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Increased expression of IL-1R8 and a possible immunomodulatory role of its ligand IL-37 in allergic rhinitis patients

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ABSTRACT

Allergic rhinitis (AR) is a chronic inflammatory airway disease that is caused by an abnormal T cell response. T helper (Th)-17 cells and Th2 cells are the $CD4^+$ T cell subsets implicated in the pathogenesis of AR. The suppression of excessive responses of these Th17 and Th2 cells has been reported to be an effective therapeutic approach to treat AR patients, and continuous efforts are being undertaken to find new methods to modulate the function of these cells. Recent studies have shown that IL-1R8 and its ligand IL-37 negatively regulate the immune response. In this study, we investigated the immunomodulatory the roles of IL-37/IL-1R8 axis in AR patients. We found that IL-1R8 expression was very low on dendritic cells (DCs) and resting $CD4^+$ T cells but increased strongly on $CD4^+$ T cells following T cell activation. Furthermore, IL-1R8 expression on $CD4^+$ T cells was markedly higher in AR patients than in healthy controls. The IL-1R8 ligand IL-37 could act on $CD4^+$ T cells to inhibit IL-17 and IL-4 production but could not influence DC-induced IL-17- and IL-4-producing $CD4^+$ T cell responses. Meanwhile, recombinant IL-37 (rIL-37) did not influence IL-6, IL-1β, and IL-10 production by DCs and expression of co-stimulatory molecules (including CD80, CD40, CD86 and HLA-DR) in DCs. Thus, IL-37 may regulate aberrant T cell immune response of allergic rhinitis mainly through $CD4^+$ T cells, not DCs. The immunomodulatory roles of the IL-37/IL-1R8 axis indicate the therapeutic potential of this axis in AR.

1. Introduction

Allergic rhinitis (AR), an allergic inflammatory reaction of the nasal mucosa, is an important health problem because of its serious impact on patients' quality of life and productivity. The incidence rate of AR is estimated at 10–20% worldwide and is still increasing and reaching almost 50% in some populations [1,2]. Although AR has been studied for decades, the details of its pathogenic mechanisms have not been clearly elucidated.

AR is classically characterized by tissue eosinophilia and the production of allergen-specific IgE, both of which are under the regulation of T helper (Th)-2 cells. In recent years, researchers have revealed that Th17 cells play an important role in the development of allergic airway inflammation and can contribute to increasing the severity of inflammatory diseases [3,4].

Interleukin-1 receptor 8 (IL-1R8) [also known as single IgG IL-1-related receptor (SIGIRR)] is a member of the TIR domain-containing family of receptors, and it negatively regulates Toll-like receptor/IL-1R signalling. The anti-inflammatory role of IL-1R8 in mouse models has been revealed by several studies. IL-1R8 is highly expressed on in vitro-polarized Th17 and Th2 cells and plays an important role in the suppression of Th17 and Th2 responses [5,6].

Recently, IL-1R8 has been shown to be the receptor of Interleukin-37 (IL-37) and indispensable for carrying out the anti-inflammatory functions of IL-37 [7]. IL-37, a newly discovered anti-inflammatory cytokine, broadly down-regulates innate inflammation and acquired immune responses [8]. In IL-37 transgenic (IL-37tg) mice, lipopolysaccharide (LPS) induces a lower expression of the co-stimulatory molecule CD86 and class II antigen-presenting molecules on dendritic cells (DCs) than in wild-type mice [9]. It has been reported that in a mouse model of asthma, via IL-1R8, the intranasal administration of recombinant IL-37 (rIL-37) could significantly attenuate the hallmarks of experimental asthma and suppress the production of Th2-associated cytokines [10].

Taken together, these results indicate that the IL-37/IL-1R8 axis may participate in feedback mechanisms and act as an attenuator of T cell-driven inflammatory responses. However, the detailed mechanism has not been elucidated. Given the differences between species and

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diseases, we investigated the immunomodulatory roles of the IL-37/IL-1R8 axis in AR patients and healthy human controls. We analysed the expression of IL-1R8 on DCs and $CD4^+$ T cells from AR patients and healthy controls; furthermore, we explored whether IL-1R8 activated by its ligand IL-37 could be exploited to inhibit the development of aberrant T cell responses in AR patients and whether the IL-37/IL-1R8 axis is a potential target for the treatment of AR.

2. Materials and methods

2.1. Study subjects

Blood samples were collected from 32 AR patients (18 men and 14 women) and 20 healthy controls (13 men and 7 women) between December 2016 and October 2017. The diagnosis of AR was made according to history and positive skin prick tests (SPTs) [11]. The SPTs were performed after blood collection in accordance with the guidelines of the European Academy of Allergy and Clinical Immunology [12]. The patient did not use corticosteroids or receive desensitization therapy for one month prior to blood collection. In addition, patients with rhinosinusitis, asthma, or systemic diseases were excluded from the study. The Clinical Ethical Research Committee of the First Affiliated Hospital of Chongqing Medical University approved this study (permit number: 2016-123), and each patient and healthy subject provided written informed consent to participate in this study.

2.2. Cell isolation and culture

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by Ficoll-Hypaque density-gradient centrifugation. $CD14^+$ monocytes and $CD4^+$ T cells were positively selected from PBMCs of AR patients and normal controls by magnetic microbeads (both purity > 96%, Miltenyi Biotec, Germany). The purified $CD4^+$ cells and $CD14^+$ cells were resuspended at 1×10^6/ml in RPMI-1640 medium supplemented with 10% FBS and cultured at a constant temperature of 37 °C in a 5% CO_2-containing humidified atmosphere. To study the effect of rIL-37 on $CD4^+$ T cells, we stimulated $CD4^+$ T cells from both groups with rIL-37 (0, 1, 10, and 100 ng/ml, R&D Systems, USA) in the presence of plates coated with anti-CD3 antibodies (2 μg/ml, eBioscience, USA) and soluble anti-CD28 antibodies (2 μg/ml, eBioscience) for 72 h.

To induce DC differentiation, we seeded isolated $CD14^+$ cells in 24-well plates (1.0×10^6 cells/well) in the presence of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (100 ng/ml, PeproTech, UK) and recombinant human IL-4 (50 ng/ml, PeproTech) for 72 h. Half of the culture medium containing recombinant cytokines was subsequently refreshed. After another 72 h, the monocyte-derived DCs were stimulated with 100 ng/ml LPS in the presence of rIL-37 (0, 1, 10, and 100 ng/ml) for 24 h. Then, the supernatants were harvested for ELISA; DCs were washed and co-cultured with allogeneic $CD4^+$ T cells at a ratio of 1:5 (1.0×10^5:5.0×10^5 cells/ml).

2.3. Quantitative real-time reverse transcription-PCR (RT-qPCR) analysis

Total RNA samples from $CD4^+$ T cells were extracted using RNAiso Plus (TaKaRa, China) and were reverse transcribed using a PrimeScript RT reagent kit (TaKaRa) according to the manufacturer's instructions. RT-qPCR was performed using SYBR Premix Ex Taq™ II (TaKaRa) with the following primers: IL-1R8 forward sequence, CCTCCTTCACTCTTC AGAGAGC and reverse sequence, ACGGCACTTGACATAGAGCAGG; GAPDH forward sequence, GGATGCCTTGCCCACAGGGT and reverse sequence, GTTGGGGGTTCTGGGGACTGGC. GAPDH was used as a housekeeping gene.

2.4. ELISA

Levels of IL-1β, IL-6, IL-4, IL-10 and IL-17 in culture media were detected using ELISA kits (eBioscience) according to the manufacturer's instructions.

2.5. Flow cytometry

For cell surface target staining, cells were pre-incubated with an Fc receptor-blocking antibody (eBioscience) at room temperature for 15 min to reduce nonspecific staining and then stained with anti-human CD86-APC, anti-human CD80-PE, anti-human CD40-PerCP CY5.5, anti-human HLA-DR-PE/Cy5, anti-human CD3-FITC, anti-human CD8-APC (eBioscience), anti-human CD14-FITC (BioLegend, USA), anti-human IL-1R8-APC (R&D Systems) antibodies and the appropriate isotype controls (eBioscience or BioLegend) for 30 min at 4 °C. For intracellular antigen staining, $CD4^+$ T cells were pre-incubated with a Cell Stimulation Cocktail (eBioscience) for 5 h at 37 °C. The cells were then washed, fixed, and permeabilized using the Cytofix/Cytoperm Kit (eBioscience) according to the manufacturer's instructions and then stained with anti-human IL-17A-PE and anti-human IL-4-PE antibodies at room temperature for 30 min. The cells were then washed twice and resuspended for flow analysis.

2.6. Statistical analysis

Independent samples test and paired sample *t*-test were performed using the SPSS 19.0 software. The differences were considered statistically significant when the *P*-values were < 0.05.

3. Result

3.1. IL-1R8 expression was significantly increased on activated $CD4^+$ T cells from AR patients

To address whether IL-1R8 expression is regulated during $CD4^+$ T cell activation, we analysed the frequency of IL-1R8-positive $CD4^+$ T cells by flow cytometry. IL-1R8 expression was very low on freshly isolated $CD4^+$ T cells, but the levels significantly increased after stimulation with anti-CD3/CD28 antibodies for three days in both AR patients ($P < 0.001$) and healthy controls ($P < 0.001$). Furthermore, the expression of IL-1R8 on resting $CD4^+$ T cells ($P < 0.05$) and activated $CD4^+$ T cells ($P < 0.05$) obtained from AR patients was higher than that on corresponding cells from healthy controls (Fig. 1a-c). Moreover, IL-1R8 mRNA expression was found to be in line with flow cytometry analysis data (Fig. 1d).

3.2. IL-1R8 was highly expressed on IL-17- and IL-4-producing cells

Since an increased expression of IL-1R8 on activated $CD4^+$ T cells was observed, we investigated the possible relationship between IL-1R8 expression and Th17 and Th2 responses. In AR patients, flow cytometric analysis revealed that the percentage of IL-1R8-positive cells was much higher in $IL\text{-}17^+$ and $IL\text{-}4^+$ populations than in corresponding $IL\text{-}17^-$ ($P < 0.01$) and $IL\text{-}4^-$ ($P < 0.01$) populations. Similar results were obtained in healthy controls ($P < 0.01$ and $P < 0.01$, respectively) (Fig. 2a, b).

3.3. rIL-37 inhibited hyperactive IL-17- and IL-4-mediated $CD4^+$ T cell responses in both AR patients and healthy controls

To investigate the effect of the IL-1R8 ligand IL-37 on IL-17- and IL-4-mediated $CD4^+$ T cell responses, we stimulated $CD4^+$ T cells from AR patients and healthy controls with various concentrations of rIL-37 (1–100 ng/ml) for 72 h. ELISA results demonstrated that the expression of IL-17 was significantly increased in AR patients ($P < 0.01$).