Involvement of mast cells in eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EE) is an emerging disorder with poorly understood pathogenesis.
Objective: Whereas prior studies have primarily focused on the role of eosinophils in disease diagnosis and pathogenesis, this study investigates the involvement of mast cells.
Methods: Total and degranulated mast cell counts were correlated to microarray and RT-PCR data to generate transcriptome expression profiles related to mast cell number and degranulation in patients with EE and healthy control subjects.
Results: Esophageal mastocytosis and mast cell degranulation were readily apparent in patients with EE compared with control subjects ($P < .01$), as assessed by staining for total mast cells and the presence of extracellular mast cell tryptase ($P < .01$). Microarray analysis revealed that mast cell levels correlated with the dysregulation of 0.8% (301 genes) of the genome, which was partially distinct from the genes that correlated with tissue eosinophilia. The expression of transcripts for the mast cell proteases carboxypeptidase A3 and tryptase, but not chymase, correlated with mast cell levels and distinguished patients with EE from control subjects. Suprabasilar mast cell counts ($P < .01$) and degranulation ($P < .01$) were proportional with *KIT* ligand mRNA expression. Treatment of patients with EE with swallowed fluticasone propionate normalized levels of mast cells and the mast cell–related transcriptome in responder patients.
Conclusion: Herein we have identified local mastocytosis and mast cell degranulation in the esophagi of patients with EE; identified an esophageal mast cell–associated transcriptome that is significantly divergent from the eosinophil-associated transcriptome, with carboxypeptidase A3 mRNA levels serving as the best mast cell surrogate marker; and provided evidence for the involvement of KIT ligand in the pathogenesis of EE. (J Allergy Clin Immunol 2010;126:140-9.)

Key words: *Mast cells/basophils, eosinophils, human*

Eosinophilic esophagitis (EE) is an emerging worldwide disease, as documented by case series from all continents except Africa, which appears to be a growing health problem with an annual incidence of at least 1:10,000 children.[1-3] The primary symptoms of EE (chest and abdominal pain, dysphagia, heartburn, vomiting, and food impaction) are also observed in patients with chronic esophagitis (CE), including those patients with gastroesophageal reflux disease (GERD).[4-6] However, in contrast to GERD, EE occurs more frequently in male subjects (80%), appears to have a common familial form, has a high rate of associated atopic disease (70%), and is typically associated with a normal pH probe recording of the esophagus.[1,7-10] Patients with EE respond inadequately to anti-GERD therapy alone but might respond to anti-inflammatory therapy, allergen elimination, or both, as determined by allergen testing or empiric dietary elimination.[11-13]

Dissection of experimental EE models in mice has revealed that EE is triggered by both food and aeroallergens, whereas pollen exposure has been associated with cases of EE in human subjects and is clearly associated with atopic disorders, such as allergic rhinitis, asthma, and eczema, in pediatric and adult patients.[12,14-17] However, nearly 25% of patients with EE are nonatopic and have no discernable evidence of allergic sensitization.[1,8,18,19] It is important to understand the relationship between the allergic and nonallergic variants of EE and whether allergic and nonallergic esophagitis involve similar effector pathways, such as localized mastocytosis and mast cell activation, which might have significant implications for therapeutic strategies.

Previously, whole genome–wide expression analysis of esophageal tissue has uncovered a striking EE transcript signature that correlated with eosinophil levels and was similar across sexes and patient ages but completely distinct from CE. Notably, the top induced transcript in patients with EE was eotaxin-3, and levels of eotaxin-3 strongly correlated with disease severity; furthermore, a single nucleotide polymorphism in the eotaxin-3 gene was associated with disease susceptibility.[20,21] Additional analyses of the EE transcriptome with respect to IL-13 demonstrated a dose-dependent increase in eotaxin-3 mRNA and protein from primary esophageal epithelial cell cultures after stimulation with IL-13,[22] which is consistent with prior reports demonstrating increased levels of antigen-specific IL-5^+ T_H2 cells in the blood of patients with EE.[23] Recently, a variant in thymic stromal lymphopoietin was also found to be associated with EE and might play a role in the male predominance found in this disorder because its receptor is encoded within a pseudoautosomal region for the X and Y chromosomes.[24]

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Abbreviations used
CCL26: CC motif ligand 26
CE: Chronic esophagitis
CPA3: Carboxypeptidase A3 gene
CXCL1: CXC motif ligand 1
CXCL6: CXC motif ligand 6
EE: Eosinophilic esophagitis
FP: Fluticasone propionate
GERD: Gastroesophageal reflux disease
H&E: Hematoxylin and eosin
HPF: High-powered field
HPRT: Hypoxanthine guanine phosphoribosyltransferase
IHC: Immunohistochemistry
KITLG: KIT ligand gene
NL: Normal
PPP2R2C: Protein phosphatase 2 gene

The high level of eosinophils in the esophagi of patients with EE, the identification of eotaxin-3 as a primary process in EE pathogenesis, and the correlation of eosinophils with the degree of epithelial cell hyperplasia all implicate the eosinophil as a primary effector cell in patients with EE.[20,21] Indeed, murine models have established that eosinophils are required for induction of allergen-induced epithelial hyperplasia and remodeling in models of EE.[14] However, mast cells might be particularly important in disease pathogenesis because they produce an abundance of cytokines that activate eosinophils and molecules that directly promote tissue remodeling, including fibrosis, a process that has been recently identified in patients with EE, even in pediatric patients.[25,26] Recently, increased levels of mast cells in the esophagi of patients with EE have been identified; however, their phenotype, regulation, and role in disease have not been explored in detail.[19,26-28] We now provide substantial evidence for the involvement of KIT ligand, esophageal mastocytosis, and mast cell activation in the pathogenesis of EE. In addition, we demonstrate that the esophageal genes associated with mast cell levels are distinct from those associated with eosinophil levels, at least in part, and that carboxypeptidase A3 *(CPA3)* mRNA serves as the best surrogate tissue marker for mast cells. Furthermore, we present molecular evidence that these processes of tissue mastocytosis and dysregulation of the mast cell transcriptomes are reversible with fluticasone propionate (FP) therapy and distinguish patients with EE from healthy control subjects and patients with CE.

METHODS

Esophageal samples

Patients were selected without regard to age, race, atopic status, or sex, and their characteristics are described in Table I. A total of 29 patients were selected for microarray analysis based on their diverse clinical features. Except when indicated, none of the patients were undergoing therapy with glucocorticoids or dietary modification. An additional 13 patients with active EE were evaluated for mast cell counts, degranulation, and the effect on mast cell transcriptomes after treatment with FP. These patients were treated with 880 μg/d swallowed FP for 3 months and are described in detail by Konikoff et al.[27] Two biopsy specimens from each patient were collected from the distal esophagus less than 5 cm from the lower esophageal sphincter, with one sample immediately fixed in formalin before hematoxylin and eosin (H&E) staining and the second stored separately for microarray and RT-PCR analysis. A pathologic diagnosis from the H&E-stained sample was determined based on the maximum eosinophil count per high-powered field (HPF; ×400 magnification; area of microscopic field, 0.22 mm^2) and basal layer expansion according to established criteria and correlated with clinical findings.[4,8,20,29] Normal biopsy specimens were obtained from patients who presented with symptoms consistent with GERD and EE but whose endoscopic and histologic appearances were ultimately normal. Patients with CE were defined as having 12 or fewer eosinophils per HPF, mild expansion of the basal layer (less than one third of the epithelium), or both and not requiring treatment with either steroid or dietary therapies. Patients with EE were defined as having 24 or more eosinophils per HPF, extensive basal layer hyperplasia (expansion to one third or more of the epithelium), and clinical evidence of disease. The maximum eosinophil counts and thickness of the basal layer were assessed after H&E staining. Mast cell counts were determined by using immunohistochemistry (IHC) for tryptase. Biopsy specimens were also assessed by staining for chymase using IHC and chloroacetate esterase activity.[30-32] This study was approved by the Institutional Review Board of the Cincinnati Children's Hospital Medical Center.

DNA microarray analysis

For each patient, a distal esophageal mucosal biopsy specimen was subjected to DNA microarray, as previously reported.[33] The genome-wide Human Genome U133 Plus 2.0 Array gene chip that encompasses 38,572 genes with 54,120 probe sets (http://www.affymetrix.com, technical note, part no. 701483 rev. 2) was used, and gene transcript levels were determined by using algorithms in the Microarray Analysis Suite and GeneSpring software (Agilent Technologies, Santa Clara, Calif). A base set of probe sets in transcriptome analyses was generated by requiring a minimum raw expression on the microarray of 250 (average difference) in at least 3 patient samples identifying 16,425 probe sets for use in all subsequent analyses.

Ontology assessment

We subjected the list of differentially expressed transcripts to gene ontology analysis by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) and the Expression Analysis Systematic Explorer (EASE), a Web-based (http://david.abcc.ncifcrf.gov) application that provides access to a relational database of functional annotations.[34,35]

RT-PCR analysis

The RNA samples (500 ng) were subjected to reverse transcription analysis by using Bioscript reverse transcriptase (Bio-Rad Laboratories, Hercules, Calif), according to the manufacturer's instructions. Human tryptase, chymase, *CPA3*, *CXCL6*, and *HPRT* were quantified by means of real-time PCR with the Roche LightCycler 480 instrument and Roche LightCycler 480 SYBR Green I master mix (Roche, Mannheim, Germany) as a ready-to-use reaction mix. Results were then normalized to *HPRT* amplified from the same cDNA mix and expressed as fold induction compared with the controls. cDNA were amplified by using the following primers: hTryptase (70 bp), gcgatgtggacaatgatgag and tccattatggggaccttcac; hChymase (64 bp), acggaactttgtgctgacg and ggctccaagggtgactgtta; *hCPA3* (94 bp), cccagatgctattgtttcccta and agaacatcagtgccaatctttg; *hCXCL6* (131 bp), agccccttttctaaagaaagtca and tccagggatctccagaaaac; and *hHPRT* (120 bp), cagactgaagagctattgtaatg and ccagtgtcaattatatcttccac.

IHC

Esophageal sections were individually immunostained with anti-tryptase (Cell Marque, CMA890), as previously reported.[36] Immunoreactive cells were counted (×400 magnification) and are expressed as maximum mast cell number per HPF.

Mast cell number and extent of degranulation were assessed in the suprabasilar epithelium from control subjects, patients with CE, and patients with EE in a blinded fashion. While blinded, each esophageal sample was assessed for overall quality, and those deemed to be of inadequate quality were excluded from our final analyses with 3 or fewer HPFs that could be evaluated. The remaining samples had between 7 and 10 HPFs counted per subject, were used in generating the mast cell transcriptomes, and were quantified for the