

Acknowledgements

Not applicable.

Authors' Contributions

JY performed the experiments, analyzed the data, and wrote the paper; LG and XT performed the experiments; YM, HP, JT, and HX analyzed the data; SW designed the study and wrote the paper. All authors read and approved the final manuscript.

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Availability of data and material

All data generated or analyzed during this study are included in the published article. The datasets used and/or analyzed in this study are available on request from the corresponding author.

Ethics approval and consent to participate

This study was approved by the ethics committee of the Affiliated People's Hospital of Jiangsu University. Patients gave written informed consent prior to collection of their blood and tissue specimens.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1
Clinical features of AR patients included in the study

	AR	HC
N	22	20
Gender (M/F)	7/15	8/12
Age (yr)	34.43 ± 2.820	30.94 ± 3.265
IgE (ng/ml)	319.1 ± 70.03 *	144.8 ± 5.729
Data represent the arithmetic mean ± SD. M, Male; F, Female. *, p < 0.05.		

Figures

Figure 1

Expression of SLAM/SAP in CD4⁺CXCR5⁺ T cells in AR patients. A, B, Gating strategy and representative flow plot. C, Frequencies of CXCR5⁺ cells among CD4⁺ T cells within PBMCs. D, Detection of SLAM/SAP expression was gated on CD3⁺CD4⁺CXCR5⁺ T cells. E, Representative SLAM plot. F, The expression of SLAM. G, Representative plot of SAP. H, The expression of SAP. I, The correlation between the expression of SLAM and SAP in CD4⁺CXCR5⁺ T cells from AR patients. J, The correlation between the expression of SLAM and IgE in AR patients. K, The correlation between the expression of SAP and IgE in AR patients.

Figure 2

High levels of IL-21 in AR patients A, Level of serum IL-21. B, The correlation between serum IL-21 and IgE. C, The correlation between SLAM and IL-21. D, The correlation between SAP and IL-21.

Figure 3

SLAM reduced the production of IL-21 in CD4+CXCR5+ T cells. A, The purity of CD4+CXCR5+ cells was analyzed by FCM. B, The levels of IL-21 secreted by CD4+CXCR5+ T cells with anti-SLAM mAb stimulation (0.5 μ g/ml, 48 h). C and D, The levels of IL-21 secreted by CD4+CXCR5+ T cells after anti-SLAM stimulation at different time points or at different concentrations.

Figure 4

Expression of SLAM on the B cell surface in AR patients. A, Representative flow plot of CD19+ B cells. B, Frequencies of CD19+ B cells in PBMCs. C, Representative plot of SLAM. D, The expression of SLAM on the CD19+ B cell surface.