Published in final edited form as:

J Pediatr Gastroenterol Nutr. 2010 November ; 51(5): 584–592. doi:10.1097/MPG.0b013e3181de7685.

Comparative analysis of FcεRI expression patterns in patients with eosinophilic and reflux esophagitis

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Abstract

Eosinophilic esophagitis (EoE) is an allergic disease of the esophagus. The IgE receptors on immune cells that infiltrate the esophagus are poorly defined. The high affinity receptor for IgE, FcεRI, may play a role in EoE.

Objectives—Identify and compare the IgE receptors in the esophageal epithelium of patients with EoE, reflux esophagitis (RE), and normal controls.

Methods—Retrospective case control study of 62 patients (19 EoE, 22 RE, 21 normal controls). Biopsies were immunostained for FcεRI, CD23, galectin-3, c-kit, CD1a, and langerin.

Results—FcεRI was the only IgE receptor present in the esophageal epithelium of patients with EoE. The FcεRI-positive cell count varied by diagnosis (proximal biopsies EoE 32.6 ±19.0 cells/HPF, RE 26.7 ±16.6, controls 15.6 ±8.3, ANOVA p=0.005; distal biopsies EoE 24.2 ±16.2, RE 35.7 ±27.6, controls 15.3 ±8.4, p=0.006). In the proximal esophagus, the FcεRI count was higher in EoE than controls (p=0.006); in the distal esophagus, the FcεRI count was higher in RE than controls (p=0.004). EoE and RE had similar FcεRI-positive cell counts. A subset of FcεRI-positive cells was similar in morphology and distribution to Langerhans cells (CD1a- and langerin-positive).

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All listed authors have seen and have approved the submitted manuscript. All authors take full responsibility for the content of the manuscript.

Samuel Nurko is the guarantor of the manuscript.

DISCLOSURES:

Elizabeth H. Yen reports no financial, professional, or personal conflicts of interest. Jason L. Hornick reports no financial, professional, or personal conflicts of interest. Maarten Dokter reports no financial, professional, or personal conflicts of interest. Eleonora Dehlink reports no financial, professional, or personal conflicts of interest. Edda Fiebiger reports no financial, professional, or personal conflicts of interest. Samuel Nurko reports no financial, professional, or personal conflicts of interest.

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Conclusion—The presence of FcεRI-positive cells in high numbers in the esophageal epithelium implies this receptor must be critical in the IgE-mediated activation of immune cells in the esophagus. Langerhans cells in the esophageal epithelium appear to express FcεRI. The role of Langerhans cells in the pathophysiology of EoE needs to be elucidated.

Keywords

eosinophilic esophagitis; food hypersensitivity; food allergy; pediatric gastroesophageal reflux

INTRODUCTION

Eosinophilic esophagitis (EoE) is an inflammatory disease of the esophagus diagnosed in children and adults with increasing prevalence in the developed world. (1) The prevalence of children with EoE in the Midwest United States increased four-fold over a period from 2000 to 2003, with a reported incidence rate of 1 per 10,000 children per year. (1) The esophageal epithelium of patients with EoE contains numerous eosinophils, defining the histological diagnosis of the disease. Cytokine and genetic expression profiling of esophageal tissue from these patients places EoE in the group of Th2-mediated immune diseases, similar to atopy and asthma. (2-4) Indeed, patients with EoE are more likely to have other atopic conditions, such as atopic dermatitis, allergic rhinitis, or asthma. (5-7) Clinically, patients improve when placed on an elemental diet, devoid of all food allergens. (8-11) Children with food allergies and EoE often have a combination of findings on allergy testing, such as positive skin prick testing and patch skin testing to common food allergens, which points to a role for IgE-mediated and cell-mediated activation of the immune system. (9)

Allergic conditions frequently are associated with high serum IgE levels and IgE receptors on effector cells of the adaptive immune system. (12,13) However, it is not known which IgE receptors are expressed by immune cells resident in the esophageal epithelium of EoE patients. Humans express three different IgE receptors: CD23, galectin 3 and FcεRI. (14) CD23 (FcεRII) is a low affinity IgE receptor that traffics IgE in epithelial cells of the gastrointestinal tract. (14) Galectin 3 is another low affinity IgE receptor with poorly defined functions for the gastrointestinal immune system. (14) FcεRI is the high-affinity receptor for IgE. (14,15) Human FcεRI is expressed on the surface of mast cells, basophils, eosinophils, macrophages, Langerhans cells and other dendritic cells, and platelets. (15) FcεRI is upregulated in allergic individuals (13) and has been shown to be upregulated in gene-expression profiling studies with tissue lesions from patients with EoE. (3) The receptor binds IgE monovalently and is only activated when allergen cross-links the IgE-FcεRI complex. FcεRI plays an important role in both immediate-type allergic reactions (Type I) and delayed-type hypersensitivity reactions (Type IV). (15)

Based on the expression pattern of FcεRI on peripheral blood cells and expression profiling data from EoE patients, we hypothesized that FcεRI should be highly expressed in tissue lesions from patients with EoE. The aims of the present study were to identify and compare the IgE receptors in the esophageal epithelium of patients with EoE, reflux esophagitis (RE), and normal controls.

METHODS

This is a retrospective case control study evaluating the expression of the high affinity IgE receptor, FcεRI, in esophageal biopsies from patients with EoE, RE, and normal controls. Patients eligible for participation in this study had undergone an esophago-gastro-duodenoscopy (EGD) between January 1, 2001, and December 31, 2007 at Children's Hospital

Boston. Biopsies used for this study had been previously obtained as part of routine clinical care. The study was approved by the Institutional Review Board of Children's Hospital, Boston.

Nineteen patients with EoE were randomly selected for inclusion in the study from a roster of 70 newly diagnosed patients during the study time period. Cases of RE and normal controls were age-matched (within one year) to EoE cases. Demographic data, medication use and comorbidities, particularly allergic conditions, were extracted from the medical record. All data were de-identified to ensure patient privacy.

Patient classification

EoE—Patients were considered to have EoE if their esophageal biopsies had greater than 15 eosinophils per high power field, basal zone hyperplasia, degranulated eosinophils, and eosinophilic microabscesses, following a minimum of 4 weeks of treatment with a proton pump inhibitor. (16-19) They all had absence of eosinophilia in gastric and duodenal biopsies. All biopsies from EoE patients were taken from their first diagnostic endoscopy prior to initiation of any EoE specific therapy, either topical steroid or specific antigen removal from their diet.

RE—Patients had evidence of abnormal acid exposure and esophageal inflammation with basal zone hyperplasia and < 15 eosinophils per high power field.

Normal controls—Control biopsies came from patients who had undergone upper endoscopy in our center for any indication other than inflammatory bowel disease, autoimmune disorders, or history of bone marrow transplant. Patients were considered controls and included if they fulfilled the following criteria: a) no reflux symptoms, b) normal upper gastrointestinal barium study without evidence of obstruction, malrotation, or hiatal hernia in those that had the study, c) normal upper endoscopy and biopsies, and d) normal pH monitoring of the distal esophagus in those that had the procedure (defined as esophageal pH <4 for less than 6% of the duration of the procedure).

Histology

It is routine practice in our institution to obtain at least two esophageal biopsies: one near the gastroesophageal junction (distal) and one at least 10 cm proximal to the GEJ (proximal). Two biopsies from each patient were evaluated: one from the distal esophagus, and one from the proximal esophagus. Initially, all esophageal mucosal biopsy specimens were formalin-fixed, routinely processed, paraffin-embedded, cut serially in 5-micron sections and stained with hematoxylin and eosin. The biopsies were classified histologically as previously described. (16,17) Quantification of intraepithelial eosinophil number was performed by counting the number of eosinophils in each biopsy in five separate high power fields (HPF) in areas with the densest inflammatory infiltrate. Data were expressed as the mean number of eosinophils per HPF (Nikon Optiphot-2, Plan 40x lens-surface area=0.196 mm²).

The distribution of eosinophils within the squamous epithelium was also assessed. Measurements of the basal zone thickness and papillary length were made only on specimens in which at least three well-oriented papillae could be identified. Measurements were considered abnormal if there was basal zone hyperplasia greater than 20% of the total epithelial thickness or lengthening of the papillae to greater than, or equal to, 75% of the epithelial height (17). Superficial layering of eosinophils was defined as preferential concentration of eosinophils in the upper one third of the esophageal epithelium. Microabscesses of eosinophils were defined as clusters of 4 or more eosinophils, typically near the mucosal surface. (16-18) Representative hematoxylin and eosin stained biopsies from each diagnostic group are shown in Figure 1.

Immunohistochemistry

Immunoperoxidase studies were performed at room temperature. Deparaffinized sections were treated with 3% hydrogen peroxide aqueous solution for 5 minutes to eliminate endogenous peroxidase activity. Heat-induced epitope retrieval was performed using 0.01 mol/L citrate buffer, pH 6.0 (FcεRI-α and galectin-3), 0.001 mol/L ethylene diamine tetra-acetic acid (EDTA) solution, pH 8.0 (CD1a and langerin), or Dako (Carpinteria, CA) retrieval solution (CD23) in a pressure cooker. No antigen retrieval was performed for c-kit. Sections were then incubated for 1 hour with monoclonal antibodies specific for FcεRI-α (clone AER-37 (CRA1); 1:100 dilution; eBioscience, San Diego, CA), CD1a (clone 010; 1:50 dilution; Immunotech, Westbrook, ME), langerin (clone 12D6; 1:100 dilution; Novocastra Laboratories, Newcastle upon Tyne, U.K., available from Vision Biosystems, Norwell, MA), CD23 (clone 1B12; 1:100 dilution; Cell Marque, Rocklin, CA), or galectin-3 (clone 9C4; 1:500 dilution; Novocastra); or a polyclonal antibody specific for c-kit (A4502; 1:250 dilution; Dako). Sections were then incubated for 30 minutes with horseradish peroxidase-labeled polymer conjugated to goat antimouse or antirabbit immunoglobulin antibodies (EnVision+ detection system, Dako). Antibody localization was effected by using a peroxidase reaction with 3,3'-diaminobenzidine tetrahydrochloride (DAB+, Dako) as the chromogen. Sections were counterstained with hematoxylin, dehydrated, and mounted. Positive controls included human tonsil (FcεRIα and CD23), human skin (CD1a and langerin), human papillary thyroid carcinoma (galectin-3), and human gastrointestinal stromal tumor (c-kit). Isotype control studies were also performed using the same protein concentrations as the primary antibodies. On selected cases, double staining with FcεRIα (Envision Plus, using 3,3'-diaminobenzidine tetrahydrochloride (DAB+, Dako), as the chromogen for a brown reaction product) and either CD1a or c-kit (alkaline phosphatase, using Fast Red (Dako) as the chromogen for a red reaction product) was performed.

Three independent investigators (JLH, AB, MD) who were blinded to the subjects' history and clinical diagnosis performed all histopathological analyses. Quantification of intraepithelial FcεRI-positive cell numbers was performed using the same method described above for eosinophils.

Statistical Analysis

The study was powered to detect a 40% difference between EoE and RE biopsies with an alpha of 0.05, and a power of 80%. Mean FcεRI counts were compared across diagnosis groups using ANOVA. Pair-wise comparisons were carried out using the Bonferroni procedure. Mean eosinophil counts were compared across groups using the Kruskal-Wallis test. Correlations between the mean number of FcεRI-positive cells and the number of eosinophils per HPF were evaluated using Spearman's correlation coefficient. Comparisons of mean FcεRI count based on atopy status or previous acid suppressing medication use were made using either the independent samples T-test or the Mann-Whitney U test. All statistical analysis was performed with SPSS v.16.0.

RESULTS

Patient Characteristics

Esophageal biopsies from 62 patients were analyzed (19 with EoE, 22 with RE, and 21 normal controls) (Table 1). The patients in all groups were of a similar age. There was a trend towards more males in the EoE group (OR 2.02, 95% C.I. 0.6, 6.6). Significantly more patients with EoE suffered from an atopic condition than non-EoE patients (OR 5.66, 95% C.I. 1.8, 18.2). As expected from the inclusion criteria EoE patients were also more likely to have been treated with acid-suppressing medication prior to endoscopy than were non-EoE patients (OR 5.04, 95% C.I. 1.03, 24.7).

FcεRI is the main IgE-binding structure on intraepithelial immune cells in esophageal biopsies from patients with EoE

We studied the presence of the three known human IgE receptors, CD23, galectin 3 and FcεRI, using immunohistochemistry in biopsies from patients with EoE. Infiltrating cells in EoE lesions react strongly with the monoclonal antibody CRA-1, which specifically recognizes the IgE-binding alpha chain of FcεRI (Figure 2 A). We could not detect any expression of CD23 in the biopsies (Figure 2 C). Galectin 3 showed weak, non-specific staining of squamous epithelial cells, but no reactivity in inflammatory cells (Figure 2 E). We included positive controls containing tissues that express the respective protein for each reagent (Figure 2 B, D, and F). The results of this set of experiments show that FcεRI is the only IgE-binding structure expressed on intraepithelial immune cells in the esophagus of EoE patients.

Expression of FcεRI in the esophagus

FcεRI was detected in biopsies from all diagnostic groups. The number and distribution of FcεRI positive cells differed between normal biopsies and EoE or RE biopsies (Fig. 3). FcεRI positive cells in normal biopsies were located in the basal zone of the epithelium (Fig. 3 C), whereas FcεRI positive cells in EoE (Fig. 3A) and RE (Fig. 3B) biopsies were distributed throughout the entire thickness of the epithelium.

Patients with EoE had higher levels of FcεRI in their proximal esophageal biopsies as compared with normal controls (32.6 ± 19.0 vs. 15.6 ± 8.3 , $p=0.006$) (Fig. 3 D). However, in the distal esophageal biopsies, no significant difference was detected between EoE and controls (24.2 ± 16.2 vs. 15.3 ± 8.4 , $p=0.39$) (Fig. 3 E). RE patients had higher FcεRI levels in distal esophageal biopsies when compared to controls (35.7 ± 27.6 vs. 15.3 ± 8.4 , $p=0.004$) (Fig. 3 E). There was no difference in the number of FcεRI positive cells between EoE and RE patients in either the proximal (32.6 ± 19.0 vs. 26.7 ± 16.6 , $p=0.7$) or distal biopsies (24.2 ± 16.2 vs. 35.7 ± 27.6 , $p=0.2$) (Fig. 3 E, D).

Additionally, we found that in all conditions the number of FcεRI positive cells was not impacted by prior therapy with acid suppressing medications (Table 2). In all conditions, there was a trend towards higher numbers of FcεRI positive cells in esophageal biopsies of patients with any atopic condition versus those with no atopic disease, but this did not reach statistical significance (Table 2).

Correlation between FcεRI-positive cells and eosinophils in esophageal biopsies

Based on the literature published about FcεRI expression on eosinophils (20-22) and the high number of eosinophils that are characteristic for EoE lesions, we expected to see a higher number of receptor-positive cells in EoE versus RE. We analyzed whether the presence of FcεRI correlated with the number of eosinophils in the biopsy specimens.

There was a weak correlation between the number of FcεRI-positive cells and intraepithelial eosinophils in proximal biopsies ($r=0.387$, $p=0.003$) (Fig. 4 A). In distal biopsies, FcεRI-positive cells were poorly correlated with intraepithelial eosinophil count ($r=0.280$, $p=0.044$) (Fig. 4 B).

FcεRI expression on intraepithelial dendritic cells, mast cells and eosinophils in the esophagus

Based on our findings that the number of FcεRI-positive cells correlated weakly with the number of eosinophils, we hypothesized that additional cell types in the esophageal epithelium must express FcεRI. The interdigitating appearance of the FcεRI-positive cells along the basal layer in normal tissue biopsies suggested that some of these cells might be dendritic cells. To investigate this possibility, we chose 5 biopsies from the entire cohort with the highest numbers

of FcεRI positive cells and 5 biopsies with the lowest numbers, and analyzed them for the presence of CD1a and langerin, both markers for Langerhans cells. As shown in Figure 5, numerous CD1a and langerin-positive dendritic cells are observed in biopsies with high numbers of FcεRI-positive cells, but not in biopsies with low numbers of FcεRI-positive cells. Figure 6 shows examples of double labeled slides for both FcεRI and CD1a (A), and FcεRI and c-kit (B). As can be observed, the CD1a and c-kit-positive cells are nearly invisible, which suggests they are essentially all staining with FcεRI.

Staining for mast cells with c-kit in biopsies with high numbers of FcεRI-positive cells revealed the presence of some mast cells in the esophageal biopsies with high FcεRI expression, with lower numbers of mast cells correlating with lower FcεRI expression (Table 3).

DISCUSSION

This is the first study describing the high affinity receptor for IgE, FcεRI, in the esophageal epithelium. We demonstrate that FcεRI is the only receptor for IgE expressed by immune cells infiltrating the esophageal epithelium of patients with EoE. Neither CD23 nor galectin-3 were expressed in the esophageal epithelium. FcεRI receptor expression in esophageal tissue was significantly up-regulated in patients with either EoE or RE when compared to esophageal tissue from healthy controls. Surprisingly, no significant difference was found in FcεRI expression between EoE and RE patients. Whether the esophageal inflammation was due to acid injury or an allergic process, the FcεRI expression in the esophagus was increased. Eosinophils are known to express FcεRI. (12,21,23,24) Our immunohistochemical studies suggest that, in addition to eosinophils, another cell type that expresses FcεRI in the inflamed esophagus is the Langerhans cell.

Increased numbers of IgE-bearing cells have been demonstrated in EoE versus normal controls. (25,26) In this study, we have established that FcεRI is highly expressed in the esophagus of patients suffering from either EoE or RE, both inflammatory conditions. Increased numbers of FcεRI-positive cells match areas of greatest inflammation. In RE, the distal esophagus is exposed to the highest amount of acid, and thus typically exhibits higher degrees of inflammation than the proximal esophagus. This pattern of inflammation is matched by the same pattern of increased FcεRI-positive cells in the RE samples. Conversely, in EoE, the pattern of inflammation is usually either the same throughout, or worse in the proximal esophagus. We show that FcεRI-positive cells are greater in the proximal than the distal esophageal biopsies from patients with EoE.

Although we found similar numbers of FcεRI-positive cells in the esophageal biopsies from patients with EoE and RE, our results cannot differentiate between cell surface FcεRI loaded with IgE and intracellular FcεRI. FcεRI is highly expressed in both EoE and RE, but not in normal controls, suggesting that its expression may be enhanced by inflammation, and not specifically by allergen binding. However, the possibility remains that the activation state of FcεRI is different between patients with EoE versus RE. Blanchard et al have shown that FcεRI is differentially up regulated in the esophageal epithelium of patients with active EoE as compared to RE and normal controls. (3) Our immunohistochemistry approach probably lacks the sensitivity to detect a modest difference in transcription or in cell surface antigen density. Eosinophils typically express a lower amount of FcεRI than mast cells, basophils, or dendritic cells. (15,24) Therefore, the increased expression of FcεRI that would be accounted for by the eosinophils in EoE may be too low to detect via immunohistochemistry. Our data show a modest correlation between eosinophils and FcεRI-positive cells in the proximal biopsies, where the majority of the inflammation is represented by EoE samples. This indicates that some of the signal from eosinophils is captured; yet the qualitative difference between EoE and RE is insufficient to reach significance. To assess the possibility of differences in

receptor numbers per individual cell between these patient groups, other methods such as flow cytometry or immunofluorescence must be used where precise quantitation is possible.

The lack of correlation between FcεRI and number of eosinophils suggested that FcεRI was also expressed by other cell types. In humans, FcεRI is found on mast cells, basophils, eosinophils, and antigen presenting cells such as Langerhans and other dendritic cells. (15) We evaluated esophageal biopsies with high levels of FcεRI for the presence of mast cells using the c-kit stain. While we found mast cells increased in biopsies with higher numbers of FcεRI, it was evident that there were many more FcεRI-positive cells than mast cells. The morphology of the FcεRI-positive cells included round cells and cells with dendritic processes. The round cells likely represent the mast cells and eosinophils, which are both expected to express FcεRI. Basophils, although not quantitated in these studies, could also account for some number of FcεRI positive cells. The dendritic-type cells were further investigated with antibodies specific for CD1a and langerin, both markers of Langerhans cells. Using immunohistochemistry on historically collected samples, we were limited in our ability to show double staining of both FcεRI and any other cellular marker. However, the presence, morphology and distribution of CD1a and langerin-positive cells seemed to match FcεRI-positive cells. This suggests that Langerhans cells in the esophageal epithelium are partly responsible for the high levels of expression of FcεRI. Langerhans cells are a subset of dendritic cells specialized in initiating primary immune responses. It is possible that Langerhans cells play a role in the pathogenesis of EoE. This argument is supported by several reports in the literature that describe an important role for Langerhans cells in IgE-mediated cell activation in several allergic diseases. (27-29) While Lucendo et al have reported equal numbers of Langerhans cells in the esophageal epithelium of patients with eosinophilic esophagitis, reflux esophagitis, or healthy controls (25), we find that Langerhans cells are increased in biopsies from patients with both eosinophilic and reflux esophagitis who also have high levels of FcεRI expression in their esophageal biopsies. .

The difference between EoE and RE may lie in the response of FcεRI-bearing dendritic cells to antigen exposure. In allergic individuals, IgE bound to FcεRI at the dendritic cell surface is poised to bind antigens with which it comes in contact. In the esophageal epithelium, this may result in dendritic cell activation and recruitment of eosinophils to the area via known mediators such as IL-5, IL-13, and eotaxin-3. (3,4,30-32)

We found a trend toward higher numbers of FcεRI-positive cells in patients with atopic conditions. Because of the uneven distribution of atopy across groups, we were unable to determine the impact of atopy on FcεRI expression within each diagnostic group. In keeping with the accepted theory that EoE is an allergic disease, patients with EoE in our study were more likely to suffer from an atopic condition than were either patients with RE or normal controls. This finding is consistent with previously described patient characteristics and supports the theory that EoE is an allergic disease. (1,5,33,34)

There are some limitations to the study. Our study was retrospective and used biopsy samples that had been obtained as part of routine clinical care. Clinical characteristics of the patients and sampling of the esophagus were therefore not completely standardized. Also, the study sample size was small and could result in a Type II error, missing a true difference between EoE and RE with respect to the number of FcεRI-positive cells in the esophageal epithelium. The study was powered to detect a difference of 40% between EoE and RE. This now appears to be an overestimation of the difference between EoE and RE, and, therefore, we may be left with the inability to detect a difference between the two groups in the current study. Despite these limitations, we found a clear distinction between the healthy state and inflammation with respect to FcεRI expression by infiltrating immune cells such as Langerhans cells and mast

cells in the esophageal epithelium. Future prospective studies to address these questions are needed.

In conclusion, FcεRI is the only receptor for IgE expressed in the esophageal epithelium of patients with EoE. Langerhans cells in the esophageal epithelium are likely one of the main bearers of FcεRI in both healthy and diseased states. Since this receptor is the only IgE binding structure on the EoE infiltrates, it must be critical for IgE-mediated activation at this tissue site. Given that we see the same receptor expressed in both EoE and RE patients, more studies are needed to better define the precise role for this receptor in Th2-mediated esophageal diseases.

Acknowledgments

FUNDING: This work was supported by a grant from the Gerber Foundation (Drs. Nurko and Fiebiger). Further support comes from a CDHNF Young Investigator Award from NASPHGAN (to Dr. Fiebiger), the APART Program of the Austrian Academy of Sciences (Dr. Dehlink) and NIH grant DK082792A (Dr. Nurko)

REFERENCES

1. Noel RJ, Putnam PE, Rothenberg ME. Eosinophilic esophagitis. *N Engl J Med* 2004;351:940–1. [PubMed: 15329438]
2. Straumann A, Bauer M, Fischer B, et al. Idiopathic eosinophilic esophagitis is associated with a T(H) 2-type allergic inflammatory response. *J Allergy Clin Immunol* 2001;108:954–61. [PubMed: 11742273]
3. Blanchard C, Wang N, Stringer KF, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest* 2006;116:536–47. [PubMed: 16453027]
4. Blanchard C, Mingler MK, Vicario M, et al. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol* 2007;120:1292–300. [PubMed: 18073124]
5. Assa'ad AH, Putnam PE, Collins MH, et al. Pediatric patients with eosinophilic esophagitis: an 8-year follow-up. *J Allergy Clin Immunol* 2007;119:731–8. [PubMed: 17258309]
6. Roy-Ghanta S, Larosa DF, Katzka DA. Atopic characteristics of adult patients with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2008;6:531–5. [PubMed: 18304887]
7. Collins MH, Blanchard C, Abonia JP, et al. Clinical, pathologic, and molecular characterization of familial eosinophilic esophagitis compared with sporadic cases. *Clin Gastroenterol Hepatol* 2008;6:621–9. [PubMed: 18434257]
8. Kagalwalla AF, Sentongo TA, Ritz S, et al. Effect of six-food elimination diet on clinical and histologic outcomes in eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2006;4:1097–102. [PubMed: 16860614]
9. Spergel JM, Andrews T, Brown-Whitehorn TF, et al. Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. *Ann Allergy Asthma Immunol* 2005;95:336–43. [PubMed: 16279563]
10. Markowitz JE, Spergel JM, Ruchelli E, et al. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. *Am J Gastroenterol* 2003;98:777–82. [PubMed: 12738455]
11. Kelly KJ, Lazenby AJ, Rowe PC, et al. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. *Gastroenterology* 1995;109:1503–12. [PubMed: 7557132]
12. Sihra BS, Kon OM, Grant JA, et al. Expression of high-affinity IgE receptors (Fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. *J Allergy Clin Immunol* 1997;99:699–706. [PubMed: 9155838]
13. Maurer D, Fiebiger E, Reininger B, et al. Expression of functional high affinity immunoglobulin E receptors (Fc epsilon RI) on monocytes of atopic individuals. *J Exp Med* 1994;179:745–50. [PubMed: 8294882]
14. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* 2008;8:205–17. [PubMed: 18301424]

15. Kraft S, Kinet JP. New developments in FcεRI regulation, function and inhibition. *Nat Rev Immunol* 2007;7:365–78. [PubMed: 17438574]
16. Nurko S, Teitelbaum JE, Husain K, et al. Association of Schatzki ring with eosinophilic esophagitis in children. *J Pediatr Gastroenterol Nutr* 2004;38:436–41. [PubMed: 15085025]
17. Walsh SV, Antonioli DA, Goldman H, et al. Allergic esophagitis in children: a clinicopathological entity. *Am J Surg Pathol* 1999;23:390–6. [PubMed: 10199468]
18. Teitelbaum JE, Fox VL, Twarog FJ, et al. Eosinophilic esophagitis in children: immunopathological analysis and response to fluticasone propionate. *Gastroenterology* 2002;122:1216–25. [PubMed: 11984507]
19. Furuta GT, Liacouras CA, Collins MH, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* 2007;133:1342–63. [PubMed: 17919504]
20. Capron M, Capron A, Dessaint JP, et al. Fc receptors for IgE on human and rat eosinophils. *J Immunol* 1981;126:2087–92. [PubMed: 7229368]
21. Capron M, Spiegelberg HL, Prin L, et al. Role of IgE receptors in effector function of human eosinophils. *J Immunol* 1984;132:462–8. [PubMed: 6228581]
22. Dehlink E, Fiebiger E. The role of the high-affinity IgE receptor, FcεRI, in eosinophilic gastrointestinal diseases. *Immunol Allergy Clin North Am* 2009;29:159–70. xii. [PubMed: 19141351]
23. Tanaka Y, Takenaka M, Matsunaga Y, et al. High affinity IgE receptor (Fc εRI) expression on eosinophils infiltrating the lesions and mite patch tested sites in atopic dermatitis. *Arch Dermatol Res* 1995;287:712–7. [PubMed: 8554381]
24. Humbert M, Grant JA, Taborda-Barata L, et al. High-affinity IgE receptor (FcεRI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. *Am J Respir Crit Care Med* 1996;153:1931–7. [PubMed: 8665058]
25. Lucendo AJ, Navarro M, Comas C, et al. Immunophenotypic characterization and quantification of the epithelial inflammatory infiltrate in eosinophilic esophagitis through stereology: an analysis of the cellular mechanisms of the disease and the immunologic capacity of the esophagus. *Am J Surg Pathol* 2007;31:598–606. [PubMed: 17414108]
26. Kirsch R, Bokhary R, Marcon MA, et al. Activated mucosal mast cells differentiate eosinophilic (allergic) esophagitis from gastroesophageal reflux disease. *J Pediatr Gastroenterol Nutr* 2007;44:20–6. [PubMed: 17204948]
27. Bieber T. The pro- and anti-inflammatory properties of human antigen-presenting cells expressing the high affinity receptor for IgE (Fc εRI). *Immunobiology* 2007;212:499–503. [PubMed: 17544834]
28. Novak N, Bieber T. The role of dendritic cell subtypes in the pathophysiology of atopic dermatitis. *J Am Acad Dermatol* 2005;53:S171–6. [PubMed: 16021172]
29. Novak N, Bieber T, Kraft S. Immunoglobulin E-bearing antigen-presenting cells in atopic dermatitis. *Curr Allergy Asthma Rep* 2004;4:263–9. [PubMed: 15175139]
30. Mishra A, Hogan SP, Brandt EB, et al. IL-5 promotes eosinophil trafficking to the esophagus. *J Immunol* 2002;168:2464–9. [PubMed: 11859139]
31. Schmid-Grendelmeier P, Altnauer F, Fischer B, et al. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J Immunol* 2002;169:1021–7. [PubMed: 12097410]
32. Mishra A, Rothenberg ME. Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. *Gastroenterology* 2003;125:1419–27. [PubMed: 14598258]
33. Spergel JM, Brown-Whitehorn TF, Beausoleil JL, et al. 14 years of eosinophilic esophagitis: clinical features and prognosis. *J Pediatr Gastroenterol Nutr* 2009;48:30–6. [PubMed: 19172120]
34. Guajardo JR, Plotnick LM, Fende JM, et al. Eosinophil-associated gastrointestinal disorders: a world-wide-web based registry. *J Pediatr* 2002;141:576–81. [PubMed: 12378201]

Figure 1.

Representative esophageal biopsies from patients with eosinophilic esophagitis (A), reflux esophagitis (B), and normal controls (C), stained with hematoxylin and eosin.

Figure 2. Presence of IgE receptors in the esophagus of patients with eosinophilic esophagitis
 Left panels (A,C,E) show esophageal biopsies from patients with eosinophilic esophagitis; right panels show positive control sections from tonsils (B, D) and papillary thyroid carcinoma (F). Figures A and B show immunohistochemistry with anti-FcεRI specific monoclonal antibody, figures C and D anti-CD23 specific monoclonal antibody, and figures E and F anti-galectin-3 specific monoclonal antibody. As can be seen, FcεRI is the main IgE binding structure on intraepithelial inflammatory cells infiltrating the esophageal epithelium of patients with eosinophilic esophagitis.

Figure 3. Comparative levels of FcεRI in esophageal biopsies from patients with eosinophilic esophagitis (EoE), reflux esophagitis (RE), and normal controls

Infiltrating cells in the esophageal epithelium of patients with EoE (A), RE (B), and normal controls (C) are positive for the high affinity IgE-receptor FcεRI. There is a higher number of FcεRI-positive cells in esophageal biopsies from patients with EoE and RE, compared to healthy controls. (*) $p=0.006$ and (#) $p=0.004$ using ANOVA across groups followed by the Bonferroni procedure.

Figure 4. Scatter plot of FcεRI-positive cells and eosinophil counts in proximal (A) and distal (B) esophageal biopsies.

Figure 5. Presence of Langerhans cells in the esophagus

Upper panels (A-C) show representative esophageal biopsies with high FcεRI-positive cell counts, and lower panels (D-E) show low FcεRI-positive cell counts. Figures A and D show immunohistochemistry with anti-FcεRI specific monoclonal antibody, figures B and E anti-CD1a specific monoclonal antibody, and figures C and F anti-langerin specific monoclonal antibody. Langerhans cells are present in high numbers in the esophageal epithelium of patients with high numbers of FcεRI-positive cells, but not in those with low FcεRI positive cell counts.

Figure 6. Double labeling to evaluate the relationship between FcεRI-positive cells, Langerhans cells, and mast cells

Immunohistochemistry was performed with both (A) FcεRI (brown) and CD1a (red) and (B) FcεRI (brown) and c-kit (red). The red reaction product on the CD1a or c-kit-positive cells is nearly invisible in both images, which suggests that essentially all these cells are staining with FcεRI.

Table 1
Patient characteristics

Age, sex, diagnosis of any atopic condition, use of acid suppressing medication, and average number of eosinophils per high power field (HPF) in proximal and distal esophageal biopsies were ascertained for all patients. There were significant differences in the number of eosinophils/HPF, and the history of atopy when comparing between groups

	Eosinophilic Esophagitis (n=19)	Reflux Esophagitis (n=22)	Normal (n=21)	p-value
Age (years)	8.3	9.3	8.4	0.846 ^a
Sex (% female)	26.3	40.9	42.9	0.501 ^b
Any atopic condition (%)	54.6	27.3	18.2	0.004 ^c
Acid suppressing medication use (%)	89.5	59.1	66.7	0.088 ^b
<i>Eosinophils/HPF</i> (Median)				
Distal	29.3	5.3	0	<0.0001 ^d
Proximal	22.3	0	0	<0.0001 ^d

^aANOVA

^bPearson chi square

^cLinear by linear association

^dKruskal Wallis

Table 2
Effect of atopy and prior use of acid suppressing medications on the number of FcεRI-positive cells per high power field

Data from all diagnostic groups was united to test whether previous use of acid suppressing medications or diagnosis of atopy impacted the numbers of FcεRI-positive cells in the esophageal epithelium

	Previous acid-suppressing medication use		History of atopy	
	Yes	No	Yes	No
Proximal	24.59 (n=40)	23.25 (n=16)	31.64 (n=16)	21.23 (n=40)
Distal	23.74 (n=39)	25.10 (n=17)	27.58 (n=22)	21.94 (n=34)

^aMann-Whitney U Test

^bProximal biopsies compared with independent samples T-test; distal biopsies compared with Mann-Whitney U Test

Table 3
Comparison of eosinophils and CD1a, Langerin, and c-kit positive cells in esophageal biopsies with high versus low numbers of FcεRI positive cells

Cell Marker	High Fc epsilon RI	Low Fc epsilon RI
	Positive cells per hpf (mean ± SD)	Positive cells per hpf (mean ± SD)
Fc epsilon RI	53.6 ± 13.1	5.2 ± 2.7
Eosinophils	14.5 ± 19.2	0 ± 0
CD1a	14.2 ± 6.1	8 ± 1.6
Langerin	13.4 ± 5.0	7 ± 2.3
c-kit	6.5 ± 4.1	0.8 ± 0.8