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Original contribution

Histologic analysis of eosinophils and mast cells of the gastrointestinal tract in healthy Canadian children☆

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Summary Many gastrointestinal (GI) disorders, including GI eosinophilia and inflammatory bowel disease, can be characterized by increased mucosal eosinophils (EOs) or mast cells (MCs). Normal mucosal cellular counts along the GI tract in healthy children have not been established for a Canadian pediatric population. To establish a benchmark reference, we quantified EO and MC from 356 mucosal biopsies of the GI tract obtained during upper and lower endoscopic biopsies of 38 pediatric patients in eastern Ontario. Mean total counts of EO varied for the 11 tissues we examined, from a low of 7.6 ± 6.5/high-power field (HPF) (×40 [×400, 0.55mm^2]) in the body of the stomach to a high of 50.3 ± 17.4/HPF in the cecum. The lower GI tract (ileum, cecum, colon, sigmoid, and rectum) generally had higher total EO counts than the upper GI tract (antrum and body of stomach, duodenum, and duodenal cap) (combined average of 32.1 ± 20.6 versus 19.3 ± 15.8, respectively). Similarly, the number of mucosal MC was different in the various regions of the GI tract ranging from 0.04 ± 0.2/HPF in the duodenal cap to 0.9 ± 2.6/HPF in the ileum. Total counts for EO and MC in the lamina propria were not significantly different between sexes when adjusted for multiple testing. EO polarity was absent in many cases, irrespective of the GI region. These numeration and localization of EO and MC will provide normative data for upper and lower endoscopic GI biopsies in the pediatric population of Eastern Ontario.

1. Introduction

Eosinophils (EOs) are normally observed in the mucosa of respiratory [1], lower genitourinary, and gastrointestinal (GI) tract locations, with the exception of the normal esophagus, which has no EO localization [2]. The precise function of

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EO in the GI tract is not fully understood, but it is known that they have a role in the mucosal immune response [3]. The number of intramucosal EO is known to be widely variable among individuals depending on age [4], exposure to food allergens, and exposure to infectious agents [5]. Aeroallergens can also induce GI mucosal eosinophilia [6]. Mast cells (MCs) are thought to be prototypes of innate immune cells, and their activation has been implicated in many types of neuroinflammatory responses and related disturbances of gut motility [7]. Quantification of colorectal EO and MC in healthy pediatric populations in 2 studies from the United States [8,9] established that EO counts also vary by geographic location of residence. To the best of our knowledge, there are no studies that have quantified and qualified EO and MC from upper and lower GI tract endoscopic biopsies in a Canadian pediatric population.

The diagnosis of eosinophilic mucosal diseases is largely based on numeration of EO in GI mucosal biopsies and, to a lesser extent, their distribution. Without reference standards, this renders the diagnosis of abnormal GI eosinophilia subjective. Establishing normal values and distribution of EO and MC in a pediatric population in central Canadian geographic location is of importance and the focus of this study.

2. Materials and methods

2.1. Study design

This study was approved by the Children's Hospital of Eastern Ontario (CHEO) Ethics Board. Pathology reports and medical records of patients who underwent a GI biopsy at CHEO between April 2013 and April 2014 were collected and reviewed. Data were extracted on patient demographics (eg, sex, age) and clinical information (eg, indication for biopsy, diagnosis). Study inclusion criteria were as follows: age younger than 18 years, normal macroscopic visualization of the mucosa during endoscopy, no underlying diagnosis of a chronic disease, no history of allergic disorders including asthma, no parasitic infections, no medication/drugs 3 months before the biopsy time, and a final clinical and pathologic diagnosis that did not involve the GI system. In addition, we included 2 patients whose pathology was normal but had medical history of health concerns (eg, *Helicobacter pylori* and short bowel due to meconium peritonitis).

2.2. Biopsy handling and evaluation

Biopsies were obtained using serrated jaws with an outside closed diameter of 2.2 mm. The biopsies were obtained during upper (esophagogastroduodenoscopy) and lower (colonoscopy) endoscopy and included multiple biopsies of the gastric body and antrum, duodenum, terminal ileum, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum from 39 patients. Esophageal biopsies were also evaluated and were found to be normal but were not included due to the abundance of evidence in this topic.

Specimens were immediately immersed in 10% buffered formalin and transported to the Pathology Department whereupon they were embedded in paraffin within 24 hours. Sections (5 μm) were cut and stained with the hematoxylin-phloxine-saffron and Giemsa stain, the latter to facilitate EO and MC enumeration. Two pediatric pathologists blinded to the clinical data reviewed all GI biopsies meeting the inclusion criteria and reached a consensus on counts through discussion. On each slide, contiguous areas were scanned at ×20 (field diameter, 1.1 mm^2) for the distribution and polarity of EO. Polarity/distribution of EO and MC within the GI mucosa refers to their location predominantly in the subepithelial area or at the middle of the lamina propria or closer to the mucosa muscularis. Absence of polarity is defined when EOs or MCs were distributed evenly throughout all layers of mucosa. The area of maximum cellular density was used for the EO and MC count. In each biopsy, irrespective of its site, the presence or absence of the muscularis mucosae was noted to indicate the depth of the biopsies.

EOs were considered granulated if they contained densely packed granules (whether they had a nucleus) and were the size of normal EO cells. EOs were considered degranulated if their granules were loose and closely spaced. All granulated and degranulated EOs and MCs were counted. Fields close to the edge of the tissues were avoided as degranulated EO in those areas may represent tissue trauma [8,9,10]. The number of EO in the surface epithelium, the lamina propria, and crypt epithelium were counted separately at ×40 (×400, 0.55mm^2) and then added for a total count including granulated and degranulated EO. Similarly, MC in the surface epithelium, the lamina propria, and crypt epithelium were counted separately at ×40 (×400, 0.55mm^2) and then added up for a total count.

EOs or MCs were considered to be within the surface epithelium if they were located on the epithelial side of the basement membrane. The EOs or MCs were considered to be within the crypts if they were found within the epithelial layer of the crypt or within the crypt lumen. EOs or MCs present in the mucosa that were neither in the crypts nor in the surface epithelium were considered to be within the lamina propria. EOs or MCs that were less than 1/high-power field (HPF) (×20) away from a Peyer's patch or other lymphoid aggregates were excluded from quantification, as EO and lymphocytes may be numerous in these regions [9].

2.3. Statistical analysis

Mean EO and MC counts per ×40 HPF ± SD were calculated, and the median value and range were provided. Wilcoxon signed-rank test for nonparametric data was used to determine *P* values as the count did not have normal distribution. *P* values were also adjusted for multiple testing using Holm correction.