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ORIGINAL ARTICLE

Beyond anti-microbial properties: The role of cathelicidin in allergic rhinitis

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Abstract
Background: Cathelicidin, an anti-microbial peptide, is a component of the innate immune system. Cathelicidin has anti-microbial, anti-inflammatory and immunoregulatory functions. Knowledge about the role of the innate immune system in the pathogenesis of allergic diseases has expanded in recent years. We measured levels of the LL-37 peptide in the nasal fluids of children with allergic rhinitis (AR) and investigated the possible role of this peptide in the pathogenesis of AR.
Methods: The study population included 46 children who were newly diagnosed with AR and not taking any medication. Thirty-three healthy control subjects were also enrolled. Nasal secretions were collected from the study and control groups using a polyurethane sponge nasal secretion collector, and nasal fluid LL-37 levels were determined using the ELISA method.
Results: The levels of LL-37 in the nasal fluid of the AR patients were lower than those of the control group (median of 2.3 ng/ml [minimum–maximum, 2.1–3.2] vs. 2.6 ng/ml [2.1–5.4], respectively; $p < 0.001$), and they were significantly reduced in patients with moderate/severe AR compared with those of patients with mild AR (2.2 ng/ml [2.1–2.4] vs. 2.5 ng/ml [2.1–3.1], respectively; $p < 0.001$).
Conclusion: Our results show that children with AR have reduced nasal fluid LL-37 levels compared with healthy controls. Additionally, children with moderate/severe AR have decreased nasal fluid LL-37 levels compared with children with mild AR. These findings highlight the role of cathelicidin in the pathogenesis of AR.

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Introduction

Allergic rhinitis (AR) is characterised by rhinorrhoea, sneezing, congestion and/or naso-ocular pruritus.[1] The overall prevalence of AR in children aged 6–7 and 13–14 is 8.5% and 14.6%, respectively.[2] Appreciation of the role of nasal innate immunity has expanded in recent years, with current evidence suggesting that the innate immune system partially mediates the pathogenesis of AR.[3]

Anti-microbial peptides (AMPs) are small molecular-weight proteins, with a positive charge that enables them to interact with bacterial membranes.[4] To date, over 100 human AMPs have been documented, of which the most extensively studied are cathelicidin and defensins.[5] The human cationic anti-microbial protein (hCAP-18) seems to be the only member of the cathelicidin family expressed in humans.[6] LL-37 is an active, mature form of this precursor protein.[4] Cathelicidin is expressed in neutrophils, monocytes, macrophages, T-cells, mast cells, human respiratory epithelial cells, keratinocytes and various squamous epithelia.[6–8] It is stored in the secretory granules of neutrophils and macrophages and is released extra-cellulary following the activation of leukocytes.[9] LL-37 induces the generation of reactive oxygen species and the release of human α-defensins from neutrophils, thus increasing neutrophil functions.[10] Various studies have reported the presence of LL-37 in human airway surface epithelia, bronchial alveolar lavage fluid and nasal lavages.[6–8]

Cathelicidin shows broad-spectrum anti-microbial activity against bacteria, viruses and fungi.[9] There is substantial evidence that cathelicidin has important functions in the prevention of lung and skin infections.[6,11] In addition to its anti-microbial activity, cathelicidin has immunomodulatory properties. Studies have shown that it acts as a chemoattractant of cells via chemokine and formyl peptide receptors and that it modulates dendritic cell differentiation and T-cell polarisation.[6,12,13]

Cathelicidin also functions as an anti-inflammatory molecule. It binds and neutralises lipopolysaccharides (LPSs), thereby limiting the extent of inflammation and LPS-mediated lethality, as shown in animal models.[4,14] LL-37 can cause the permeabilisation of apoptotic leukocytes and the leakage of cytoplasmic and intra-granular molecules. The aforementioned factors are important in the termination of acute inflammation.[15]

LL-37 affects each step of mucosal immunity. Although LL-37 is known to be expressed by nasal mucosa and to be upregulated in the course inflammation, there have been no studies on the possible role of LL-37 in the pathogenesis of AR.[16] With regard to its immunoregulatory and anti-inflammatory roles, we hypothesised that insufficient secretion of LL-37 from neutrophils or nasal epithelia might lead to dysfunction of nasal mucosal immunity and, therefore, contribute to the pathogenesis of AR. In the present study, we measured levels of LL-37 in the nasal fluids of AR patients and investigated the possible association with the disease and its severity.

Materials and methods

Patients

Fifty children with AR were recruited from Bezmialem Vakif University's Paediatric Allergy and Immunology Department between July 2014 and October 2014. One child who was unable to tolerate the sampling procedure and three children from whom nasal fluid samples could not be obtained were excluded from the study. The clinical diagnosis and severity of the AR were determined using the criteria defined in the Allergic Rhinitis and its Impact on Asthma guidelines.[17] All the patients were newly diagnosed with persistent AR and had not previously received therapy. All exhibited a positive skin test reaction to at least one aeroallergen and had no signs or symptoms of any infectious disease.

Subjects with acute or chronic inflammatory diseases (except asthma) and a history of maternal or paternal smoking and those who were taking any medications (including poly-vitamins or minerals) were excluded from the study because smoking and vitamin D may affect AMP levels according to the literature.[18,19] The control group consisted of 38 healthy children who periodically attended paediatric clinics at the same hospital for regular developmental check-ups. Children were included in the control group if they had no history of any allergic disease or paternal or maternal smoking and were not taking any medication. The children in the control group had no signs or symptoms of any infectious disease. Three children from whom nasal secretions could not be obtained and two children who could not tolerate the sampling procedure, were excluded from the study. The study was approved by the Bezmialem Vakif University Ethical Committee (No. 1563). Informed consent was obtained from all study participants.

Collection of nasal secretions

Nasal secretions were collected using a polyurethane sponge nasal secretion collector (NSC) according to the method described by Lü et al. but with minor modifications.[20] Samples were collected from patients and control groups at similar times of day (between 8:00 and 10:00 a.m.) every morning to avoid possible daily variations with AR. A sixty-pore-per-inch reticulated polyurethane sponge was cut into rectangular prism shapes (base of 5 mm $\times$ 10 mm and height 20 mm) using a homemade cutting device and the pieces were sterilised by autoclaving for 20 min at 121 °C prior to use. A single use metal rod with a tip clamp was used to hold the sponge during the sample collection (Fig. 1). The sponge was inserted and placed on the floor of the nasal cavity between the septum and the inferior turbinate for at least 5 min. The sponge containing nasal secretions was pulled out from the nostril and inserted into an inner tube that was in the outer centrifuge tube (Fig. 1). We pierced the bottom of a 2 ml Eppendorf tube (Eppendorf AG, Hamburg, Germany) using a 21-gauge needle and created 15 standard holes to allow nasal secretions to pass into the outer tube during centrifugation. The outer tube was a standard 10 ml vacuum blood collection tube. Next, this device was