Correlation of gammadelta-T-cells, Th17 cells and IL-17 in peripheral blood of patients with allergic rhinitis

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Summary

Background: **Gammadelta-T-cells play an important role in the mucosal immune system of the respiratory tract. Th17 cells plays an important role in promoting inflammation and autoimmune diseases. Th17 cells mainly secrete IL-17. However, little information is available on the relation between gammadelta-T-cells and Th17 cells in allergic rhinitis (AR).**

Objective: **To explore the correlation between the prevalence of gammadelta-T-cells, the prevalence of Th17 cells and the expression of IL-17 in the peripheral blood of patients with AR.**

Methods: **Peripheral blood from the AR group (32 cases) and the control group (20 cases) was collected from March 2012 to July 2012. The percentages of gammadelta-T-cells and Th17 cells were measured by flow cytometry, and the levels of IL-17 were measured by ELISA.**

Results: **The percentage of gammadeta-T-cells and Th17 cells in the AR group were significantly higher than those in the control group ($P < 0.01$). The levels of IL-17 in the AR group were also significantly higher than those in the control group ($P < 0.01$). There were positive correlations between the gammadelta-T-cell percentage and the Th17 cell percentage in the peripheral blood of AR patients ($r = 0.597$, $P < 0.01$); and between the gammadelta-T-cells percentage and the levels of IL-17 ($r = 0.469$, $P < 0.01$). However, no correlation was found between the Th17 cell percentage and the levels of IL-17 ($r = 0.100$, $P > 0.05$).**

Conclusion: **Gammadelta-T-cells and Th17 cells may be involved in the pathogenesis of AR; gammadelta-T-cells may primarily be associated with the secretion of IL-17.** *(Asian Pac J Allergy Immunol 2014;32:235-9)*

Introduction

Allergic rhinitis (AR) is caused by exposure to allergens in atopic individuals leading to the IgE-mediated release of histamine. Various immune cells and cytokines are involved in non-infectious nasal mucosal and chronic inflammatory diseases. The AR incidence rate has gradually increased in recent years. The disease seriously impacts patient health and quality of life.[1] It has been reported that T-cell immunity is closely associated with the pathogenesis of AR.[2] T-cells are categorized as αβT-cells or γδT-cells based on their T-cell receptors (TCR) for antigen recognition. αβT-cells play an important role in the adaptive immune response, whereas γδT-cells are not restricted by the major histocompatibility complex (MHC) and are not dependent on antigen processing and presentation. γδT-cells function as a bridge between innate and adaptive immunity and play an important role in the mucosal immune system of the respiratory tract. In addition, γδT-cells provide bidirectional immune regulation through the secretion of various cytokines (e.g. IL-17 and interferon-gamma [INFγ]).[3] Th17 cells are a new αβT-cell subset that plays an important role in promoting inflammation and autoimmune diseases. Th17 cells mainly secrete IL-17 and express RORγt, which is a key nuclear transcription factor.[4,5] Our previous studies showed that expression of IL-17 is significantly higher in the peripheral blood of AR patients than in that of the control group,[6] and patients with persistent AR showed a significant difference in the percentage of peripheral blood γδT-cells before and after treatment with specific immunotherapy (SIT).[7] The aim of this study was to investigate the γδT- and Th17 cells, IL-17 by flow

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cytometry and ELISA in the peripheral blood of AR patients. Our data suggest that γδT-cells and Th17 cells may be involved in the pathogenesis of AR.

Methods

Research subjects

Blood samples from thirty-two AR patients that received treatment in our department were collected from March 2012 to July 2012. All patients were adults, aged from 18 to 45 years. The patients included 20 males and 12 females with an average age of 29.6 years. The diagnosis of all AR patients met the standard criteria.[1] Inclusion criteria were moderate/severe allergic rhinitis at least for one year and a skin prick test positive only for the dust mite allergen (ALK, Hørsholm, Denmark). Exclusion criteria were nonallergic inflammatory nasal pathology, chronic sinusitis, asthma and aspirin intolerance, active tuberculosis, an autoimmune disorder, or an immunodeficiency disorder. No patients received systemic or topical corticosteroids, antihistamines or immunotherapy within a month of enrolment. Twenty healthy volunteers (control group, age from 20 to 43 years) from our hospital with no symptoms of allergic rhinitis and negative results in a skin prick test were used in this study, including 9 males and 11 females with an average age of 30.9 years. All subjects had skin prick test for a series of aeroallergens, including dust mite, cockroach, cat hair, dog dander, molds, ragweed, mugwort, pollen. The IRB had approved the research. All participants signed informed consent forms before participation in the experiments.

Instruments and reagents

Flow cytometry was performed with a BD FACSCalibur™ model from BD biosciences, USA. Cellquest software (BD Bioscience, USA) was used for data analysis of the flow cytometry. A Bio-Tek ELX-800 microplate reader (Bio-Tek, USA) and the following reagents were also purchased for this study: the γδ TCR kit (BD Bioscience, USA); PMA (phorbol ester), ionomycin calcium (ionomycin) and BFA (MultiSciences Co.); APC-labeled anti-human CD8 monoclonal antibody (BD Biosciences, USA); PerCP-Cy5.5-labeled anti-human CD3 monoclonal antibody, PE-labeled anti-human IL-17 monoclonal antibody and its matched isotype control (eBioscience Co., USA); fixative and lysis buffer (Invitrogen, USA); and the anti-human IL-17 ELISA kit (eBioscience Co., USA).

Sample collection

Four-milliliter samples of peripheral blood in heparin solution were collected in the early morning. Two milliliters of blood sample were centrifuged at 300g to collect serum for the measurement of the serum IL-17 concentrations and were stored in a -20°C freezer. Two milliliters of the sample were used to perform flow cytometry of γδ T cells and Th17 cells within three hours after sample collection.

γδ T cell assessment by flow cytometry

Fifty microliters of anticoagulated whole blood was gently vortexed with 20 μl of CD3 or γδ TCR antibody and incubated at room temperature in the dark for 15 minutes. To each tube, 1 ml of 1xFACS lysis solution was added. The tubes were gently vortexed and incubated at room temperature in the dark for 10 minutes, followed by 300g centrifugation for 5 minutes and resuspension in 500 μl of PBS. Immune flow cytometry was then performed and CellQuest software analysis was used for the data analysis.

Th17 cell assessment by flow cytometry

Two hundred and fifty microliters of peripheral blood was combined with 50 μg/L PMA, 2.0 μmol/L blocking agent (monensin, Gorky Scientific), and 750 μmol/L ionomycin, and then incubated at 37 °C in a 5% CO_2 incubator for 4 hours. Cell suspensions were transferred to 1.5 ml EP tubes and centrifuged at 300g for 6 minutes. Supernatants were removed and the pellets were washed twice with PBS before performing the flow cytometry analysis. Ten microliters of PECy5-anti-CD3 and 10 μl of FITC-anti-CD8 were added into the cell suspensions, followed by incubation at room temperature for 30 minutes. After washing with PBS twice, 300 μl of fixative was added, and the suspensions were incubated in the dark at 4 °C for 15 minutes. After centrifugation, the supernatant was removed and cells were washed twice with PBS and aliquoted into two tubes. To each tube 20 μl PE-anti-IL-17 or 10 μl isotype control PE-IgG1 was added, followed by further incubation at room temperature for 30 minutes. After washing twice in PBS, the cells were resuspended in 300 μl and analyzed using a flow cytometer. CellQuest software was used for data analyses.
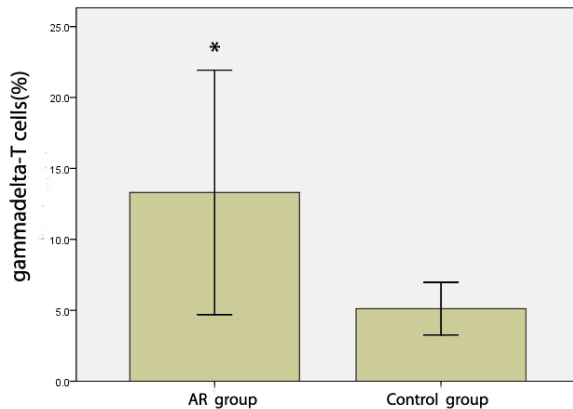
Figure 1. The percentage of γδT cells in the peripheral blood of the AR group and the control group, *, p <0.01, compared with the "control group"
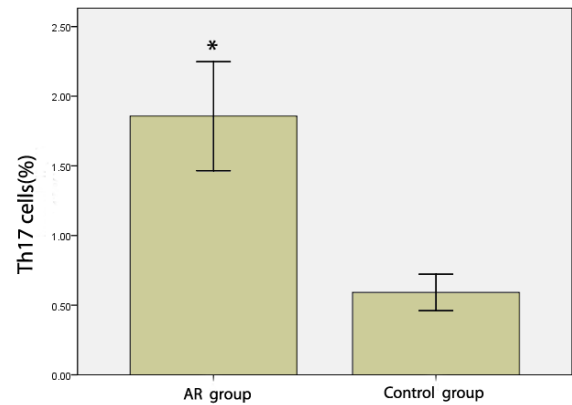
Figure 2. The percentage of Th17 cells in the peripheral blood of the AR group and the control group, *, p <0.01, compared with the "control group"

ELISA for the measurement of serum IL-17 concentration

Measurement of IL-17 was done in accordance with the instruction manual of the ELISA kit. The sensitivity of the IL-17 ELISA was determined to be 0.5 pg/ml.

Statistical analysis

SPSS 16.0 software was used for statistical analysis. Normally distributed data are presented as mean ± standard deviation (χ±s). Correlations between parameters were analyzed using a Pearson correlation test. $P < 0.05$ represents statistically significant differences.

Results

Flow cytometry analysis of the percentage of γδT cell and Th17 cell in the AR and control groups

The percentage of γδT-cells in the AR group (13.30 ± 8.62%) was higher than that in the control group (5.18 ± 1.86%), with statistically a significant difference between the groups ($t = 5.18$; $P < 0.01$; Figure 1).

The percentage of Th17 cells in the peripheral blood of AR patients (1.86 ± 0.39%) was significantly higher than that in the control group (0.59 ± 0.26) ($t = 13.92$; $P < 0.01$, Figure 2).

Analysis of the serum IL-17 levels in the AR and control groups

The serum IL-17 levels in the AR and control groups were (668.55 ± 45.15 pg/ml) and (573.53 ± 17.42 pg/ml), respectively. The IL-17 level in the AR group was significantly higher than that in the control group ($t = 8.97$; $P < 0.01$, Figure 3).

Correlation analyses of the γδT-cell percentage, Th17 cell percentage and IL-17 levels in the peripheral blood of AR patients

A Pearson correlation analysis showed that the percentages of γδT-cells and Th17 cells in the peripheral blood of AR patents were positively correlated ($r = 0.597$; $P < 0.01$; Figure 4). The percentage of γδT-cells and the IL-17 level was also positively correlated ($r = 0.469$; $P < 0.01$; Figure 25). However, no significant correlation was observed between the IL-17 level and the percentage of Th17 cells ($r = 0.100$, $P < 0.05$).

Discussion

γδT-cells mainly reside in the epidermis and mucosa and account for only 1% to 10% of T-cells in the peripheral blood. According to the different combinations of TCRγ and δ chains of the variable (V) region in γδT-cells, γδT-cells are divided into various subsets which carry different functions. There are two main subgroups of human γδT-cells: Vδ2[+] T-cells and Vδ1[+] T-cells. The Vδ2[+] T-cell subset, which primarily includes Vγ2/Vδ2 T-cells, accounts for 80% to 90% of the total circulating γδT-cells, whereas the Vδ1[+] T-cell subset mostly resides in the epithelial mucosa. These subsets share similarities in their receptors and antigens and provide a rapid response to generate large amounts of IL-17 and IFN-γ cytokines.[3,8] In an asthma model, (TCRVδ knockout mice) it was demonstrated that there was reduced serum IgE levels and airway reactivity.[9] Reyes et al.[10] showed that the absence of γδTCR in animal models of allergic conjunctivitis was associated with reduced clinical symptoms,
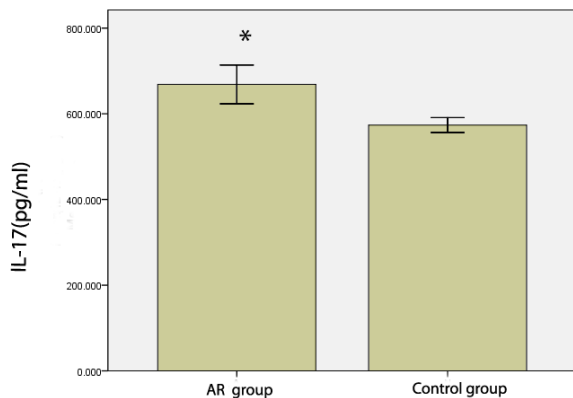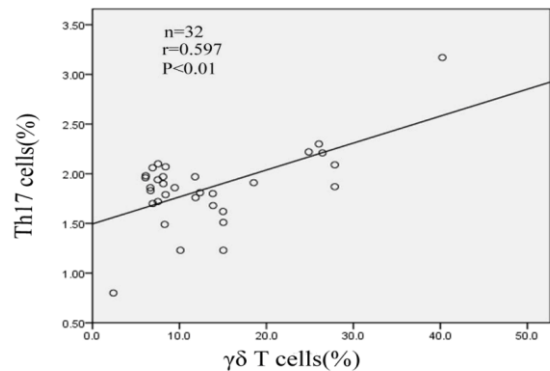
Figure 3. The level of IL-17 cells in the peripheral blood of the AR group and the control group, *, $p < 0.01$, compared with the "control group"

Figure 4. Scatter plot of the percentages of γδ T cells and Th17 cells in the peripheral blood of AR patients.

eosinophil invasion and Th2 cytokine secretion. γδT-cells are increased in the peripheral blood of patients with eczema and dermatitis syndrome [11]. Additionally, our previous study showed that patients with persistent AR had a significant difference in the percentage of peripheral blood γδT-cells before and after treatment with specific immunotherapy (SIT). After SIT treatment, the Vδ1+ and Vδ2+ subsets of γδT-cells were significantly reduced, while the Vδ6 subset of γδT-cells increased selectively.[7] This present study also revealed an increase of γδT-cells in the peripheral blood of AR patients, suggesting that γδT-cells may be involved in the inflammation of AR, and further study for the function of subset of γδT-cells in AR would be worthwhile.

Studies have demonstrated that γδT-cells promote a Th17 cell response after administration of complete Freund's adjuvant in mice,[12] and IL-17 (+) γδT-cells enhance Th17 cell differentiation in T-cell mediated colitis.[13] Ex-vivo PBMNC evaluation has shown that allergic patients have higher frequencies of IL-17 producing T cells.[14] The results of this study also showed that the percentage of Th17 cell in AR was significantly higher than the control group and there was a positive correlation between the Th17 percentage and γδT-cells. These results suggest that Th17 cell may be involve in AR pathogenesis and that γδT-cells may have an immunomodulatory function on Th17 cells in the peripheral blood of AR patients.

IL-17 is an important effector of Th17 cells with a strong pro-inflammatory role in rheumatoid arthritis. Increased expression of IL-17 has been observed in multiple sclerosis, asthma, systemic lupus erythematosus and transplant rejection.[15] IL-17 may play a pathogenic role in an established AR mouse model.[16,17] Expression of IL-17 is significantly higher in the peripheral blood of AR patients than in the control group.[6] In a study by Ciprandi et al.,[18] a positive correlation was demonstrated between IL-17 expression in the peripheral blood of AR patients and the severity of AR clinical symptoms. Nasal allergen challenge induces the enhanced secretion of IL-17 in the lower airways of nonasthmatic patients with AR.[19] Studies in recent years have shown that γδT-cells are an important source of IL-17 production. In infectious disease models of *Listeria monocytogenes*[20] and *Bacillus subtilis*-induced hypersensitivity pneumonitis,[21] γδT-cells rapidly produces large amounts of IL-17 after the dendritic cells produce IL-23 following infection. Our results demonstrate an increased level of IL-17 and Th17 cells in the peripheral blood of AR patients. Increased levels of IL-17 showed a positive correlation with γδT-cells but no significant correlation with Th17 cells, suggesting that IL-17 in the peripheral blood of AR may be primarily generated by the γδT-cells. Further in-depth research is needed to determine which subset of γδT-cells is involved in the secretion of IL-17.

In conclusion, the present study showed higher expression of γδT-cells, Th17 cells and IL-17and positive correlations between Th17 cells, IL-17 level and γδT-cells in the peripheral blood of AR patients. This study provides preliminary evidence that γδT-cells and Th17 cells may be involved in the pathogenesis of AR. More studies with a large sample size are required to confirm the role of γδT-
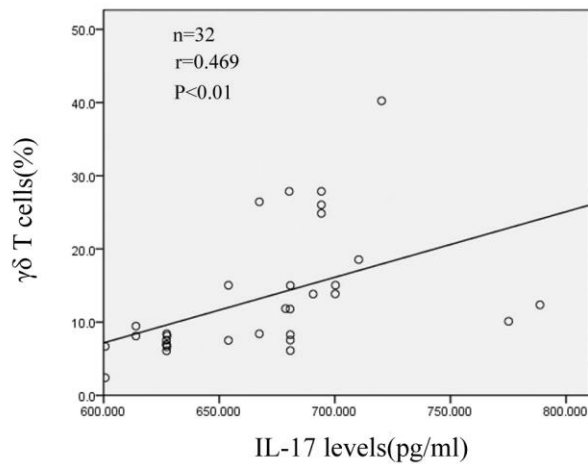
Figure 5. Scatter plot of the percentages of $\gamma\delta$T cells and IL-17 levels in the peripheral blood of AR patients.

cells and Th17 cells in the AR and assess IL-17 expression at a cell level.

Acknowledgement

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