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Original contribution

Increased expression of eotaxin-3 distinguishes between eosinophilic esophagitis and gastroesophageal reflux disease☆

Baishali Bhattacharya MD, MPH[a,*], James Carlsten MD[c], Edmond Sabo MD[a], Sripathi Kethu MD[b], Patricia Meitner PhD[a], Rosemarie Tavares BS[a], Shriram Jakate MD, FRCPath[d], Shamlal Mangray MD[a], Bassam Aswad MD[a], Murray B Resnick MD, PhD[a]

[a]*Department of Pathology, Rhode Island Hospital, Brown Medical School, Lifespan Academic Center, Providence, RI 02903, USA*
[b]*Department of Gastroenterology, Rhode Island Hospital, Brown Medical School, Lifespan Academic Center, Providence, RI 02903, USA*
[c]*Department of Pathology, Memorial Hospital, Pawtucket, RI 02860, USA*
[d]*Department of Pathology, Rush-Presbyterian St. Luke's Medical Center, Rush Medical College, Chicago, IL 60612, USA*

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Summary Differentiating eosinophilic esophagitis from gastroesophageal reflux disease is important given their pathogenetic differences and responses to therapy. Eotaxins are a family of chemokines important for activation and recruitment of eosinophils mediated by their receptor, chemokine receptor-3 (CCR-3). Interleukin 5 (IL-5) is a key cytokine involved in many steps of eosinophil production and recruitment. The aim of this study was to compare the messenger RNA expression of the eotaxins, CCR-3, and IL-5 between well-characterized groups of patients with eosinophilic esophagitis, patients with gastroesophageal reflux disease, and healthy individuals. This was a retrospective study using esophageal biopsies from 33 patients with eosinophilic esophagitis, 20 patients with gastroesophageal reflux disease, and 17 healthy controls. Parameters studied included demographic features, presenting symptoms, endoscopic findings, histopathologic features, and messenger RNA levels of eotaxins 1, 2, and 3, CCR-3, and IL-5 by quantitative real-time polymerase chain reaction using formalin-fixed, paraffin-embedded tissue. Patients with eosinophilic esophagitis were predominantly males (M/F = 3:1), with a mean age of 15.9 years and a mean eosinophil count of 55 per ×400 high-power field. Patients with gastroesophageal reflux disease had a mean age of 31.5 years and a mean eosinophil count of 5.8 per high-power field. Total intraepithelial eosinophil and lymphocyte counts, the presence of superficial eosinophil clusters, microabscesses, and basal cell hyperplasia were all significantly associated with eosinophilic esophagitis as opposed to gastroesophageal reflux disease ($P < .0001$). The mean expression levels of eotaxin-3 were

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* Corresponding author.
E-mail address: bbhattacharya@lifespan.org (B. Bhattacharya).

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markedly elevated in patients with eosinophilic esophagitis as compared with the gastroesophageal reflux disease and healthy control groups (731 ± 276, 31 ± 12, and 1.5 ± 0.4 pg/ng β-actin, respectively; $P < .001$). Mean expression levels of eotaxins 1 and 2, IL-5, and CCR-3 were also significantly increased in the patients with eosinophilic esophagitis, albeit at lower levels than eotaxin-3. In conclusion, our results highlight the important contribution of eotaxin-3 in the pathogenesis of eosinophilic esophagitis. Determination of eotaxin-3 levels by real-time polymerase chain reaction on paraffinized, formalin-fixed tissue may be a useful test in the differentiation of eosinophilic esophagitis from gastroesophageal reflux disease.

1. Introduction

Eosinophilic esophagitis (EE), also known as allergic or idiopathic esophagitis, is an increasingly recognized chronic inflammatory disorder restricted to the esophagus, seen predominantly in children and young adults with a male preponderance [1-3]. Typically, patients present with food impaction and dysphagia to solid food [1-3]. Esophageal biopsies from the proximal and distal esophagus of EE case patients demonstrate a striking intraepithelial eosinophilic infiltration of greater than 20 to 24 eosinophils per high-power field (HPF) [2-4]. Gastroesophageal reflux disease (GERD), on the other hand, is predominant in an older population with no sex predilection. Patients typically complain of heartburn and may have abnormal findings from pH probe studies. GERD is present in the distal esophagus with eosinophil counts of less than 20/HPF [2,5]. Patients respond to acid suppressive medications such as histamine$_2$ blockers, proton-pump inhibitors (PPIs), and fundoplication. In contrast, patients with EE show a dramatic response to topical or oral steroids and are usually unresponsive to anti-reflux medications [4,6].

Although the exact etiology of EE is unknown, allergy is thought to play an important role. Many factors support the association between EE and allergy, including the concomitant presence of bronchial asthma in many patients with EE, elevated peripheral blood eosinophilia or serum immunoglobulin E levels, response to elemental diet, systemic and topical corticosteroids, and sodium cromoglycate [7,8]. The esophagus normally lacks eosinophils; therefore, the presence of intraepithelial eosinophils suggests a pathologic condition [9]. However, their presence within the esophagus is by no means restricted to EE because it has been linked with numerous pathologic entities including GERD, eosinophilic gastroenteritis, hypereosinophilic syndrome, parasitic and fungal infections, inflammatory bowel disease, collagen vascular diseases, and so on [9].

Eosinophil recruitment into this region of the gastrointestinal tract is an area of intense study, and much of our understanding of eosinophil recruitment is derived from studies of asthma and allergy models. This process of eosinophil recruitment involves multiple steps, including eosinophil proliferation in the bone marrow, transmigration from the bone marrow into the circulation, activation within the bloodstream, adhesion to the vascular endothelium, diapedesis through the vascular wall into the tissue, and migration toward the targeted tissue area [10]. Each of these steps is influenced by a different subset of cytokines/mediators. The number of potential mediators is ever growing and includes interleukin (IL) 1, IL-3, IL-5, IL-8, IL-13, RANTES, eotaxins, tumor necrosis factor α, monocyte chemoattractant proteins, and macrophage inflammatory protein 1α [11,12]. Of the mediators mentioned, there has been an increasing focus on IL-5 and the eotaxin subfamily of chemokines in EE [13]. In a recent study using gene array analysis, Blanchard et al [14] identified eotaxin-3 as the most highly induced gene in a cohort of patients with EE compared with its expression level in healthy individuals. A single nucleotide polymorphism in the human eotaxin-3 gene was associated with disease susceptibility. Furthermore, mice deficient in the eotaxin-3 receptor (chemokine receptor 3 [CCR-3]) were protected from experimental EE.

The primary aim of this study was to determine whether the expression levels of specific chemokines (and their receptors) involved in eosinophilic recruitment would assist in the differentiation of EE from GERD in a well-characterized cohort of patients.

2. Materials and methods

2.1. Case selection

This was a retrospective study and included esophageal biopsies from consecutive patients diagnosed with EE between January 2001 and January 2007 that were retrieved from the archives of the Pathology Department at the Rhode Island Hospital, Providence. Esophageal biopsies from consecutive patients diagnosed with GERD and esophageal biopsies from controls diagnosed as being healthy at the Rhode Island Hospital were also studied. None of the study patients had evidence of Barrett esophagus, inflammatory bowel disease, or gastrointestinal malignancy. None of the EE cases had evidence of eosinophilic gastroenteritis when biopsies from other sites were available for study. This study was approved by the institutional review board of the Rhode Island Hospital.