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Original article

The effect of calprotectin on TSLP and IL-25 production from airway epithelial cells

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Abbreviations:
AR, allergic rhinitis; DAMPs, damage-associated molecular patterns; ECRS, eosinophilic chronic rhinosinusitis; IL, interleukin; IT, inferior turbinate; NECRS, non-eosinophilic chronic rhinosinusitis; NHBE, normal human bronchial epithelial; NPs, nasal polyps; PNE, primary nasal epithelial; RAGE, receptor for advanced glycation end products; TLR, Toll-like receptor; TSLP, thymic stromal lymphopoietin; UT, uncinate process tissues

ABSTRACT

Background: Calprotectin is a heterodimer complex of the S100A8 and S100A9 proteins, and has various functions as an innate mediator at the sites of inflammation.
The aim of this study was to elucidate the roles of calprotectin in the eosinophilic chronic rhinosinusitis (ECRS).
Methods: Allergen-induced production of calprotectin was evaluated in cultured normal human bronchial epithelial (NHBE) cells by ELISA and RT-PCR. We then examined the roles of calprotectin on *Alternaria alternata* (*Alternaria*)-induced production of thymic stromal lymphopoietin (TSLP) and IL-25 in NHBE cells. The extracellular concentration and allergen-induced secretion of calprotectin in cultured primary nasal epithelial (PNE) cells were examined and compared between patients with ECRS and non-eosinophilic chronic rhinosinusitis (NECRS).
Results: *Alternaria*, house dust mites, protease from *Staphylococcus aureus*, papain, trypsin, poly-inosinic:polycytidylic acid and lipopolysaccharide stimulated calprotectin production in the cultured NHBE cells. The combination of calprotectin and ATP stimulated the production of TSLP and IL-25 in NHBE cells, and calprotectin stimulated *Alternaria*-induced production of TSLP and IL-25, which was suppressed by blocking P2 purinergic receptors and by treatment with siRNA for S100A8, S100A9 or calprotectin receptors (Toll-like receptor 4 or receptor for advanced glycation end products). Allergen-induced calprotectin production was significantly stimulated in PNE cells from patients with ECRS.
Conclusions: These results indicate that calprotectin enhances the allergen-induced Th2-type inflammatory responses in airway epithelial cells via the secretion of TSLP and IL-25, and that calprotectin secreted by the epithelial cells may be involved in the pathogenesis of ECRS.

Introduction

Calprotectin is a heterodimer complex of the S100A8 and S100A9 proteins, which are members of the S100 family of proteins. S100 family proteins play various roles in inflammation, barrier function, cancer and innate immunity. Intracellularly, calprotectin promotes phagocyte migration by promoting the polymerization and stabilization of tubulin microfilaments.[1] Extracellular calprotectin is released by neutrophils, monocytes and epithelial cells, and exhibits proinflammatory functions, such as antimicrobial activity against bacteria and fungi, and chemoattractant activity for neutrophils and monocytes.[2] Calprotectin stimulates the production of proinflammatory cytokines, such as IL-8 and tumor necrosis factor (TNF)-α, in keratinocytes and airway epithelial cells.[3,4] Calprotectin also has anti-inflammatory and tissue-protective functions as it can inhibit matrix metalloproteinase activity and the production of reactive oxygen species (ROS).[5–7] Thus, calprotectin may have diverse functions as an innate mediator at the sites of inflammation.

Toll-like receptor 4 (TLR4) and receptor for advanced glycation end products (RAGE) are specific receptors for calprotectin.[8,9] Calprotectin is found in inflammatory tissues and exudates, including nasal polyps (NPs) and nasal lavage fluids,[10] and is

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thought to be involved in the pathogenesis of various inflammatory diseases, such as rheumatoid arthritis, autoimmune diseases, inflammatory bowel diseases and anti-neutrophil cytoplasmic autoantibody-associated vasculitis.[11–15] However, the role of calprotectin in upper airway Th2-type inflammation is not well understood.

Newly discovered epithelial-derived cytokines, i.e., thymic stromal lymphopoietin (TSLP) and IL-25, induce Th2 cytokine-dependent inflammation, and play key roles in innate and adaptive immune responses in airway mucosa.[16] Allergens, such as *Alternaria alternata* (*Alternaria*) and house dust mites (HDM), stimulate the production of TSLP and IL-25 in airway epithelial cells via protease-activated receptor (PAR)-2,[17,18] and TSLP and IL-25 contribute to the initiation and development of allergic inflammation in allergic rhinitis, asthma and atopic dermatitis.[19–22] Increased expression levels of TSLP and IL-25 have been reported in the NPs of patients with chronic rhinosinusitis (CRS), and were shown to exacerbate airway inflammation, and contribute to CRS disease progression.[23,24]

We hypothesized that calprotectin also plays an important role in the pathogenesis of eosinophilic chronic rhinosinusitis (ECRS). In the present study, we evaluated the effects of allergens on the release of calprotectin from airway epithelial cells, and the role of calprotectin in the secretion of TSLP and IL-25 induced by an airborne allergen, *Alternaria*. Finally, we examined the intracellular concentration and allergen-induced secretion of calprotectin in cultured primary nasal epithelial (PNE) cells of CRS patients.

Methods

Human subjects

Sixty-six patients with nasal or paranasal sinus diseases were enrolled in this study (age, 20–77 years; mean age, 49.1 years) (Table 1). Total IgE, allergen-specific IgE and blood eosinophil counts were performed for all the patients. Computed tomography (CT) assessment by Lund–Mackay score, the number of the nasal polyp and endoscopy were performed for the CRS patients. The CRS patients were classified into ECRS and non-eosinophilic chronic rhinosinusitis (NECRS) groups based on JESREC study.[25] ECRS was defined histologically as an averaged eosinophil count of more than 70 per microscopic field (400× magnification) in three fields of the subepithelial area of NPs; these were counted by two of the authors independently under light microscopy.[25] The diagnosis of asthma was based on the criteria of the Global Initiative for Asthma published in 2006.[26] Allergic rhinitis was diagnosed based on the Practical Guidelines for the Management of Allergic Rhinitis in Japan published in 2009.[27]

Inferior turbinates (IT) were obtained from patients with allergic rhinitis (AR) or patients with deflected nasal septum during conchotomy. NPs of patients with NECRS or ECRS, and uncinate process tissues (UT) of patients with NECRS or control subjects were obtained during endoscopic sinus surgery. None of the patients had been treated with systemic corticosteroids or topical corticosteroids for at least 4 weeks prior to the surgery. Informed consent was obtained from all subjects before sampling. This clinical protocol was approved by the Shiga University of Medical Science Institutional Review Board for Clinical Investigation.

Reagents

Crude allergens of *Alternaria* and HDM were purchased from Greer Laboratories (Lenoir, NC, USA). Protease from *Staphylococcus aureus* (*S. aureus*) was obtained from Abnova (Taipei, Taiwan). Polyinosinic:polycytidylic acid (poly(I:C)) was obtained from InvivoGen (San Diego, CA, USA). Trypsin from bovine pancreas, lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4, adenosine 5′-triphosphate disodium salt hydrate (ATP), adenosine 5′-triphosphate periodate oxidized sodium salt (oATP), trans-epoxysuccinyl-L-leucylamide (4-guanidino) butane (E-64), 4-amidinophenylmethanesulfonyl fluoride (APMSF) and nonidet P-40 were obtained from Sigma–Aldrich (St. Louis, MO, USA). Papain from *Carica papaya* and suramin were obtained from Calbiochem (Charlottesville, VA, USA). Recombinant calprotectin was obtained from Hycult Biotech (Uden, Netherlands). Small interfering RNA (siRNA) for TLR4, S100A8, and S100A9, and Allstars Negative Control siRNA were obtained from Qiagen (Hilden, Netherland), and siRNA for RAGE was obtained from Santa Cruz Biotechnology (Dallas, TX, USA).

Cell culture, treatment, and transfection

Normal human bronchial epithelial (NHBE) cells were obtained from Lonza (Walkersville, MD, USA) and were then transfected with the catalytic component of telomerase, the human catalytic subunit of the telomerase reverse transcriptase gene, which was kindly provided by Professor H. Kita (Mayo Clinic, Rochester, MN, USA). PNE cells were obtained by scratching the UT or NPs by curettage with a Rhinoprobe (Arlington Scientific, Inc., Springville, UT, USA). The NHBE cells or PNE cells were suspended in supplemented basal epithelial growth medium (BEGM) (Lonza, Basel, Switzerland) and cultured in a 24-well tissue culture plate (Falcon; Corning, Inc., NY,

Table 1
Patient characteristics.

Subject characteristics		IT		UT	UT or NP	NP
		Control	AR	Control	NECRS	ECRS
Total		8 (6M/2F)	17 (9M/8F)	10 (8M/2F)	15 (11M/4F)	16 (14M/2F)
Average age (range)		50.4 (38–66)	33.4 (20–64)	54.7 (46–76)	58.3 (20–77)	53.3 (26–75)
Asthma (n)		0	5	1	2	8
Atopy (n)		0	17	5	5	14
Blood eosinophils (%)		2.13 ± 1.33	5.17 ± 3.27**	4.10 ± 2.27	5.01 ± 5.01	8.42 ± 5.14*
Total IgE (U/ml)		76.2 ± 66.9	254.2 ± 239.2	209.4 ± 209.4	250.3 ± 258.4	343.6 ± 190.2*
Specific IgE (U/ml)	HDM	0.13 ± 0.01	17.5 ± 2.22**	6.21 ± 3.04	1.29 ± 4.01	7.11 ± 2.27
	Alternaria	0.1	0.15 ± 0.01	0.14 ± 0.11	0.13 ± 0.10	0.70 ± 0.29
CT score					8.47 ± 7.17	15.8 ± 6.62*
Nasal polyps (%)	Mono				20% (3/15)	0% (0/16)
	Multi				40% (6/15)	100% (16/16)

IT, inferior turbinate; UT, uncinate process; NP, nasal polyp; AR, allergic rhinitis; ECRS, eosinophilic chronic rhinosinusitis; NECRS, non-eosinophilic chronic rhinosinusitis; F, female; M, male.
P values were evaluated from the Mann–Whitney U test comparing 2 groups (IT) and the Kruskal–Wallis test comparing all 3 groups (UT/NP). $^{*}P < 0.05$ and $^{**}P < 0.01$.