Effects of IL-17 on Expression of GRO-α and IL-8 in Fibroblasts from Nasal Polyps

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Summary: Recent studies indicated that interleukin (IL)-17, growth-related oncogene (GRO)-α and IL-8 play an important role in the pathogenesis of nasal polyps. However, the effects of the increased amount of IL-17 and the production of GRO-α and IL-8 in human nasal polyp fibroblasts are not completely understood. This study aimed to determine the effects of the increased IL-17 on the changes of GRO-α and IL-8 expression in human nasal polyp fibroblasts and further investigate the mechanism of neutrophil infiltration in nasal polyps. Nasal polyp fibroblasts were isolated from six cases of human nasal polyps, and the cells were stimulated with five different concentrations of IL-17. Real-time fluorescence quantitative polymerase chain reaction (RT-PCR) was used to detect the mRNA expression of GRO-α and IL-8. The mRNA of GRO-α and IL-8 was expressed in unstimulated controls and remarkably increased by stimulation with IL-17. Moreover, the levels of GRO-α and IL-8 produced by fibroblasts were increased gradually with the increases in IL-17 concentrations. The present study showed that nasal fibroblasts can produce GRO-α and IL-8, and their production is remarkably enhanced by IL-17 stimulation, thereby clarifying the mechanism of the IL-17 mediated neutrophil infiltration in nasal polyps. These findings might provide a rationale for using IL-17 inhibitors as a treatment for nasal inflammatory diseases such as nasal polyps.

Key words: nasal polyps; neutrophil infiltration; growth-related oncogene-α; interleukin-8; interleukin-17

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common clinical disease characterized by persistent inflammation of the nasal and paranasal mucosa and the protrusion of benign ‘grape-like’ formations in the nasal cavity; these NPs are visible to otolaryngologists during nasal endoscopy[1, 2]. CRSwNP is a major public health concern because of its high incidence worldwide (2%–5%) and the considerable economic burden associated with the disease[3]. However, the pathogenesis of CRSwNP is still poorly understood.

Earlier research on CRSwNP mainly focused on eosinophils, but recent studies have suggested that neutrophils play an important role in chronic inflammation of CRSwNP in Chinese patients[4, 5]. Interleukin (IL)-17 is a key inflammatory cytokine correlated with inflammatory lung disorders by triggering an accumulation of neutrophils[6]. Recent studies have revealed that IL-17 is significantly up-regulated in CRSwNP[7-9], and may modulate the survival of neutrophils in nasal polyps[9]. Therefore, further understanding the precise function of IL-17 in CRSwNP will be of great value in elucidating the pathogenesis of CRSwNP.

The fibroblast, one of the major cell types of nasal polyps, is thought to be a target cell of various cytokines, producing several chemokines and cytokines[10, 11]. In addition, previous studies have demonstrated that growth-related oncogene (GRO)-α and IL-8 can be produced by nasal fibroblasts and play a role in neutrophil infiltration and retention in the connective tissue of nasal polyps[12-14]. However, IL-17-stimulated expression of GRO-α and IL-8 from nasal polyp fibroblasts remains unknown.

In the present study, we examined the GRO-α and IL-8 mRNA levels from the nasal polyp fibroblasts and the effects of IL-17 on the expression of GRO-α and IL-8 in the nasal polyp fibroblasts.

1 MATERIALS AND METHODS

1.1 Patients
Six cellular cultures derived from nasal polyps were used in all the experiments. Nasal polyps were obtained by functional endoscopic sinus surgery in the Department of Otolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (China). All the subjects (4 males and 2 females; aged 23–60 years; median age, 35.8 years) were diagnosed based on the criteria of the European position...
The diagnosis of NPs was established on the basis of the medical history, clinical examination, endoscopic examination and CT imaging. None had taken topical or systemic corticosteroids or other immune-modulating medications for at least one month before surgery. The informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the Institution.

1.2 Isolation and Culture of Primary Nasal Polyp Tissue-Derived Fibroblasts

Resected polyp samples were immediately rinsed to wash out mucus with phosphate buffered saline (PBS) (Wuhan Boster Co., China) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (Wuhan Beyotime Institute of Biotechnology, China) and then nasal polyps were cut into small fragments. Small pieces of nasal polyps were enzymatically dissociated in Dulbecco’s Modified Eagle’s Medium (DMEM; Gibco® Life Technologies, China) containing a mixture of 2 mg/mL collagenase type IV (Gibco, USA) and 10 U/mL DNAse (Invitrogen, USA) for 1.5 h at 37°C, and agitated every 15 min. Cell suspensions were washed and resuspended in the complete medium [DMEM supplemented with 10% fetal bovine serum (FBS; Gibco® Life Technologies, Australia), 100 U/mL penicillin and 100 μg/mL streptomycin]. The cells were then cultured in standard conditions: 5% CO₂, 37°C and constant air humidity. After 1.5 h, the DMEM was removed to exclude the surface epithelial cells and the freshly complete medium was added. The culture was allowed to continue at 37°C and 5% CO₂ for one week, and the medium was changed every three days. After the fibroblast-like cells had reached confluence, they were subcultured until passage 3 or frozen at −80°C until they were used.

1.3 Identification of Primary Nasal Polyp Tissue-Derived Fibroblasts

There was no contamination of epithelial cells and white blood cells under the phase contrast microscope. The monolayer of fibroblasts was morphologically recognized (fig. 1A). Further, the cells were characterized by flow cytometry (FACScan; BD Biosciences, USA) using an anti-human Thy-1 antibody (eBioscience, USA). The cells were trypsinized with 0.25% trypsin and 0.02% EDTA (Wuhan Beyotime Institute of Biotechnology, China) and detached from cell culture flasks. After washing twice with buffer, the cells were pre-incubated with 4% BSA for 1 h at 4°C and then incubated with PE-conjugated anti-human Thy-1 antibody (eBioscience, USA) or with isotype control mouse IgG1 K (eBioscience, USA) for 30 min in the dark at 4°C. Finally, the cells were washed twice with the buffer and analyzed. Approximately 100% of the cells were Thy-1-positive, indicating that the culture was composed of nasal fibroblasts (fig. 1B).

1.4 Total RNA Extraction and cDNA Synthesis

Fibroblasts were seeded in 6-well plates reaching confluence and the complete medium was removed and the fibroblasts were stimulated with various concentrations (1, 5, 25, and 125 ng/mL) of recombinant human IL-17 (rhIL-17; PeproTech, USA) diluted in serum-free DMEM for 24 h. The negative control consisted of cells cultured without stimulation. Appropriate amount of RNAiso Plus (Takara Co., Dalian, China) was added to lyse and homogenize these fibroblasts. The total RNA in the lysate was extracted with chloroform, precipitated with isopropanol, and washed with 75% ethanol. Isolated RNA was dissolved with appropriate amount of RNase-free water. Purity and integrity of RNA were analyzed by spectrophotometry (260/280 nm) and agarase gel electrophoresis, respectively.

Genomic DNA elimination reaction was performed