

CORRESPONDENCE

Mucosal mast cell counts in pediatric eosinophilic gastrointestinal disease

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To the Editor,

Primary eosinophilic gastrointestinal disorders (EGID) include a spectrum of conditions where increased collection of eosinophil leukocytes occurs within the intestinal tract in the setting of symptomatic gastrointestinal (GI) disease. Disorders falling under this broad category include eosinophilic esophagitis (EoE), eosinophilic gastroenteritis, eosinophilic colitis, and eosinophilic proctitis.(1).

With the emergence of EoE, mutual efforts of allergists and gastroenterologists continue to identify candidate biomarkers of EGID, including mast cell-associated ones.(2) These markers may help to understand the pathophysiology of the disorders, guide their diagnosis and treatments, as well as aid the monitoring of treatment response. Increased numbers of mast cells have been described in adults and pediatrics patients with EoE (3), but there is limited knowledge of these cells in other EGID conditions.

We conducted a retrospective study to investigate correlation between eosinophil and mast cell numbers in pediatric EGID (excluding EoE) and controls.

Patients with EGID were identified from the EGID clinic at Texas Children's Hospital (TCH). The diagnosis was based on increased number of eosinophils above empirically set internal reference ranges at TCH in stomach, duodenum, terminal ileum, right, or left colon. Control patients included those that underwent endoscopic evaluation for constipation and suspected polyp. Patients with any other conditions where mucosal eosinophilia has been observed (e.g., inflammatory bowel disease, irritable bowel syndrome, diarrhea, abdominal pain, and celiac disease) were excluded from the control group. Age, gender, and allergies were recorded for the purpose of this study. Percent of peripheral eosinophils within 2 months of the endoscopy was documented.

The biopsy specimens were examined by a pediatric gastrointestinal pathologist, who was blinded to the clinical diagnosis, and the previously reported histology reports. The biopsy specimens were fixed in 10% neutral-buffered formalin immediately following endoscopy. Paraffin sections (3 micron) were cut and stained with hematoxylin and eosin (H&E) with 8 tissue sections on 1 slide. They were reviewed for eosinophil count at $\times 400$ magnification. Duplicated 3-micron sections were stained for mast cell tryptase (MCT) via immunohistochemistry using a monoclonal mouse antihuman MCT antibody (clone AA1, code number 7052, DAKO, Glostrup, Denmark). After pre-treatment with trypsin for 5 min at room temperature, the specimens were incubated with the primary antibody dilution 1:200 at room temperature for 15 minutes. Novocastra Bond Polymer Refine Detection System (biotin-free, peroxide conjugated) was used (from LEICA, Newcastle upon Tyne, United Kingdom) with diaminobenzidine

tetrahydrochloride (DAB) as the chromogen and hematoxylin as the counter stain.

Nonparametric Mann–Whitney U-test (Prism software version 2.0 [Graph Pad Inc., San Diego, California, USA]) was employed for group comparisons. Pearson correlation coefficient was calculated in Excel (Microsoft Office Excel 2007). Significance for correlation was calculated with public Statistics Calculator version 3.0 (<http://www.danielsoper.com/statcalc3/calc.aspx?id=44>). Statistical significance was determined at $p < 0.05$. The study was approved by the IRB of Baylor College of Medicine (protocol number H-32539).

Twenty-six patients were in the study; 14 controls and 12 with EGID. Intestinal biopsies from stomach, duodenum, terminal ileum, right, and left colon were examined. Not all

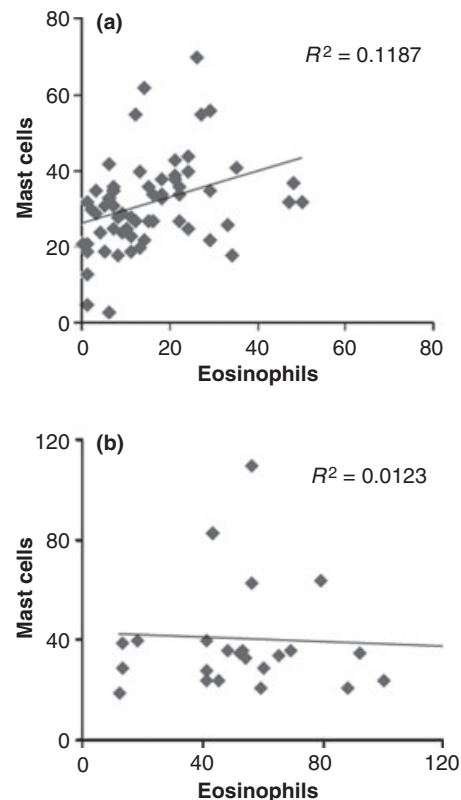


Figure 1 Scatter plots between mast cell–eosinophil counts. a. Control samples from stomach, duodenum, terminal ileum, and colon showed a positive correlation ($n = 63$; $r = 0.34$; $p = 0.0057$). b. EGID patient biopsies where eosinophil counts superseded the normal reference range showed no significant correlation between eosinophil and mast cell numbers ($n = 26$; $r = -0.11$; $p = 0.59$).

the 26 patients had tissue samples from the 5 mentioned anatomic locations. A total of 126 slides were reviewed (63 from each group) for eosinophil and MCT count. The average age of the patients in the EGID group was 10.4 yr (2.4–16.11 yr) with 7 females and 5 males, compared with control group average age of 12.3 yr (3.7–17.4 yr, $p = 0.37$) with 10 females and 4 males ($p = 0.18$). Only 2 patients in the EGID group had positive skin prick test, and the remaining had no documented evidence of food or environmental allergies.

The average percent of peripheral eosinophils within a 2-month period of the endoscopy in the EGID group was 2.96% compared to control with 1.82% ($p = 0.43$).

We investigated the correlation between tissue mast cells and eosinophils. There was a significant positive correlation ($r = 0.34$; $p = 0.0057$) between eosinophil and mast cell counts in the control mucosal samples (Fig. 1a). However, in those biopsy samples from the patients with EGID where the eosinophil counts were above normal limits ($n = 26$), there was no significant correlation ($r = -0.11$; $p = 0.59$) between eosinophils and mast cell numbers (Fig. 1b).

Next, we examined the difference between control and EGID mast cells numbers according to anatomic locations (stomach, duodenum, terminal ileum, right/left colon). Only those EGID biopsies were compared, where eosinophil counts fulfilled the criteria for disease. In none of the areas, did we find a significant differences between control and EGID (stomach $p = 0.32$; duodenum $p = 0.57$; terminal ileum $p = 0.12$; and colon $p = 0.92$).

EGID is histologically characterized by increased infiltration of eosinophils in the gastrointestinal tract and a wide range of common symptoms such as abdominal pain, vomiting, and diarrhea. As it is relatively uncommon condition, the prevalence and clinical associations of pediatric EGID are unclear (4). However, approximately 2–8% children manifest IgE and non-IgE food allergies.

There is limited understanding of the key mediators that may play a role in the pathogenesis and symptoms of EGID. Mucosal mast cell infiltration has been described in other

gastrointestinal diseases, such as celiac disease, inflammatory bowel disease, and diarrhea predominant irritable bowel syndrome (5–7) as well. However, standardized reference ranges for these cells in the GI tract do not exist. One adult study defined 20 mast cells/high power field as the upper normal limit in colonic biopsy specimens (8). The number of mast cells in gastric tissue has shown to be increased in pediatric patients with EGID (9), but this finding was not consistent in the duodenum regardless of eosinophilia. In the meantime, the significance of mast cells with eosinophilia is uncertain. The mast cell–eosinophil interaction is a complex phenomenon where IL-13 produced by mast cells in recruiting eosinophils may play an important role.(10).

To our knowledge, this is the first study to investigate the association between mast cell and eosinophil numbers not only in the upper, but also in the lower gastrointestinal tract of children. Although our cohorts are small, those surpass other pediatric studies on this topic (9) and have provided significant results. Interestingly, there was a significant positive correlation between eosinophil and mast cell counts in the control mucosal samples. This correlation was observed to be absent in EGID raising the potential possibility for disrupted mast cell–eosinophil interaction playing a role in pathogenesis. It should be noted that the MCT staining utilized (while being a sensitive and specific for detection of tissue mast cells) only reveals granulated mast cells. Therefore, it is possible that augmented mast cell degranulation occurred in the patients with EGID where increased eosinophils were present, and this lead to the lack of positive correlation between the cell types studied. These possibilities warrant further studies to increase our understanding of EGID in children.

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