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Involvement of interleukin-18 in the pathogenesis of human eosinophilic esophagitis

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Abstract IL-18 is induced in food allergy and EoE is food allergen-induced disease. Therefore, we tested the hypothesis whether IL-18 is involved in food allergen-induced EoE pathogenesis. Accordingly, we examined normal SPT+ and SPT− EoE patient blood and biopsy samples for IL-18, IL-18Rα, ICAM and VCAM expression. Herein, we show increased IL-18 level is highly significant in food allergen SPT+ compared to SPT− EoE patients. We also report that IL-18Rα+ cells and mRNA levels are induced in the esophageal biopsies of EoE patients and blood IL-18 levels correlate with esophageal eosinophilia ($P < 0.01$). Additionally, we report that the levels of esophageal eosinophil and mast cells correlate with ICAM expression in human EoE. Mechanistically, we show that IL-18 in vitro stimulates iNKT cells and endothelial cells and induce eosinophil active cytokines IL-5 and IL-13. We provide the evidence that IL-18 is critical cytokine involved in activation of iNKT cells and ICAM in promoting human EoE.

Abbreviations: CE, chronic esophagitis; DAPI, 4′,6-diamidino-2-phenylindole fluorescent mounting material; EoE, eosinophilic esophagitis; ELISA, enzyme-linked immunosorbent assay; GERD, gastroesophageal reflux disease; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; IL, interleukin; iNKT, invariant natural killer T cell; PCR, polymerase chain reaction; HMVEC, primary human microvascular endothelial cells; SPT+, skin allergen positive test

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1. Introduction

In recent years, major advances have occurred in understanding eosinophil and mast cells (MCs) accumulation [1–3] and remodeling in EoE [4,5]; however, the mechanisms of EoE induction, progression, diagnosis and treatment are still not clearly understood. EoE patients that have <15 eosinophils/hpf in esophageal biopsies are hard to differentiate from gastroesophageal reflux disease (GERD), as some EoE patients may have PPI-responsiveness [6]. Experimental modeling has established that Th2 cytokine signaling is required for induction of EoE. Considerable evidence supports a critical role for the Th2 cytokines interleukin (IL)-5, IL-13 and IL-15 in EoE pathogenesis [7,8]. The gene exhibiting the highest increase of expression in the esophagus of EoE patients is eotaxin-3, an eosinophil chemoattractant and activator produced by esophageal epithelial cells [2]. Additionally, we, and others, reported that IL-15 responsive iNKT cells are induced in EoE and neutralization of iNKT cells ameliorates the severity in pediatric EoE [9–13]. Earlier, our microarray analyses showed increased levels of the IL-18Rα transcript in EoE patients [2]. IL-18 activates B cells and natural killer (NK) T cells in an antigen-independent manner [14,15] and this process has been shown to contribute in a number of intestinal allergic responses including celiac disease, a disease that shares features with EoE [16–19]. IL-18 is a pleiotropic cytokine that is elevated in a number of eosinophilic allergic diseases such as food allergy, dermatitis, asthma, and colitis [20–22]. Inflammatory cells involved in innate immunity secrete IL-18 [16,23] and it has been reported that IL-18 stimulates iNKT cells without T cell receptor (TCR) engagement [24,25]. The present study indicates that IL-18 may be involved in the pathogenesis of EoE. Herein, we demonstrate that IL-18 and its specific receptor IL-18Rα are increased in the blood and esophagus of EoE patients, respectively. Notably, IL-18Rα transcript levels correlate significantly with esophageal eosinophilia in active and treated EoE patients. Mechanistically, we show that IL-18 activate iNKT cells to produce eosinophil active Th2 cytokines (e.g. IL-5, IL-13) that promotes EoE pathogenesis. Taken together, our current findings define a novel role for IL-18 in Th2 responses and provide evidence that IL-18 may have an important role in EoE pathogenesis by activating iNKT cells. Therefore, in the current study we tested the hypothesis that the induction of IL-18 is associated with the esophageal eosinophilia in human EoE. Herein, we report that indeed IL-18 may have a significant role in food allergic (SPT+) EoE patients.

2. Materials and methods

2.1. Selection of EoE and non-EoE patients and tissue collection

Formalin-fixed, paraffin-embedded biopsy samples were obtained from the esophagus of normal individuals or EoE patients as per an Institutional Review Board (IRB)-approved protocol. The comparison control normal subjects (non-EoE), chronic esophagitis (CE) and EoE patients were selected without regard to age, atopic status or gender. Diagnosis was established based on the maximum eosinophil count per high power field (hpf) (×400). Control individuals (non-EoE or CE) were defined as having 0–2 esophageal eosinophils/hpf and no basal layer expansion. The normal biopsies were obtained from patients who showed symptoms typical of gastroesophageal reflux disease (GERD) and EoE but were found normal upon microscopic analyses of esophageal biopsies. Typically, these patients had abdominal pain, and some had allergic diseases including asthma and rhinitis. Patients with EoE were defined as having ≥15-esophageal eosinophils/hpf and included other allergic diseases such as asthma and atopic dermatitis. The CE (chronic esophagitis) patients define, as the group of patient does not qualify with the definition of EoE or normal. They have esophageal eosinophilia <15eosinophil/hpf with basal cell hyperplasia. The hospital pathologist as consistent with reflux esophagitis evaluated the eosinophils and mast cell level in the esophageal biopsies of non-EoE and EoE patients. The fresh biopsy samples were collected in RNA latter buffer for RNA isolation and blood was drawn from each normal, EoE and non-EoE patients at the time of scheduled biopsy (including before and after treatment of EoE patients) at Cincinnati Children's and Tulane University School of Medicine in a citrate-coated tubes. The blood plasma was isolated by centrifugation and stored at −20C for cytokine analysis. All samples were used according to the patients' consent and IRB approved protocol of CCHMC, Cincinnati, OH (Year 2011–12) and TUSM, New Orleans, LA (Year 2013–14). The pool EoE and non-EoE patient data generated at both hospital centers are presented and the patient's detailed clinical characteristics are shown in Table 1. Our current study was performed on heterogeneous patient population, as PPI trial was not performed on all EoE patients, and is our limitation.

2.2. Quantification of serum IL-18

IL-18 protein concentrations in the serum of normal and EoE patient samples were quantified by using a DuoSet enzyme-linked immunosorbent assay (ELISA) Development kit (R & D Systems). The detection limit was 0.9 pg/ml.

2.3. Quantitative PCR

The RNA samples (500 ng) were subjected to reverse transcription using Bioscript reverse transcriptase (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. IL-18Rα, ICAM, VCAM, IL-5 and IL-13 were quantified by real-time PCR using IQ5 (Bio-Rad). Results were then normalized to human or mouse GAPDH amplified from the same cDNA mix and expressed as relative gene expression. cDNAs were amplified using the primers listed in Table 2.

2.4. Immunofluorescence staining of biopsy samples for IL-18Rα+ cell detection

Cryostat sections from frozen esophageal biopsies of normal subjects (non-EoE) and EoE patients were fixed, blocked with normal goat serum to reduce non-specific binding, and incubated with anti-IL-18Rα+ antibody (eBioscience). The IL-18Rα+ immunostained sections were mounted with DAPI mounting material. The images were captured using an Olympus BX51 microscope with appropriate filters. IL-18Rα+ positive cells were counted on each stained tissue sections per high