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Original article

# IL-22/IL-22R1 signaling regulates the pathophysiology of chronic rhinosinusitis with nasal polyps via alteration of MUC1 expression

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*Abbreviations:*
AIA, aspirin-intolerant asthma; AT, alpha-toxins; ATA, aspirin-tolerant asthma; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; DNPCs, dispersed nasal polyp cells; DUTCs, dispersed uncinate tissue cells; NP, nasal polyps; SEB, staphylococcal enterotoxin B; UT, uncinate tissues

ABSTRACT

*Background:* IL-22 is an IL-10-family cytokine that regulates chronic inflammation. We investigated the role of IL-22 and its receptor, IL-22R1, in the pathophysiology of chronic rhinosinusitis with nasal polyps (CRSwNP).
*Methods:* IL-22 and IL-22R1 protein and mRNA expression in NP and in uncinate tissues (UT) from CRS and non-CRS patients was examined using immunohistochemistry and real-time PCR, respectively. Dispersed NP and UT cells were cultured with the *Staphylococcus aureus* exotoxins, staphylococcal enterotoxin B and alpha-toxin, following which exotoxin-induced IL-22 levels and their association with clinicopathological factors were analyzed. Effects of IL-22 on MUC1 expression and cytokine release in NP cells were also determined.
*Results:* IL-22 and IL-22R1 in NP were mainly expressed in infiltrating inflammatory cells and in epithelial cells, respectively. IL-22 mRNA levels in NP were significantly higher than those in UTs from non-CRS patients whereas IL-22R1 levels were conversely lower in NPs. NP cells produced substantial amounts of IL-22 in response to exotoxins. Exotoxin-induced IL-22 production by NP cells significantly and negatively correlated with the degree of local eosinophilia and postoperative computed tomography (CT) score, whereas conversely it positively correlated with the forced expiratory volume in 1s ($FEV_1$)/forced vital capacity (FVC) ratio. IL-22 significantly enhanced MUC1 mRNA expression in NP cells. IL-22-induced MUC1 mRNA levels were significantly and positively correlated with IL-22R1 mRNA levels in NPs.
*Conclusions:* These data suggest that imbalance of IL-22/IL-22R1 signaling regulates the pathogenesis of CRSwNP, including local eosinophilia, via alteration of MUC1 expression.


## Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by mucosal inflammation and remodeling. The condition is often associated with asthma and substantially impairs quality of life due to longstanding symptoms including nasal congestion, headache, and loss of smell.[1,2] While the precise pathogenesis underlying this disease remains poorly understood, imbalances in expression of local cytokines including IL-5 and TGF-β appear to be involved.[3]

IL-22 is an IL-10-family cytokine produced by a variety of cells that include not only $CD4^+$ T cells of the Th0, Th17 and Th22 linage but also innate immune cells such as NK cells and type 3 innate

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lymphoid cells.[4,5] IL-22 has versatile effects on airway inflammation via binding to the IL-22 receptor, which consists of IL-22R1 and IL-10R2.[6] For example, IL-22 promotes the migration of airway smooth muscle cells and, in skin, IL-22 and TNF-α synergistically promote the production of chemokines including eotaxin-1 and eotaxin-2 by keratinocytes.[7,8] In contrast, IL-22 attenuates IL-25 production by airway epithelial cells and inhibits antigen-induced eosinophilic airway inflammation.[9] IL-22 also suppresses IFN-γ-induced expression of MHC class I, MHC class II, ICAM-1, RANTES and IP-10 in bronchial epithelial cells from asthmatic patients.[10] Gene delivery of IL-22 suppressed antigen-induced immune responses and eosinophilic airway inflammation via an IL-10-associated mechanism in a murine model of asthma.[11] In the intestine, IL-22 enhanced the expression of mucin genes including MUC1, which is known to display a regulatory role in mucosal immunity.[12,13] In addition, recent reports showed a dual role of IL-22 in airway inflammation in the mouse.[14,15] Thus, IL-22 is required for the onset of allergic inflammation but functions as a negative regulator of established allergic inflammation.[14] The pro-inflammatory and tissue-protective roles of IL-22 in bleomycin-induced airway inflammation are dependent on the presence or absence of IL-17A, respectively.[15]

To date, only a few reports have demonstrated an association between IL-22 and the severity of CRSwNP.[16,17] Ramanathan *et al.* reported that local mRNA expression of IL-22R1 but not of IL-22 was significantly lower in treatment-recalcitrant CRSwNP compared to responsive CRSwNP, suggesting that refractoriness of CRSwNP is associated with decreased expression of mucosal IL-22R1.[16] Endam *et al.* showed an association between three single nucleotide polymorphisms in IL-22R1 and severe CRS.[17] However, it remains unclear how IL-22 regulates the pathogenesis of this condition.

In the present study, we investigated the local production of IL-22 in NP using a recently developed *ex vivo* system.[18] Local expression of IL-22 and IL-22R1 was compared among various CRS phenotypes, and the role of IL-22/IL-22R1 signaling in the pathogenesis of CRS is discussed. The present findings provide novel insights into the pathogenesis of chronic eosinophilic airway diseases regulated by IL-22, in addition to providing a basis for the regulatory effect of IL-22 in airways via induction of MUC1 expression.

## Methods

### *Patients*

Sixty-six Japanese patients with CRS were enrolled for the quantification of IL-22 and IL-22R1 mRNA in sinonasal mucosa. Briefly, 53 of the 66 CRS patients exhibited NP (CRSwNP). The remainder of the CRS patients demonstrated no visible NP in the middle meatus (CRSsNP: $n = 13$). The diagnosis of CRSsNP and CRSwNP was defined using the criteria reported in a European position paper on rhinosinusitis and nasal polyps.[19] In order to eliminate the effect of macrolides and corticosteroids on the expression of IL-22 and IL-22R1, patients were excluded when they received systemic corticosteroids for at least eight weeks prior to surgery or they received pharmacotherapy for rhinosinusitis, such as macrolide antibiotics or intranasal glucocorticoids for at least three weeks prior to surgery. Thirty-seven patients were asthmatic and had NPs. Of these, 16 patients were considered to exhibit aspirin sensitivity based on their history of asthma attacks precipitated by non-steroidal anti-inflammatory drugs (aspirin-intolerant asthma: AIA). The remainder of the asthmatic patients were diagnosed as aspirin-tolerant asthma (ATA, $n = 21$). During surgery, the NP and uncinate process tissues (UT) were sampled from patients with CRSwNP and CRSsNP, respectively. In addition, 19 non-CRS patients (e.g. blowout fracture or sphenoidal cyst) with normal UT at inspection were enrolled as a control. The clinical characteristics of the patients are presented in Table 1. All patients provided informed consent prior to their participation, and the study was pre-approved by the Human Research Committee of the Okayama University Graduate School of Medicine and Dentistry.

### *Quantification of IL-22, IL-22R1 and MUC1 mRNA in sinonasal mucosa*

Surgically excised NP and UT tissues were soaked in RNAlater™ RNA stabilization reagent (Qiagen, Hilden, Germany) and were stored at −30 °C until use. Extraction of total cellular RNA, reverse transcription to generate cDNA, and real-time quantitative PCR for IL-22 and IL-22R1 were then performed, as described previously.[18] Primers for analysis of GAPDH levels, which were used as an internal control, were purchased from Toyobo (Osaka, Japan). The absolute copy number was calculated for each sample, and samples are reported as copy numbers relative to GAPDH. The sequences and product size of the primers used for PCR were as follows: IL-22, forward 5′-GCTGCCTCCTTCTCTTGG-3′ and reverse 5′-GTGCGGTTGGTGATATAGG-3′ (112 bp); IL-22R1, forward 5′-TCTGCTCCAGCACGTGAAAT-3′ and reverse 5′-GTCCCTCTCTCCGTAC GTCT-3′ (124 bp); MUC1, forward 5′-TTTCCAGCCCGGGATACCTA-3′ and reverse 5′-AGAGGCTGCTGCCACCATTA-3′ (PCR product size; 136 bp).

### *Immunohistochemistry*

Immunohistochemical staining for IL-22 and IL-22R1 was performed according to a previously described protocol.[20] Briefly, 4-μm sections were collected from paraffin-embedded tissue blocks, deparaffinized and rehydrated. The sections were heated in sodium citrate buffer (pH 6.5) in a microwave oven for antigen retrieval and were incubated with primary antibodies including

**Table 1**
Subjects' characteristics.

| Groups | Non-CRS | CRSsNP | CRSwNP without asthma | CRSwNP with ATA | CRSwNP with AIA |
|---|---|---|---|---|---|
| Number | 19 | 13 | 16 | 21 | 16 |
| Sex (male/Female) | 6/13 | 8/5 | 11/5 | 15/6 | 9/7 |
| Age (y) | 54.7 ± 19.0 | 44.0 ± 17.7 | 41.1 ± 19.6 | 50.4 ± 16.7 | 52.3 ± 17.9 |
| Age range (y) | 14–81 | 14–74 | 13–65 | 21–77 | 21–79 |
| Serum IgE (IU/mL) | 204 ± 415 | 283 ± 291 | 243 ± 414 | 701 ± 1047 | 249 ± 252 |
| Blood eosinophil count ($\times 10^2/\mu L$) | 1.56 ± 1.24 | 2.94 ± 2.06 | 3.40 ± 2.52 | 7.17 ± 4.68 | 5.25 ± 3.19 |
| CT grading score (Lund–Mackay) | 0 | 5.8 ± 3.9 | 11.7 ± 6.7 | 18.0 ± 5.4 | 15.6 ± 6.7 |
| $FEV_1$/FVC ratio (%) | 79.9 ± 8.9 | 81.3 ± 5.3 | 80.4 ± 8.5 | 71.3 ± 12.9 | 64.0 ± 12.0 |

ATA, aspirin-tolerant asthma; AIA, aspirin-intolerant asthma; CT, computed tomography; FEV1, forced expiratory volume in one second per forced vital capacity ratio.
Results were shown as a mean ± standard deviation.