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Review article

Interleukin-33 induces mucin gene expression and goblet cell hyperplasia in human nasal epithelial cells

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ABSTRACT

We investigated whether IL-33 is involved in mucus overproduction and goblet cell hyperplasia in eosinophilic chronic rhinosinusitis (ECRS). *IL-33* mRNA was significantly higher in the eosinophilic CRS group than in the non-eosinophilic CRS group from human nasal polyps. IL-33 induced *MUC5AC* mRNA and MUC5AC protein, and also goblet cell hyperplasia at air liquid interface culture in human nasal epithelial cells. In addition to that, IL-33 induced *MUC5B* and *FOXA3*, and reduces *FOXJ*mRNA.

In conclusion, our present study demonstrated that the direct evidence of IL-33 which lead to increase mucin gene and protein expression, as well as goblet cell hyperplasia. This study provides novel insights into the role of IL-33 on mucus overproduction in eosinophilic inflammation of human airways.

Contents

1. Introduction

Chronic rhinosinusitis (CRS) is a common chronic sinus inflammatory disease characterized by distinct cytokine production profiles and tissue-remodelling patterns. CRS can be classified into two types according to the presence of nasal polyps [1]. CRS with nasal polyps (CRSwNP) is often accompanied by Th2-cell-skewed

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eosinophilic inflammation, whereas CRS without nasal polyps by Th1-cell-skewed response especially in the United States and European countries [2]. In contrast, much more heterogeneity of CRSwNP has been reported in East Asian countries such as Japan, Korea, and China [3–5], and therefore, CRSwNP has been further subdivided into two types of clinical forms based on the extent of eosinophilic inflammation: eosinophilic CRS(ECRS) and non-eosinophilic rhinosinusitis (NECRS) [3]. ECRS is an intractable form of rhinosinusitis with mucus hypersecretion and persistent airway inflammation.

The pathophysiologic mechanism of ECRS remains unclear. The Th2-mediated inflammatory response with the effector cytokines interleukin (IL) 4, IL-5, and IL-13 is the classic mediator of eosinophilia. Factors that preferentially induce the Th2 pathway over other inflammatory pathways are currently a focus of many investigations. IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) are cytokines produced predominantly by epithelial cells that are believed to be initiators of the Th2 inflammatory response [6–9]. Although some recent studies detected the expression of TSLP, IL-25, and IL-33 in CRSwNP [10–12], the role of these cytokines in the pathogenesis of CRSwNP remains poorly understood.

Mucins, the major components of mucus, are very-high-molecular-weight glycoproteins that can either be membrane associated or secreted into the extracellular space [13]. MUC5AC is considered to be the predominant mucin in the airway, and can be induced by various pathogens and cytokines including *Pseudomonas aeruginosa* [14], nontypeable *Haemophilus influenzae* [15] and TGF-α [16]. During Th2 inflammation, it is well-known that IL-13 increases *MUC5AC* gene expression in airway epithelial cells [17–19]. Mucus production is a primary defense mechanism for maintaining airway health. However, overproduction of mucin is a common pathological feature in chronic rhinosinusitis, asthma and chronic obstructive pulmonary disease leading to mucociliary dysfunction in the upper and lower airways. Recent studies demonstrated the role of IL-33 in eosinophilic CRS [10,20], but whether IL-33 is involved in mucus overproduction and goblet cell hyperplasia in human nasal epithelial cells still remains unknown.

In the present study, by using human nasal samples, we provide clear evidence that the expression of *IL-33* mRNA is significantly upregulated in ECRS compared to NECRS. In addition, we also showed that IL-33 induces *MUC5AC* gene transcription and protein as well as goblet cell hyperplasia in human nasal epithelial cells. These studies may lead to development of novel therapeutic strategies for controlling mucus overproduction in respiratory eosinophilic inflammation.

2. Materials and methods

2.1. Materials

Human recombinant IL-33 and IL-13 were purchased from R&D systems Inc (Minneapolis, USA).

2.2. Subjects

All samples were taken from patients undergoing surgery at the Department of Otorhinolaryngology-Head & Neck Surgery, Mie University Hospital between 2013 and 2015. Nasal polyps from 16 patients with eosinophilic rhinosinusitis and 6 patients with non-eosinophilic rhinosinusitis were collected (Table 1). Patients were diagnosed as having ECRS according to the JESREC SCORE [21] and then divided into ECRS or Non-ECRS based on the results of nasal endoscopy, a computed tomography scan and blood eosinophils. The study protocols were approved by the Review Board for Human Studies of the Mie University (approval number 2497) and informed written consent was obtained from all participants.

Table 1
Baseline characteristics of the participants.

	NECRS	ECRS
Total no.	6	16
Mean age (y)	63.2	59.1
Gender (male:female)	5:1	10:6
Eosinophil count in blood (average, %)	1.7	10.5
Asthma	0/6	10/16
Smoking status (smoker:non-smoker)	5:1	8:8
Oral steroid usage	0/6	2/16

2.3. Cell culture

Primary human nasal epithelial cells (HNEpC) were purchased from Promo Cell (Heidelberg, Germany) and cultured as follows. Briefly, passage-2 HNEpC cells (1×10^5 cells/culture) were seeded in 0.5 mL of culture medium on Transwell clear culture inserts (24.5-mm, with a 0.45-mm pore size; Costar Co., Cambridge, MA, USA). Cells were cultured using an airway epithelial cell growth medium Kit (Promo Cell, Heidelberg, Germany) under submersion for the first 4 days. The culture medium was changed on day 1, and every other day thereafter. An air-liquid interface (ALI) was created on day 5 by removing the apical medium and feeding the culture only from the basal compartment. The culture medium was changed every other day after the initiation of ALI. The media were supplemented with antibiotics (1% penicillin-streptomycin) during subculture and culture stages and an the antifungal agent, fungizone (1 mL/1000 mL media) (Life technologies, Grand island, NY, USA) after filtering the media. All experiments described here used cultured nasal epithelial cells at 14 days after the creation of the ALI. Either IL-33 or IL-13 with 20 ng/mL were added to the basolateral side and exchanged every other day during the 14 day stimulation.

2.4. PCR

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, California, USA), according to the manufacturer's instructions.

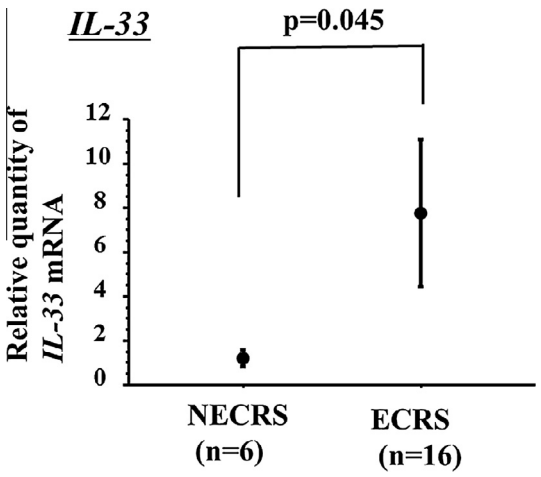

Fig. 1. *IL-33* mRNA levels in nasal polyps from ECRS and NECRS patients. The relative mRNA level of *IL-33* increase in ECRS patients compared to NECRS patients. The nasal polyps obtained from 6 NCRS subjects and 14 ECRS patients were used for the assessment of *IL-33* mRNA by quantitative PCR. Results are expressed as medians. $p < 0.05$ ($p = 0.045$).